FAMILIAL FRONTOTEMPORAL LOBAR DEGENERATION

PHENOTYPICAL CHARACTERIZATION OF PRESYMPTOMATIC AND CLINICAL DISEASE STAGES

ELISE DOPPER

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Familial Frontotemporal Lobar Degeneration

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Fenotypische karakterisering van de presymptomatische en klinische ziektestadia

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CONTENTS

CHAPTER 1

General introduction

Frontotemporal lobar degeneration (FTLD) is a devastating neurodegenerative disorder, characterized by destruction of the frontal and temporal lobes, leading to behavioral disorders and language deficits that typically present before the age of 65 years. Most patients present with either the behavioral variant of frontotemporal dementia or primary progressive aphasia. Some patients exhibit additional symptoms of amyotrophic lateral sclerosis, corticobasal syndrome or progressive supranuclear palsy. Moreover, some patients exhibit an amnestic syndrome or prominent psychiatric disturbances. This large clinical diversity hampers diagnostic accuracy in the initial phase of the disease. Several causative genetic defects are associated with FTLD, of which mutations in the *microtubule-associated protein tau* and *progranulin* genes and the *C9orf72* repeat expansion are the most common. There currently is no therapy available to treat or cure the disease. Treatment strategies for FTLD largely rely on symptom management without evidence from randomized controlled trials. To come to a disease-modifying therapy, knowledge of the pathophysiology of the disease is essential. Hereditary forms of FTLD have learned us a lot about the FTLD disease process, which is crucial for the development of disease-modifying agents. For setting up clinical trials with such agents, proper biomarkers to detect the disease and track disease progression are crucial. Presymptomatic carriers of genetic mutations that cause FTLD provide a unique population to study the transition from the preclinical to the clinical disease stage and to identify suitable biomarkers for the earliest stage of the disease. In this chapter current knowledge about genetics and MRI biomarkers in FTLD is presented after shortly outlining the main clinical and pathological subtypes of FTLD.

CLINICAL SYNDROMES

FTLD patients typically present with frontotemporal dementia (FTD), which is an umbrella term for the behavioral variant of FTD (bvFTD) and the language variants named primary progressive aphasia (PPA). Clinical criteria for both bvFTD and PPA have recently been revised.[1, 2]

BvFTD is characterized by slowly progressive behavioral disturbances. The revised criteria include imaging characteristics and functional decline in order to separate probable from possible bvFTD.[2] This separation facilitates better differentiation of

bvFTD from other conditions, including the phenocopy syndrome. Patients with the phenocopy syndrome exhibit behavioral disturbances that are characteristic for bvFTD, but without the typical disease progression and imaging abnormalities. It remains to be elucidated whether a neurodegenerative disease underlies this phenocopy syndrome, or that it is merely a psychiatric disorder.[3] A diagnosis of possible bvFTD requires the presence of at least three out of six features, including disinhibition, apathy, loss of empathy, compulsive behavior, hyperorality, and executive dysfunction. Patients with additional presence of functional decline and frontotemporal atrophy or hypometabolism on neuroimaging fulfill probable bvFTD criteria. A definite diagnosis requires the presence of a known pathogenic mutation or pathological confirmation of FTLD.[2] Deficits in social cognition, such as impaired emotion recognition, theory of mind or decision-making, have not been incorporated in the current bvFTD criteria, since neuropsychological tests assessing such deficits are not widely incorporated in clinical practice yet. However, it is increasingly recognized that such tests can be of great value for early diagnosis of bvFTD.[2, 4, 5]

Patients with prominent language deficits fulfilling PPA criteria can be subdivided into three language subtypes. Progressive non-fluent aphasia (PNFA) is characterized by agrammatism and hesitant speech, semantic dementia (SD) presents with impaired naming and word comprehension, and logopenic progressive aphasia (LPA) is defined by difficulties in word retrieval and repetition of sentences.[1] All three variants can be associated with both FTLD and Alzheimer's disease pathology, although PNFA and SD are more frequent in FTLD and LPA is more common in patients with pathological Alzheimer's disease.[1]

A minority of patients presents with or later develops symptoms of amyotrophic lateral sclerosis, which is characterized by rapidly progressive muscular atrophy and weakness, leading to death within a few years.[6] Other patients develop extrapyramidal symptoms, sometimes fulfilling criteria for the Parkinsonian disorders corticobasal syndrome (CBS), characterized by asymmetric rigidity and apraxia,[7] or progressive supranuclear palsy (PSP), with falls and vertical supranuclear gaze palsy.[8]

NEUROPATHOLOGY

FTLD is characterized by focal atrophy most prominent in the frontal and temporal lobes. Atrophy of the basal ganglia and depigmentation of the substantia nigra can also be found. Microscopically, spongiosis, neuronal loss, and gliosis are typically found. Using immunohistochemistry FTLD can be further classified based on the major constituent of protein accumulations in the brain.[9] More than 90% of FTLD patients show accumulation of either tau or transactive response DNA binding protein of 43 kDa (TDP-43).

FTLD-tau includes Pick's disease, corticobasal degeneration (CBD), progressive supranuclear palsy (PSP), argyrophilic grain disease (AGD), multiple system tauopathy with dementia (MSTD), neurofibrillary-tangle predominant dementia (NFTD), and white matter tauopathy with globular glial inclusions (WMT-GGI).[10] There are six isoforms of tau in the brain: three isoforms with three microtubule-binding repeats (3R tau) and three isoforms with four microtubule-binding repeats (4R tau). Tau accumulations in Pick's disease predominantly contain 3R tau, while CBD, PSP, AGD, MSTD, and WMT-GGI inclusions are composed of 4R tau, and NFTD is characterized by a mixture of 3R and 4R tau.[9]

FTLD with TDP-43-positive inclusions (FTLD-TDP) can be further classified into four subtypes based on the type of inclusions in the brain: type A is characterized by numerous dystrophic neurites and neuronal cytoplasmic inclusions and moderate numbers of neuronal intranuclear inclusions; type B shows moderate numbers of neuronal cytoplasmic inclusions, but few dystrophic neurites; in type C long dystrophic neurites are found, with few neuronal cytoplasmic inclusions; type D is characterized by numerous short dystrophic neurites and frequent neuronal intranuclear inclusions.[11]

Of the remaining 10% of FTLD without tau- or TDP-43-positive inclusions, the most common subtype is FTLD-FET, which is a group of disorders with accumulation of fused in sarcoma (FUS), Ewing's sarcoma protein (EWS), and TATA-binding protein associated factor 15 (TAF15).[12] The group of FTLD-FET includes atypical FTLD with ubiquinated inclusions (aFTLD-U), basophilic inclusion body disease (BIBD), and neuronal intermediate filament inclusion disease (NIFID), with distinct patterns

of pathology.[11, 12] Finally, a small group of patients only show positive staining for ubiquitin protease system, without tau-, TDP-43-, or FET-pathology, named FTLD-UPS.[11]

GENETICS OF FTLD

Around 40% of all clinical FTD cases have at least one other affected family member with dementia or a related disorder, such as ALS or Parkinsonism.[13-16] In 10-30% there is an autosomal dominant pattern of inheritance of the disease, which is defined as at least three affected persons in two generations, with one person being a first-degree relative of the other two.[13, 15, 16] However, the heritability varies per clinical subtype. BvFTD is associated with a positive family history most frequently, whereas SD is a sporadic syndrome in general.[13, 15, 16] Heritability estimates for FTD with motor neuron disease (FTD-MND) are inconsistent between different cohorts, which might be due to small sample sizes, but might also reflect actual geographical differences in heritability.[13, 15, 16] Over the past twenty years several genetic defects have been discovered that can cause an autosomal dominant familial form of FTLD. Mutations in the *microtubule-associated protein tau*, *progranulin* and *C9orf72* genes are responsible for most familial FTD cases, together accounting for 10 - 20% of all FTLD cases.

Microtubule-associated protein tau

Since 1994 several studies linked families with a variety of neurodegenerative disorders to chromosome 17, together named FTDP-17.[17-25] Finally, in 1998 mutations in the *microtubule-associated protein tau* (*MAPT*) gene on chromosome 17q21.11 were identified as the first genetic causes of FTLD.[26] To date, 44 different mutations in the *MAPT* gene have been reported in 134 families.[27] All currently known *MAPT* mutations are located in exons 1 or 9-13, or in the splice sites of exons 9 or 10.[27] The *MAPT* gene encodes the protein tau, which pays a role in microtubule stabilization and neuronal integrity. In the human brain, six isoforms of the tau protein exist, resulting from alternative splicing of exons 2, 3 and 10. Depending on splicing of exon 10 these isoforms contain either 3 or 4 repeats (3R and 4R), and in normal brain the ratio of 3R and 4R isoforms is close to 1:1.[26] Most exonic mutations affect normal tau function, by altering the affinity of tau for microtubules, and thereby alter microtubule assembly. Some mutations have

additional pro-fibrillogenic effects leading to tau filament formation. Most intronic and some exonic mutations affect splicing of exon 10 leading to altered ratios of tau isoforms. Some mutations lead to an increase of 4R tau isoforms by increase of splicing-in of exon 10 whereas other mutations reduce exon 10 splicing and thereby increase 3R tau. Some mutations exhibit their effect by a combination of these mechanisms.[28]

The most frequent clinical presentation of *MAPT* mutations is bvFTD, characterized by disinhibition, loss of initiative and apathy.[15, 16, 28] Most patients develop semantic language deficits, but a true SD presentation is rare.[21, 23, 25] In some cases the initial presentation suggests a psychiatric disorder or Alzheimer's disease.[25] Some *MAPT* mutations typically present with a parkinsonism-prominent phenotype, sometimes fulfilling clinical criteria for PSP or CBS.[21, 25] Motor neuron disease is uncommon in patients with *MAPT* mutations.[16, 29] *MAPT* mutations are highly penetrant,[30] although incomplete penetrance has occasionally been found, for example in the L315R mutation.[31] Symptoms usually develop around the age of 50, but phenotypical variation exists even within families ranging from 40-65 years.[25, 28] Specific mutations are associated with a very young onset age between 20 and 40 years, whereas other mutations generally have an onset age of higher than 70 years.[28]

Pathology of *MAPT* mutations is characterized by neuronal loss and gliosis most prominent in the frontal and temporal lobes with tau-positive neuronal inclusions, mainly in the form of pretangles, neurofibrillary tangles or Pick bodies.[14, 25, 28] Glial tau-positive inclusions are also frequent and can even be more severe than neuronal pathology.[25, 28] Subcortical nuclei and substantia nigra can be severely affected in some patients, but can be normal in other cases.[28] Amyloid plaques can be found, but mostly in older patients, probably reflecting normal effects of aging.[25, 28] Tufted astrocytes, characteristic for PSP, can be found in some mutations.[28]

Progranulin

After the discovery of *MAPT* mutations, several other FTLD families were linked to chromosome 17, but lacked mutations in the *MAPT* gene. Neuropathological examination in these families did not show tau-positive inclusions, but revealed

ubiquitin-positive neuronal cytoplasmic and intranuclear inclusions.[32] In 2006 two groups discovered mutations in the *GRN* gene, which is located 1.7 Mb centromeric from the *MAPT* gene,[32] as the genetic cause of the disease in these families.[32, 33] Although *GRN* and *MAPT* are part of the same chromosomal region, no link between these genes has been found and the fact that mutations in both genes can cause the same clinical phenotype is considered to be an extraordinary coincidence.[32] At this moment, 69 different *GRN* mutations have been reported in 231 families.[27] Most *GRN* mutations, including nonsense, frameshift and splice-site mutations, result in a premature stop codon, thereby creating null alleles, which leads to haploinsufficiency of the progranulin protein.[32-34] Not surprisingly, serum and plasma levels of progranulin are reduced in *GRN* mutation carriers.[35-38] The progranulin protein is a growth factor and has a role in brain development, wound repair, inflammation, and tumorigenesis. The presence of progranulin appears to be crucial for neuronal survival in the cerebral cortex, and loss of progranulin eventually leads to neurodegeneration, although the exact mechanism is unknown.[32-34, 39, 40] The antibody against progranulin does not stain the ubiquitin-positive neuronal inclusions.[32] TDP-43 was discovered to be the major constituent of the ubiquitinated inclusions.[41,42] Normal nuclear staining with TDP-43 is reduced, suggesting translocation of TDP-43 from nucleus to cytoplasm.[39] Suppressed *GRN* expression was found to cause accumulation of TDP-43, but the exact mechanism remains unknown.

Pathologically, FTLD due to *GRN* mutations is characterized by neuronal loss and gliosis most prominent in the frontal and temporal cortex, with TDP-43 positive dystrophic neurites and cytoplasmatic and intranuclear inclusions, named FTLD-TDP type A.[26, 34, 39] The parietal cortex and basal ganglia can also be involved.[39] Astrocytic gliosis and myelin loss in the white matter and hippocampal sclerosis are frequently found.[34, 39]

Although most patients with *GRN* mutations have a positive family history with an autosomal dominant pattern of inheritance, *GRN* mutations have occasionally been found in patients with apparently sporadic disease. Possible explanations include de novo mutations, non-paternity, or incomplete or age-dependent penetrance.[24, 34] The latter possibility is supported by the large variation in onset age, both between and within families.[16, 40, 43] The mean onset age lies around

60 years, but the onset age can vary from 35 - 89 years.[39] Gass *et al* reported a penetrance of 50% at the age of 60 years, whereas at the age of 70 penetrance was over 90%.[34] This large variation in onset age cannot be explained by the type of mutation, since all mutations exert their effect by producing a null allele.[32-34] However, the risk allele of rs1990622 on *TMEM106B* has been associated with a decrease in age of onset and lower plasma progranulin levels. Therefore it is now assumed that genetic variation in *TMEM106B* influences the age of onset in *GRN* mutation carriers by modifying progranulin levels.[44]

The clinical presentation is also highly variable. Most patients present with bvFTD, with apathy as most prominent feature.[34, 36, 39, 45] However, language deficits are frequent, sometimes fulfilling a clinical diagnosis of PNFA.[34, 36, 41, 45] Moreover, prominent memory deficits can lead to an initial misdiagnosis of AD,[36, 41, 45] whereas early hallucinations and delusions can be suggestive of schizophrenia.[41] Finally, extrapyramidal features and apraxia are frequent and several cases of CBS have been reported, [15, 16, 39, 45] and a case of clinical Lewy body dementia was recently described.[46] Despite extensive analyses, ALS has only been reported in the A9D *GRN* mutation,[47] and one other mutation of which the pathogenic nature remains uncertain.[39]

C9orf72

There still remained a large number of families with FTLD with an autosomal dominant pattern of inheritance, not explained by *MAPT* or *GRN* mutations. In many of these families the clinical presentation varied between FTD, ALS or a combination of both. The existence of familial FTD-ALS, the co-occurrence of FTD and ALS in patients,[48] and the presence of TDP-43 pathology in the central nervous system in both disorders,[42] led to the hypothesis that ALS and FTD represent a clinicopathological disease spectrum. Several linkage and genome-wide association studies had already shown that a locus on chromosome 9p21 was associated with both FTD and ALS.[49-57] Finally, in 2011 an intronic expanded GGGGCC hexanucleotide repeat in *chromosome 9 open reading frame 72* (*C9orf72*) was identified as the responsible genetic defect and proved to be present in 30 - 50% of patients with familial ALS or FTD, and in 4 - 10% of sporadic cases.[58, 59] The repeat expansion is located between non-coding exons 1a and 1b. Exons 2 - 11 of *C9orf72* encode the C9orf72 protein, with a currently unknown function.

The repeat length in healthy individuals usually varies from 2 - 25 repeats, while a repeat size greater than 30 repeats is considered to be pathogenic. However, measurement of the exact repeat size remains challenging and the optimal cut-off to define pathogenic repeat expansions remains to be elucidated.[58, 59]

The mechanism by which the repeat expansion causes disease is currently unknown. A loss-of-function mechanism has been suggested by reduced expression of one of the transcript of *C9orf72*, however no reduction in C9orf72 protein level was found in the brains of patients.[13] Another possibility is a toxic-gain of function of abnormal messenger RNA, which is supported by the presence of nuclear RNA foci in brains of patients with the repeat expansion.[13]

Other genetic causes of FTLD

A mutation in the *charged multivesicular body protein 2B* gene (*CHMP2B*) on chromosome 3 was found in a large Danish family with FTLD in 2005.[60] Other studies have shown a very low frequency or even absence of this genetic defect in FTD cohorts.[15, 16] To date, four mutations have been found in five families.[11] The gene encodes a component of the ESCRTIII (endosomal sorting complex required for transport-III) complex, which has a role in two protein degradation pathways.[61] Patients typically present with bvFTD in their late 50s,[60, 61] whereas language problems and motor deficits, including parkinsonism, dystonia, pyramidal signs and myoclonus, can arise at a later stage.[61] A presentation with motor neuron disease can also be seen, in particular primary muscular atrophy, mainly affecting lower motor neurons.[62] Neuropathological investigation of patients with a *CHMP2B* mutation shows ubiquitin- and p62-positive neuronal cyptolasmic inclusions, negative for tau, TDP-43, FUS, α-synuclein, β-amyloid, prion protein and neurofilament,[63, 64] which is named FTLD-UPS.[10]

Missense mutations in the *valosin-containing protein* (*VCP*) gene on chromosome 9 can cause inclusion body myopathy associated with Paget disease of bone and FTD (IBMPFD). This is a rare autosomal dominant hereditary disorder in which most patients develop myopathy, about half develop Paget bone disease and 30% develop early-onset FTD, with a mean onset age of 53 years.[65] However, *VCP* mutations are rare in families with pure FTD.[15, 16, 66] At this moment 18 different mutations have been identified in 48 families.[12] *VCP* mutations are pathologically

characterized by FTLD-TDP type D with numerous short dystrophic neurites and neuronal intranuclear inclusions.[36]

Other genetic causes of ALS and FTLD

Mutations in *fused in sarcoma* (*FUS*) and *transactive response DNA binding protein* (*TARDBP*) have convincingly been associated with ALS, but seem to be very rare in pure FTLD.[48, 50, 67-69] Mutations in *UBQLN2* have been demonstrated to cause dominant X-linked familial ALS, with or without FTLD.[70] Mutations in *SQSTM1*, coding for p62, have been reported in approximately 3% of familial ALS and FTLD, and had previously been associated with Paget disease of bone.[71, 72] *Optineurin* mutations can cause both autosomal dominant and recessive ALS, but seem very rare in pure FTLD.[73]

MRI BIOMARKERS FOR FTLD

The typical frontotemporal atrophy in FTLD can be visualized by means of volumetric MRI, which is widely used in clinical practice. The characteristic atrophy patterns for each clinical subtype have been incorporated in the clinical criteria.[1, 2] A recent meta-analysis reported the bilateral medial frontal cortex, dorsolateral prefrontal cortex, anterior cingulate cortex, insula, and striatum as the most consistently affected brain regions in bvFTD.[74] PNFA is typically associated with left inferior frontal and anterior insular atrophy, SD is characterized by atrophy of bilateral anterior temporal lobes, and left temporoparietal atrophy is characteristic of LPA.[1]

Regarding the major genetic subtypes of FTLD, *MAPT* mutations are associated with symmetric atrophy most prominent in temporal lobes, whereas involvement of the posterior temporal and parietal lobes, posterior cingulate cortex and precuneus is characteristic of *GRN* mutations.[75, 76] Whereas a few small studies or case reports demonstrated grey matter atrophy in carriers of *GRN* or *MAPT* mutations before symptom onset,[77-82] other studies reported no presymptomatic atrophy.[83-85] Larger studies are needed to investigate this. Moreover, the specific imaging abnormalities for *C9orf72* repeat expansions remain to be elucidated.

Diffusion tensor imaging

Besides grey matter atrophy, structural imaging has also demonstrated white

matter impairment in FTLD.[86] However, conventional MRI is not capable of defining the exact regional distribution of white matter pathology. Diffusion tensor imaging (DTI) allows for microstructural investigation of white matter by measuring directionality of water diffusivity. The most common measure of white matter integrity is fractional anisoptropy (FA), which ranges from zero (indicating undirected water diffusion) to one (indicating exclusive water diffusion along one direction). Well-structured white matter tracts restrict water diffusion along the direction of axonal fibers and are therefore associated with high FA values. As white matter tracts degenerate diffusion becomes less directional, leading to lower FA values. Mean diffusivity (MD) represents diffusivity to all directions within a voxel, with higher values indicating less restricted diffusion and thereby poorer white matter integrity. Finally, axial (DA) and radial (DR) diffusivity represent water diffusivity parallel and perpendicular to the axis of the fiber tract, respectively. Animal models have suggested that changes in DA are associated with axonal damage, whereas DR alterations implicate myelin breakdown,[87, 88] however the exact interpretation of the different diffusion parameters remains controversial.[89]

DTI studies in patients with FTD have consistently shown widespread disruption of white matter tracts, most prominent in the frontal and temporal lobes.[90-98] Alterations within the uncinate fasciculus, forceps minor, anterior cingulum and inferior longitudinal fasciculus are found in most studies, but the extension of white matter changes differs between studies, and can include the superior longitudinal fasciculus, inferior fronto-occipital fasciculus, corticospinal tract and, posterior cingulum.[90-98] Conversely, one study found increased FA in the corticospinal tract.[95] Several studies have demonstrated a correlation between white matter integrity and behavioral symptoms or cognitive performance.[91, 92, 99, 100] The different clinical subtypes of FTD can be discriminated from each other using DTI with more involvement of the forceps minor in bvFTD, prominent disruption of the inferior longitudinal fasciculus in SD, and impairment of the superior longitudinal fasciculus in PNFA.[90, 95, 96] The uncinate fasciculus has been shown to best differentiate FTLD from controls across all syndromic groups.[90, 97] One study also reported the uncinate fasciculus as the tract that most accurately discriminated FTLD from AD,[97] whereas the forceps minor was suggested in another study.[94] Regarding the different DTI measures, several studies have suggested that DR is the most sensitive measure for FTLD,[95-97] whereas FA was most accurate in

discriminating FTLD from AD.[97] Thus far, only two studies investigated white matter integrity in the presymptomatic stage of FTLD, in small groups of healthy *GRN* mutation carriers, and showed reduced FA in the left uncinate and inferior fronto-occipital fasciculus and increased DA in the right cingulum, superior longitudinal fasciculus and corticospinal tract, respectively.[81, 83]

DTI changes in FTD largely co-localize with grey matter atrophy, with predominant white matter impairment in tracts connecting regions of grey matter atrophy (measured by voxel-based morphometry), and a correlation between these measures has been demonstrated.[90, 95, 101, 102] Therefore it has been suggested that DTI findings reflect axonal damage secondary to neuronal loss.[1] However, it has also consistently been shown that white matter impairment strongly exceeds the distribution of grey matter atrophy.[83, 90, 96, 97] Therefore, it has been proposed that DTI findings might also directly reflect protein accumulation within the white matter,[90, 98] consistent with previous pathological findings.[103, 104] Moreover, postmortem DTI revealed a strong correlation between DTI findings and WM gliosis and demyelination.[105]

Resting-state fMRI

Resting-state functional MRI (fMRI) can be used to detect anatomically distinct, but functionally related brain regions with temporally correlated patterns of brain activation at rest, together constituting functional networks.[106] These functional networks closely match the networks found to activate or deactivate during task-related fMRI.[107] Resting-state fMRI has major advantages over task-related fMRI, since no complicated design is needed, and results are not influenced by task compliance and performance, which is especially important for FTD patients exhibiting behavioral problems. Patients can simply be instructed to lie still, with their eyes open or closed and not to fall asleep. Moreover, resting-state fMRI provides more detailed information about interactions between brain regions, which have been shown to be crucial for brain function.[108] The functional networks show great similarity with the distinct atrophy patterns in the various neurodegenerative syndromes.[109] The spatial distribution of atrophy in bvFTD closely resembles the so-called salience network (SN), which is anchored by the anterior and midcingulate cortex and frontoinsula, and is involved in processing emotional stimuli.[110] Several resting-state fMRI studies have indeed shown

reduced functional connectivity within this network, [84, 85, 111-115] which was found to correlate with disease severity on the clinical dementia rating scale,[112] and with behavioral disturbances.[116] The default mode network (DMN), involving the anterior and posterior cingulate cortex, precuneus, bilateral parietal cortex, and medial prefrontal cortex, is typically affected in Alzheimer's disease,[117] and several studies demonstrated enhanced connectivity within this network in patients with FTD.[83, 85, 111, 112, 114, 115] It has therefore been suggested that the reduced SN connectivity leads to an increase in DMN connectivity.[114] However, one study also reported increased DMN connectivity without SN alterations in FTD patients,[115] whereas others reported decreased DMN connectivity.[85, 111, 113] Several small resting-state fMRI studies have been conducted in presymptomatic FTD, showing both increased and decreased DMN connectivity, without SN alterations, in *MAPT* mutation carriers,[85] and a large variation in results across three studies in *GRN* mutation carriers.[81, 84, 118] This large diversity in exact resting-state fMRI results may reflect differences in analytical techniques, including seed-based analyses, graph theory, and independent component analysis, and differences in which networks are investigated.[81, 84, 85, 111-115, 118]

Arterial spin labeling

Arterial spin labeling MRI (ASL) is a non-invasive method to measure brain perfusion by magnetically labeling arterial water protons using radiofrequency pulses as an endogenous tracer for cerebral blood flow (CBF).[119] Blood is labeled when passing through the carotid and vertebral arteries, and after a short delay 'tag' images are acquired and compared with control images without prior labeling. The difference between control and tag images reflects the flow of labeled arterial blood and thereby represents brain perfusion. Direct comparison of ASL with positron emission tomography with 18F-fluorodeoxyglucose (FDG-PET), which is often used to diagnose dementia in clinical practice, have shown highly comparable results in patients with AD.[120, 121] However, ASL has several major advantages over FDG-PET, including that ASL is less expensive, less invasive for patients, does not require exposure to a radioactive tracer, and MRI scanners are more widely available compared with PET-scanners. Moreover, ASL can be easily combined with other MRI techniques in one single session.

Hypoperfusion in bilateral frontal lobes, including prefrontal, inferior, middle, and

superior frontal cortex, and the anterior cingulate cortex, insula and thalamus was demonstrated in FTD patients compared to controls using ASL.[98, 122-125] One study also found regions of hyperperfusion, including medial parietal cortex, precuneus, and PCC.[123] No studies have performed a comparison of hypoperfusion patterns between bvFTD and PPA using ASL thus far. Alterations in perfusion can still be identified after correction for grey matter volume, indicating that hypoperfusion can be seen independent of volume loss.[30, 50] Conversely, two studies have shown more widespread grey matter atrophy compared to hypoperfusion, and suggested that loss of brain tissue other than neurons contributed to grey matter atrophy and that the surviving neurons were able to maintain brain function as reflected by normal brain perfusion.[98, 122] A correlation between hypoperfusion and judgment and problem solving on the clinical dementia rating scale suggested an association with disease severity, however longitudinal studies are lacking.[125] Thus far, only one study has investigated brain perfusion in presymptomatic FTLD, in carriers of *CHMP2B* mutations, by means of spin-echo, and found widespread hypoperfusion in hippocampus, temporal, parietal, and occipital lobes.[126]

SCOPE OF THIS THESIS

To conclude, genetic forms of FTLD have provided abundant information about the pathophysiological process, which is crucial for the development of disease-modifying therapies. Further exploration of the clinical and pathological characteristics of each genetic subtype of FTLD can increase our insight into the disease process. Moreover, for the evaluation of disease-modifying therapies in clinical trials, the development of sensitive biomarkers for the earliest stages of FTLD is crucial.

The aim of the current study was twofold:

1) To expand current knowledge on phenotypical characteristics in the clinical stage of genetic FTLD.

   Chapter 2.1 describes an atypical corticobasal syndrome presentation in a patient with a novel *GRN* mutation and FTLD-TDP pathology. In **Chapter 2.2** we investigated the clinicopathological characteristics of the *C9orf72* repeat expansion in a Dutch FTD cohort. In **Chapter 2.3** we estimated the global frequency of this repeat expansion.

2) To investigate presymptomatic changes in familial FTLD using neuropsychological assessment and MRI.
   Chapter 3.1 describes findings on neuropsychological investigation, volumetric MRI, DTI and resting-state fMRI in a large group of presymptomatic carriers of *GRN* and *MAPT* mutations. **Chapter 3.2** reports longitudinal findings over two years follow-up in this same group of subjects. In **Chapter 3.3** we investigated presymptomatic alterations in CBF using ASL in the same population.

In **Chapter 4** the main findings and implications of the current study are discussed in light of literature and suggestions for future research are given.

REFERENCES

1. Gorno-Tempini ML, Hillis AE, Weintraub S, Kertesz A, Mendez M, Cappa SF, et al. Classification of primary progressive aphasia and its variants. Neurology. 2011 Mar 15;76(11):1006-14.
2. Rascovsky K, Hodges JR, Knopman D, Mendez MF, Kramer JH, Neuhaus J, et al. Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. Brain. 2011 Sep;134(Pt 9):2456-77.
3. Kipps CM, Hodges JR, Hornberger M. Nonprogressive behavioural frontotemporal dementia: recent developments and clinical implications of the 'bvFTD phenocopy syndrome'. Curr Opin Neurol. 2010 Dec;23(6):628-32.
4. Harciarek M, Cosentino S. Language, executive function and social cognition in the diagnosis of frontotemporal dementia syndromes. Int Rev Psychiatry. 2013 Apr;25(2):178-96.
5. Torralva T, Roca M, Gleichgerrcht E, Bekinschtein T, Manes F. A neuropsychological battery to detect specific executive and social cognitive impairments in early frontotemporal dementia. Brain. 2009 May;132(Pt 5):1299-309.
6. Rowland LP, Shneider NA. Amyotrophic lateral sclerosis. N Engl J Med. 2001 May 31;344(22):1688-700.
7. Boeve BF, Lang AE, Litvan I. Corticobasal degeneration and its relationship to progressive supranuclear palsy and frontotemporal dementia. Ann Neurol. 2003;54 Suppl 5:S15-9.
8. Kaat DL, Chiu WZ, Boon AJ, van Swieten JC. Recent advances in progressive supranuclear palsy: a review. Curr Alzheimer Res. 2011 May;8(3):295-302.
9. Cairns NJ, Bigio EH, Mackenzie IR, Neumann M, Lee VM, Hatanpaa KJ, et al. Neuropathologic diagnostic and nosologic criteria for frontotemporal lobar degeneration: consensus of the Consortium for Frontotemporal Lobar Degeneration. Acta Neuropathol. 2007 Jul;114(1):5-22.
10. Mackenzie IR, Neumann M, Bigio EH, Cairns NJ, Alafuzoff I, Kril J, et al. Nomenclature and nosology for neuropathologic subtypes of frontotemporal lobar degeneration: an update. Acta Neuropathol. 2010 Jan;119(1):1-4.
11. Mackenzie IR, Neumann M, Baborie A, Sampathu DM, Du Plessis D, Jaros E, et al. A harmonized classification system for FTLD-TDP pathology. Acta Neuropathol. 2011 Jul;122(1):111-3.
12. Mackenzie IR, Neumann M. FET proteins in frontotemporal dementia and amyotrophic lateral sclerosis. Brain Res. 2012 Jun 26;1462:40-3.
13. Goldman JS, Farmer JM, Wood EM, Johnson JK, Boxer A, Neuhaus J, et al. Comparison of family histories in FTLD subtypes and related tauopathies. Neurology. 2005 Dec 13;65(11):1817-9.
14. Chow TW, Miller BL, Hayashi VN, Geschwind DH. Inheritance of frontotemporal dementia. Arch Neurol. 1999 Jul;56(7):817-22.
15. Rohrer JD, Guerreiro R, Vandrovcova J, Uphill J, Reiman D, Beck J, et al. The heritability and genetics of frontotemporal lobar degeneration. Neurology. 2009 Nov 3;73(18):1451-6.
16. Seelaar H, Kamphorst W, Rosso SM, Azmani A, Masdjedi R, de Koning I, et al. Distinct genetic forms of frontotemporal dementia. Neurology. 2008 Oct 14;71(16):1220-6.
17. Foster NL, Wilhelmsen K, Sima AA, Jones MZ, D'Amato CJ, Gilman S. Frontotemporal dementia

and parkinsonism linked to chromosome 17: a consensus conference. Conference Participants. Ann Neurol. 1997 Jun;41(6):706-15.

18. Wilhelmsen KC, Lynch T, Pavlou E, Higgins M, Nygaard TG. Localization of disinhibition-dementia-parkinsonism-amyotrophy complex to 17q21-22. Am J Hum Genet. 1994 Dec;55(6):1159-65.
19. Baker M, Kwok JB, Kucera S, Crook R, Farrer M, Houlden H, et al. Localization of frontotemporal dementia with parkinsonism in an Australian kindred to chromosome 17q21-22. Ann Neurol. 1997 Nov;42(5):794-8.
20. Froelich S, Basun H, Forsell C, Lilius L, Axelman K, Andreadis A, et al. Mapping of a disease locus for familial rapidly progressive frontotemporal dementia to chromosome 17q12-21. Am J Med Genet. 1997 Jul 25;74(4):380-5.
21. Murrell JR, Koller D, Foroud T, Goedert M, Spillantini MG, Edenberg HJ, et al. Familial multiple-system tauopathy with presenile dementia is localized to chromosome 17. Am J Hum Genet. 1997 Nov;61(5):1131-8.
22. Wijker M, Wszolek ZK, Wolters EC, Rooimans MA, Pals G, Pfeiffer RF, et al. Localization of the gene for rapidly progressive autosomal dominant parkinsonism and dementia with pallido-ponto-nigral degeneration to chromosome 17q21. Hum Mol Genet. 1996 Jan;5(1):151-4.
23. Heutink P, Stevens M, Rizzu P, Bakker E, Kros JM, Tibben A, et al. Hereditary frontotemporal dementia is linked to chromosome 17q21-q22: a genetic and clinicopathological study of three Dutch families. Ann Neurol. 1997 Feb;41(2):150-9.
24. Yamaoka LH, Welsh-Bohmer KA, Hulette CM, Gaskell PC, Jr., Murray M, Rimmler JL, et al. Linkage of frontotemporal dementia to chromosome 17: clinical and neuropathological characterization of phenotype. Am J Hum Genet. 1996 Dec;59(6):1306-12.
25. Dark F. A family with autosomal dominant, non-Alzheimer's presenile dementia. Aust N Z J Psychiatry. 1997 Feb;31(1):139-44.
26. Hutton M, Lendon CL, Rizzu P, Baker M, Froelich S, Houlden H, et al. Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. Nature. 1998 Jun 18;393(6686):702-5.
27. Cruts M, Theuns J, Van Broeckhoven C. Locus-specific mutation databases for neurodegenerative brain diseases. Hum Mutat. 2012 Sep;33(9):1340-4.
28. van Swieten J, Spillantini MG. Hereditary frontotemporal dementia caused by Tau gene mutations. Brain Pathol. 2007 Jan;17(1):63-73.
29. Goldman JS, Rademakers R, Huey ED, Boxer AL, Mayeux R, Miller BL, et al. An algorithm for genetic testing of frontotemporal lobar degeneration. Neurology. 2011 Feb 1;76(5):475-83.
30. Poorkaj P, Grossman M, Steinbart E, Payami H, Sadovnick A, Nochlin D, et al. Frequency of tau gene mutations in familial and sporadic cases of non-Alzheimer dementia. Arch Neurol. 2001 Mar;58(3):383-7.
31. van Herpen E, Rosso SM, Serverijnen LA, Yoshida H, Breedveld G, van de Graaf R, et al. Variable phenotypic expression and extensive tau pathology in two families with the novel tau mutation L315R. Ann Neurol. 2003 Nov;54(5):573-81.
32. Baker M, Mackenzie IR, Pickering-Brown SM, Gass J, Rademakers R, Lindholm C, et al. Mutations

in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. Nature. 2006 Aug 24;442(7105):916-9.

33. Cruts M, Gijselinck I, van der Zee J, Engelborghs S, Wils H, Pirici D, et al. Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. Nature. 2006 Aug 24;442(7105):920-4.

34. Gass J, Cannon A, Mackenzie IR, Boeve B, Baker M, Adamson J, et al. Mutations in progranulin are a major cause of ubiquitin-positive frontotemporal lobar degeneration. Hum Mol Genet. 2006 Oct 15;15(20):2988-3001.

35. Coppola G, Karydas A, Rademakers R, Wang Q, Baker M, Hutton M, et al. Gene expression study on peripheral blood identifies progranulin mutations. Ann Neurol. 2008 Jul;64(1):92-6.

36. Ghidoni R, Benussi L, Glionna M, Franzoni M, Binetti G. Low plasma progranulin levels predict progranulin mutations in frontotemporal lobar degeneration. Neurology. 2008 Oct 14;71(16):1235-9.

37. Sleegers K, Brouwers N, Van Damme P, Engelborghs S, Gijselinck I, van der Zee J, et al. Serum biomarker for progranulin-associated frontotemporal lobar degeneration. Ann Neurol. 2009 May;65(5):603-9.

38. Finch N, Baker M, Crook R, Swanson K, Kuntz K, Surtees R, et al. Plasma progranulin levels predict progranulin mutation status in frontotemporal dementia patients and asymptomatic family members. Brain. 2009 Mar;132(Pt 3):583-91.

39. van Swieten JC, Heutink P. Mutations in progranulin (GRN) within the spectrum of clinical and pathological phenotypes of frontotemporal dementia. Lancet Neurol. 2008 Oct;7(10):965-74.

40. Le Ber I, van der Zee J, Hannequin D, Gijselinck I, Campion D, Puel M, et al. Progranulin null mutations in both sporadic and familial frontotemporal dementia. Hum Mutat. 2007 Sep;28(9):846-55.

41. Arai T, Hasegawa M, Akiyama H, Ikeda K, Nonaka T, Mori H, et al. TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Biochem Biophys Res Commun. 2006 Dec 22;351(3):602-11.

42. Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science. 2006 Oct 6;314(5796):130-3.

43. Pietroboni AM, Fumagalli GG, Ghezzi L, Fenoglio C, Cortini F, Serpente M, et al. Phenotypic heterogeneity of the GRN Asp22fs mutation in a large Italian kindred. J Alzheimers Dis. 2011;24(2):253-9.

44. Cruchaga C, Graff C, Chiang HH, Wang J, Hinrichs AL, Spiegel N, et al. Association of TMEM106B gene polymorphism with age at onset in granulin mutation carriers and plasma granulin protein levels. Arch Neurol. 2011 May;68(5):581-6.

45. Yu CE, Bird TD, Bekris LM, Montine TJ, Leverenz JB, Steinbart E, et al. The spectrum of mutations in progranulin: a collaborative study screening 545 cases of neurodegeneration. Arch Neurol. 2010 Feb;67(2):161-70.

46. Arosio B, Abbate C, Galimberti D, Rossi PD, Inglese S, Fenoglio C, et al. GRN Thr272fs clinical

heterogeneity: a case with atypical late onset presenting with a dementia with Lewy bodies phenotype. J Alzheimers Dis. 2013;35(4):669-74.

47. Cannon A, Fujioka S, Rutherford NJ, Ferman TJ, Broderick DF, Boylan KB, et al. Clinicopathologic variability of the GRN A9D mutation, including amyotrophic lateral sclerosis. Neurology. 2013 May 7;80(19):1771-7.

48. Lomen-Hoerth C, Anderson T, Miller B. The overlap of amyotrophic lateral sclerosis and frontotemporal dementia. Neurology. 2002 Oct 8;59(7):1077-9.

49. Morita M, Al-Chalabi A, Andersen PM, Hosler B, Sapp P, Englund E, et al. A locus on chromosome 9p confers susceptibility to ALS and frontotemporal dementia. Neurology. 2006 Mar 28;66(6):839-44.

50. Vance C, Al-Chalabi A, Ruddy D, Smith BN, Hu X, Sreedharan J, et al. Familial amyotrophic lateral sclerosis with frontotemporal dementia is linked to a locus on chromosome 9p13.2-21.3. Brain. 2006 Apr;129(Pt 4):868-76.

51. Valdmanis PN, Dupre N, Bouchard JP, Camu W, Salachas F, Meininger V, et al. Three families with amyotrophic lateral sclerosis and frontotemporal dementia with evidence of linkage to chromosome 9p. Arch Neurol. 2007 Feb;64(2):240-5.

52. Le Ber I, Camuzat A, Berger E, Hannequin D, Laquerriere A, Golfier V, et al. Chromosome 9p-linked families with frontotemporal dementia associated with motor neuron disease. Neurology. 2009 May 12;72(19):1669-76.

53. Boxer AL, Mackenzie IR, Boeve BF, Baker M, Seeley WW, Crook R, et al. Clinical, neuroimaging and neuropathological features of a new chromosome 9p-linked FTD-ALS family. J Neurol Neurosurg Psychiatry. 2011 Feb;82(2):196-203.

54. Pearson JP, Williams NM, Majounie E, Waite A, Stott J, Newsway V, et al. Familial frontotemporal dementia with amyotrophic lateral sclerosis and a shared haplotype on chromosome 9p. J Neurol. 2011 Apr;258(4):647-55.

55. van Es MA, Veldink JH, Saris CG, Blauw HM, van Vught PW, Birve A, et al. Genome-wide association study identifies 19p13.3 (UNC13A) and 9p21.2 as susceptibility loci for sporadic amyotrophic lateral sclerosis. Nat Genet. 2009 Oct;41(10):1083-7.

56. Laaksovirta H, Peuralinna T, Schymick JC, Scholz SW, Lai SL, Myllykangas L, et al. Chromosome 9p21 in amyotrophic lateral sclerosis in Finland: a genome-wide association study. Lancet Neurol. 2010 Oct;9(10):978-85.

57. Van Deerlin VM, Sleiman PM, Martinez-Lage M, Chen-Plotkin A, Wang LS, Graff-Radford NR, et al. Common variants at 7p21 are associated with frontotemporal lobar degeneration with TDP-43 inclusions. Nat Genet. 2010 Mar;42(3):234-9.

58. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron. 2011 Oct 20;72(2):245-56.

59. Renton AE, Majounie E, Waite A, Simon-Sanchez J, Rollinson S, Gibbs JR, et al. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron. 2011 Oct 20;72(2):257-68.

60. Skibinski G, Parkinson NJ, Brown JM, Chakrabarti L, Lloyd SL, Hummerich H, et al. Mutations in the endosomal ESCRTIII-complex subunit CHMP2B in frontotemporal dementia. Nat Genet. 2005 Aug;37(8):806-8.

61. Isaacs AM, Johannsen P, Holm I, Nielsen JE, consortium FR. Frontotemporal dementia caused by CHMP2B mutations. Curr Alzheimer Res. 2011 May;8(3):246-51.

62. Cox LE, Ferraiuolo L, Goodall EF, Heath PR, Higginbottom A, Mortiboys H, et al. Mutations in CHMP2B in lower motor neuron predominant amyotrophic lateral sclerosis (ALS). PLoS One. 2010;5(3):e9872.

63. Holm IE, Englund E, Mackenzie IR, Johannsen P, Isaacs AM. A reassessment of the neuropathology of frontotemporal dementia linked to chromosome 3. J Neuropathol Exp Neurol. 2007 Oct;66(10):884-91.

64. Holm IE, Isaacs AM, Mackenzie IR. Absence of FUS-immunoreactive pathology in frontotemporal dementia linked to chromosome 3 (FTD-3) caused by mutation in the CHMP2B gene. Acta Neuropathol. 2009 Nov;118(5):719-20.

65. Watts GD, Wymer J, Kovach MJ, Mehta SG, Mumm S, Darvish D, et al. Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia is caused by mutant valosin-containing protein. Nat Genet. 2004 Apr;36(4):377-81.

66. van der Zee J, Pirici D, Van Langenhove T, Engelborghs S, Vandenberghe R, Hoffmann M, et al. Clinical heterogeneity in 3 unrelated families linked to VCP p.Arg159His. Neurology. 2009 Aug 25;73(8):626-32.

67. Broustal O, Camuzat A, Guillot-Noel L, Guy N, Millecamps S, Deffond D, et al. FUS mutations in frontotemporal lobar degeneration with amyotrophic lateral sclerosis. J Alzheimers Dis. 2010;22(3):765-9.

68. Van Langenhove T, van der Zee J, Sleegers K, Engelborghs S, Vandenberghe R, Gijselinck I, et al. Genetic contribution of FUS to frontotemporal lobar degeneration. Neurology. 2010 Feb 2;74(5):366-71.

69. Borroni B, Archetti S, Del Bo R, Papetti A, Buratti E, Bonvicini C, et al. TARDBP mutations in frontotemporal lobar degeneration: frequency, clinical features, and disease course. Rejuvenation Res. 2010 Oct;13(5):509-17.

70. Deng HX, Chen W, Hong ST, Boycott KM, Gorrie GH, Siddique N, et al. Mutations in UBQLN2 cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia. Nature. 2011 Sep 8;477(7363):211-5.

71. Le Ber I, Camuzat A, Guerreiro R, Bouya-Ahmed K, Bras J, Nicolas G, et al. SQSTM1 mutations in French patients with frontotemporal dementia or frontotemporal dementia with amyotrophic lateral sclerosis. JAMA Neurol. 2013 Nov;70(11):1403-10.

72. Rubino E, Rainero I, Chio A, Rogaeva E, Galimberti D, Fenoglio P, et al. SQSTM1 mutations in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Neurology. 2012 Oct 9;79(15):1556-62.

73. Rollinson S, Bennion J, Toulson G, Halliwell N, Usher S, Snowden J, et al. Analysis of optineurin in frontotemporal lobar degeneration. Neurobiol Aging. 2012 Feb;33(2):425 e1-2.

74. Pan PL, Song W, Yang J, Huang R, Chen K, Gong QY, et al. Gray matter atrophy in behavioral variant frontotemporal dementia: a meta-analysis of voxel-based morphometry studies. Dement Geriatr Cogn Disord. 2012;33(2-3):141-8.

75. Rohrer JD, Ridgway GR, Modat M, Ourselin S, Mead S, Fox NC, et al. Distinct profiles of brain atrophy in frontotemporal lobar degeneration caused by progranulin and tau mutations. Neuroimage. 2010 Nov 15;53(3):1070-6.

76. Whitwell JL, Jack CR, Jr., Boeve BF, Senjem ML, Baker M, Rademakers R, et al. Voxel-based morphometry patterns of atrophy in FTLD with mutations in MAPT or PGRN. Neurology. 2009 Mar 3;72(9):813-20.

77. Cruchaga C, Fernandez-Seara MA, Seijo-Martinez M, Samaranch L, Lorenzo E, Hinrichs A, et al. Cortical atrophy and language network reorganization associated with a novel progranulin mutation. Cereb Cortex. 2009 Aug;19(8):1751-60.

78. Rohrer JD, Warren JD, Barnes J, Mead S, Beck J, Pepple T, et al. Mapping the progression of progranulin-associated frontotemporal lobar degeneration. Nat Clin Pract Neurol. 2008 Aug;4(8):455-60.

79. Janssen JC, Schott JM, Cipolotti L, Fox NC, Scahill RI, Josephs KA, et al. Mapping the onset and progression of atrophy in familial frontotemporal lobar degeneration. J Neurol Neurosurg Psychiatry. 2005 Feb;76(2):162-8.

80. Moreno F, Sala-Llonch R, Barandiaran M, Sanchez-Valle R, Estanga A, Bartres-Faz D, et al. Distinctive age-related temporal cortical thinning in asymptomatic granulin gene mutation carriers. Neurobiol Aging. 2013 May;34(5):1462-8.

81. Pievani M, Paternico D, Benussi L, Binetti G, Orlandini A, Cobelli M, et al. Pattern of structural and functional brain abnormalities in asymptomatic granulin mutation carriers. Alzheimers Dement. 2014 Oct;10(5 Suppl):S354-S63 e1.

82. Miyoshi M, Shinotoh H, Wszolek ZK, Strongosky AJ, Shimada H, Arakawa R, et al. In vivo detection of neuropathologic changes in presymptomatic MAPT mutation carriers: a PET and MRI study. Parkinsonism Relat Disord. 2010 Jul;16(6):404-8.

83. Borroni B, Alberici A, Premi E, Archetti S, Garibotto V, Agosti C, et al. Brain magnetic resonance imaging structural changes in a pedigree of asymptomatic progranulin mutation carriers. Rejuvenation Res. 2008 Jun;11(3):585-95.

84. Borroni B, Alberici A, Cercignani M, Premi E, Serra L, Cerini C, et al. Granulin mutation drives brain damage and reorganization from preclinical to symptomatic FTLD. Neurobiol Aging. 2012 Oct;33(10):2506-20.

85. Whitwell JL, Josephs KA, Avula R, Tosakulwong N, Weigand SD, Senjem ML, et al. Altered functional connectivity in asymptomatic MAPT subjects: a comparison to bvFTD. Neurology. 2011 Aug 30;77(9):866-74.

86. Chao LL, Schuff N, Clevenger EM, Mueller SG, Rosen HJ, Gorno-Tempini ML, et al. Patterns of white matter atrophy in frontotemporal lobar degeneration. Arch Neurol. 2007 Nov;64(11):1619-24.

87. Song SK, Sun SW, Ju WK, Lin SJ, Cross AH, Neufeld AH. Diffusion tensor imaging detects and differentiates axon and myelin degeneration in mouse optic nerve after retinal ischemia.

Neuroimage. 2003 Nov;20(3):1714-22.

88. Song SK, Sun SW, Ramsbottom MJ, Chang C, Russell J, Cross AH. Dysmyelination revealed through MRI as increased radial (but unchanged axial) diffusion of water. Neuroimage. 2002 Nov;17(3):1429-36.

89. Haller S, Nguyen D, Rodriguez C, Emch J, Gold G, Bartsch A, et al. Individual prediction of cognitive decline in mild cognitive impairment using support vector machine-based analysis of diffusion tensor imaging data. J Alzheimers Dis. 2010;22(1):315-27.

90. Agosta F, Scola E, Canu E, Marcone A, Magnani G, Sarro L, et al. White matter damage in frontotemporal lobar degeneration spectrum. Cereb Cortex. 2012 Dec;22(12):2705-14.

91. Borroni B, Brambati SM, Agosti C, Gipponi S, Bellelli G, Gasparotti R, et al. Evidence of white matter changes on diffusion tensor imaging in frontotemporal dementia. Arch Neurol. 2007 Feb;64(2):246-51.

92. Hornberger M, Geng J, Hodges JR. Convergent grey and white matter evidence of orbitofrontal cortex changes related to disinhibition in behavioural variant frontotemporal dementia. Brain. 2011 Sep;134(Pt 9):2502-12.

93. Lillo P, Mioshi E, Burrell JR, Kiernan MC, Hodges JR, Hornberger M. Grey and white matter changes across the amyotrophic lateral sclerosis-frontotemporal dementia continuum. PLoS One. 2012;7(8):e43993.

94. McMillan CT, Brun C, Siddiqui S, Churgin M, Libon D, Yushkevich P, et al. White matter imaging contributes to the multimodal diagnosis of frontotemporal lobar degeneration. Neurology. 2012 May 29;78(22):1761-8.

95. Whitwell JL, Avula R, Senjem ML, Kantarci K, Weigand SD, Samikoglu A, et al. Gray and white matter water diffusion in the syndromic variants of frontotemporal dementia. Neurology. 2010 Apr 20;74(16):1279-87.

96. Zhang Y, Tartaglia MC, Schuff N, Chiang GC, Ching C, Rosen HJ, et al. MRI signatures of brain macrostructural atrophy and microstructural degradation in frontotemporal lobar degeneration subtypes. J Alzheimers Dis. 2013;33(2):431-44.

97. Mahoney CJ, Ridgway GR, Malone IB, Downey LE, Beck J, Kinnunen KM, et al. Profiles of white matter tract pathology in frontotemporal dementia. Hum Brain Mapp. 2014 Aug;35(8):4163-79.

98. Zhang Y, Schuff N, Ching C, Tosun D, Zhan W, Nezamzadeh M, et al. Joint assessment of structural, perfusion, and diffusion MRI in Alzheimer's disease and frontotemporal dementia. Int J Alzheimers Dis. 2011;2011:546871.

99. Lu PH, Lee GJ, Shapira J, Jimenez E, Mather MJ, Thompson PM, et al. Regional differences in white matter breakdown between frontotemporal dementia and early-onset Alzheimer's disease. J Alzheimers Dis. 2014;39(2):261-9.

100. Tartaglia MC, Zhang Y, Racine C, Laluz V, Neuhaus J, Chao L, et al. Executive dysfunction in frontotemporal dementia is related to abnormalities in frontal white matter tracts. J Neurol. 2012 Jun;259(6):1071-80.

101. Kuceyeski A, Zhang Y, Raj A. Linking white matter integrity loss to associated cortical regions using structural connectivity information in Alzheimer's disease and fronto-temporal dementia:

the Loss in Connectivity (LoCo) score. Neuroimage. 2012 Jul 16;61(4):1311-23.

102. Avants BB, Cook PA, Ungar L, Gee JC, Grossman M. Dementia induces correlated reductions in white matter integrity and cortical thickness: a multivariate neuroimaging study with sparse canonical correlation analysis. Neuroimage. 2010 Apr 15;50(3):1004-16.

103. Schofield E, Kersaitis C, Shepherd CE, Kril JJ, Halliday GM. Severity of gliosis in Pick's disease and frontotemporal lobar degeneration: tau-positive glia differentiate these disorders. Brain. 2003 Apr;126(Pt 4):827-40.

104. Neumann M, Kwong LK, Truax AC, Vanmassenhove B, Kretzschmar HA, Van Deerlin VM, et al. TDP-43-positive white matter pathology in frontotemporal lobar degeneration with ubiquitin-positive inclusions. J Neuropathol Exp Neurol. 2007 Mar;66(3):177-83.

105. Larsson EM, Englund E, Sjobeck M, Latt J, Brockstedt S. MRI with diffusion tensor imaging post-mortem at 3.0 T in a patient with frontotemporal dementia. Dement Geriatr Cogn Disord. 2004;17(4):316-9.

106. Damoiseaux JS, Rombouts SA, Barkhof F, Scheltens P, Stam CJ, Smith SM, et al. Consistent resting-state networks across healthy subjects. Proc Natl Acad Sci U S A. 2006 Sep 12;103(37):13848-53.

107. Smith SM, Fox PT, Miller KL, Glahn DC, Fox PM, Mackay CE, et al. Correspondence of the brain's functional architecture during activation and rest. Proc Natl Acad Sci U S A. 2009 Aug 4;106(31):13040-5.

108. Mesulam MM. From sensation to cognition. Brain. 1998 Jun;121 (Pt 6):1013-52.

109. Seeley WW, Crawford RK, Zhou J, Miller BL, Greicius MD. Neurodegenerative diseases target large-scale human brain networks. Neuron. 2009 Apr 16;62(1):42-52.

110. Seeley WW, Menon V, Schatzberg AF, Keller J, Glover GH, Kenna H, et al. Dissociable intrinsic connectivity networks for salience processing and executive control. J Neurosci. 2007 Feb 28;27(9):2349-56.

111. Agosta F, Sala S, Valsasina P, Meani A, Canu E, Magnani G, et al. Brain network connectivity assessed using graph theory in frontotemporal dementia. Neurology. 2013 Jul 9;81(2):134-43.

112. Zhou J, Greicius MD, Gennatas ED, Growdon ME, Jang JY, Rabinovici GD, et al. Divergent network connectivity changes in behavioural variant frontotemporal dementia and Alzheimer's disease. Brain. 2010 May;133(Pt 5):1352-67.

113. Filippi M, Agosta F, Scola E, Canu E, Magnani G, Marcone A, et al. Functional network connectivity in the behavioral variant of frontotemporal dementia. Cortex. 2013 Oct;49(9):2389-401.

114. Farb NA, Grady CL, Strother S, Tang-Wai DF, Masellis M, Black S, et al. Abnormal network connectivity in frontotemporal dementia: evidence for prefrontal isolation. Cortex. 2013 Jul-Aug;49(7):1856-73.

115. Rytty R, Nikkinen J, Paavola L, Abou Elseoud A, Moilanen V, Visuri A, et al. GroupICA dual regression analysis of resting state networks in a behavioral variant of frontotemporal dementia. Front Hum Neurosci. 2013;7:461.

116. Day GS, Farb NA, Tang-Wai DF, Masellis M, Black SE, Freedman M, et al. Salience network resting-state activity: prediction of frontotemporal dementia progression. JAMA Neurol. 2013 Oct;70(10):1249-53.

117. Hafkemeijer A, van der Grond J, Rombouts SA. Imaging the default mode network in aging and dementia. Biochim Biophys Acta. 2012 Mar;1822(3):431-41.

118. Premi E, Formenti A, Gazzina S, Archetti S, Gasparotti R, Padovani A, et al. Effect of TMEM106B polymorphism on functional network connectivity in asymptomatic GRN mutation carriers. JAMA Neurol. 2014 Feb;71(2):216-21.

119. Roberts DA, Detre JA, Bolinger L, Insko EK, Leigh JS, Jr. Quantitative magnetic resonance imaging of human brain perfusion at 1.5 T using steady-state inversion of arterial water. Proc Natl Acad Sci U S A. 1994 Jan 4;91(1):33-7.

120. Chen Y, Wolk DA, Reddin JS, Korczykowski M, Martinez PM, Musiek ES, et al. Voxel-level comparison of arterial spin-labeled perfusion MRI and FDG-PET in Alzheimer disease. Neurology. 2011 Nov 29;77(22):1977-85.

121. Musiek ES, Chen Y, Korczykowski M, Saboury B, Martinez PM, Reddin JS, et al. Direct comparison of fluorodeoxyglucose positron emission tomography and arterial spin labeling magnetic resonance imaging in Alzheimer's disease. Alzheimers Dement. 2012 Jan;8(1):51-9.

122. Shimizu S, Zhang Y, Laxamana J, Miller BL, Kramer JH, Weiner MW, et al. Concordance and discordance between brain perfusion and atrophy in frontotemporal dementia. Brain Imaging Behav. 2010 Mar;4(1):46-54.

123. Hu WT, Wang Z, Lee VM, Trojanowski JQ, Detre JA, Grossman M. Distinct cerebral perfusion patterns in FTLD and AD. Neurology. 2010 Sep 7;75(10):881-8.

124. Binnewijzend MA, Kuijer JP, van der Flier WM, Benedictus MR, Moller CM, Pijnenburg YA, et al. Distinct perfusion patterns in Alzheimer's disease, frontotemporal dementia and dementia with Lewy bodies. Eur Radiol. 2014 Sep;24(9):2326-33.

125. Du AT, Jahng GH, Hayasaka S, Kramer JH, Rosen HJ, Gorno-Tempini ML, et al. Hypoperfusion in frontotemporal dementia and Alzheimer disease by arterial spin labeling MRI. Neurology. 2006 Oct 10;67(7):1215-20.

126. Lunau L, Mouridsen K, Rodell A, Ostergaard L, Nielsen JE, Isaacs A, et al. Presymptomatic cerebral blood flow changes in CHMP2B mutation carriers of familial frontotemporal dementia (FTD-3), measured with MRI. BMJ Open. 2012;2(2):e000368.

CHAPTER 2.1

Symmetrical corticobasal syndrome caused by a novel c.314dup *progranulin* mutation

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ABSTRACT

Corticobasal syndrome (CBS) is characterized by asymmetrical Parkinsonism and cognitive impairment. The underlying pathology varies between corticobasal degeneration, progressive supranuclear palsy, Alzheimer's disease, Creutzfeldt-Jakob disease, and frontotemporal lobar degeneration sometimes in association with *GRN* mutations. A 61-year-old male underwent neurological examination, neuropsychological assessment, MRI, and HMPAO-SPECT at our medical Centre. After his death at the age of 63, brain autopsy, genetic screening and mRNA expression analysis were performed. The patient presented with slowly progressive walking disabilities, non-fluent language problems, behavioral changes and forgetfulness. His family history was negative. He had primitive reflexes, rigidity of his arms and postural instability. Later in the disease course he developed dystonia of his left leg, pathological crying, mutism and dysphagia. Neuropsychological assessment revealed prominent ideomotor and ideational apraxia, executive dysfunction, non-fluent aphasia and memory deficits. Neuroimaging showed symmetrical predominant frontoparietal atrophy and hypoperfusion. Frontotemporal lobar degeneration (FTLD)-TDP type 3 pathology was found at autopsy. *GRN* sequencing revealed a novel frameshift mutation c.314dup, p.Cys105fs and *GRN* mRNA levels showed a 50% decrease. We found a novel *GRN* mutation in a patient with an atypical CBS presentation with symmetric neuroimaging findings. *GRN* mutations are an important cause of CBS associated with FTLD-TDP type 3 pathology, sometimes in sporadic cases. Screening for *GRN* mutations should be considered in CBS patients without a positive family history.

INTRODUCTION

Corticobasal syndrome (CBS) is characterized by progressive asymmetric rigidity and apraxia, accompanied by additional signs of cortical and extrapyramidal dysfunction such as alien limb phenomenon, cortical sensory loss, myoclonus, bradykinesia, and focal dystonia unresponsive to dopaminergic treatment.[1] Although corticobasal degeneration (CBD) is the most frequent underlying pathology of CBS, other neuropathological causes including frontotemporal lobar degeneration (FTLD), Alzheimer's disease (AD), progressive supranuclear palsy (PSP), and Creutzfeldt-Jakob disease (CJD) have been reported.[2-4]

Although family history is usually negative in CBS,[5] a familial form has been described which can be caused by *progranulin* (*GRN*) mutations.[2, 6] *GRN* mutations show a high phenotypic variability reflected by diagnoses of frontotemporal dementia (FTD), progressive non-fluent aphasia (PNFA), or AD within the same families.[6-10] The underlying pathology of *GRN* mutations is FTLD with TAR DNA-binding protein of 43 kDA (TDP-43) positive neuronal cytoplasmic and intranuclear inclusions,[7-9, 11] which is known as FTLD-TDP type 3.[12]

In this report we describe the clinical, radiological, pathological, and genetic features of a 61-year-old patient with an initial presentation of symmetric CBS caused by a novel *GRN* c.314dup mutation in the absence of a positive family history.

MATERIALS AND METHODS

Subjects

A 61-year-old male was referred to the outpatient clinic of our neurology department. On the basis of neurological examination, neuropsychological assessment, brain MRI, and single photon emission computed tomography with ^{99m}Tc-hexamethyl propyleneamine oxine (HMPAO-SPECT) he was diagnosed with atypical Parkinsonism with dementia. He died from bronchopneumonia at the age of 63.

Neuropathological analysis

Brain autopsy was performed by the Netherlands Brain Bank according to their legal and ethical code of conduct. Paraffin-embedded sections from all brain regions were stained with haematoxylin and eosin, Bodian, methenamine silver, and Congo Red. Immunostaining with antibodies for tau (Innogenetics), ubiquitin (DAKO), β-amyloid (DAKO), α-synuclein (Zymed Laboratories), p62 (BD Biosciences Pharmingen), FUS (Sigma-Aldrich anti-FUS) and TDP-43 (Biotech) was performed. Neuropathological diagnosis was made by a neuropathologist (A.J.M.R.).

Genetic analysis

DNA was extracted from peripheral blood using the DNA Blood Kit Special (Chemagen, Baesweiler), which is based on DNA extraction and purification with

magnetic beads.[13] All coding exons (2-13) and exon/intron boundaries of *GRN* (NM_002087.2) were amplified from genomic DNA by PCR and directly sequenced in both strands using the ABI 3730XL automated sequencer (Applied Biosystems, Foster City, CA). Data was analyzed using SeqScape software (version 2.6; Applied Biosystems).

mRNA expression analysis

Total RNA was extracted from cerebellar brain tissue using the RNeasy Plus Mini Kit (Qiagen, Valencia, CA), and quality and quantity was assessed on the 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). RNA samples were normalized to 50 ng/µl and using 200 ng as the template a reverse transcription reaction was performed using a 1:1 mix of random hexamers and oligo(dT) primers, and the SuperScript III system (Invitrogen, Carlsbad, CA). Gene expression assays were ordered from Applied Biosystems for *GRN* (Hs00963703_g1), and *GAPDH* (Hs00266705_g1) as an endogenous control. Real-time PCR was performed on an ABI 7900 using the TaqMan method. Reactions contained 1 µl of cDNA amplified with 0.25 µl primer/probe mix and 2.5 µl TaqMan 2x Universal PCR Master Mix (Applied Biosystems, Foster City, CA). The cycling parameters recommended by the manufacturer were followed. All samples were run in triplicate and normalized to *GAPDH*. The carboxyfluorescein (FAM)-fluorescent signal was analyzed using SDS2.2.2 software, and relative quantities of *GRN* mRNA were determined using the ΔΔct method.

RESULTS

Clinical, neuropsychological and imaging features

A 61-year-old male barber presented with two-year duration of progressive walking disability, frequent falls, and difficulty with rising from a chair. This motor decline was later followed by loss of initiative, emotional lability, more compliant behavior, and increasing forgetfulness. He made inappropriate sexual remarks, and his speech became non-fluent with perseverations, word-finding difficulties and comprehension deficits. Financial matters were taken over by his wife. Furthermore, he needed assistance for dressing and personal hygiene, and was occasionally incontinent of urine and faeces. His symptoms were unresponsive to galantamine and levodopa. The patient's family history was negative for

neurodegenerative disorders. His father died at the age of 72 from a stroke and his mother at the age of 53 due to liver cancer.

Initial neurological examination showed normal eye movements, a mask-like facial expression, hypokinetic dysarthria, positive primitive reflexes, subtle axial rigidity, hypokinetic-rigid syndrome with cog-wheeling of the upper extremities, mildly increased tone of the lower extremities, postural instability and impaired tandem gait.

Neuropsychological testing revealed a Mini-Mental State Examination (MMSE) score of 24/30 with intact orientation in time and place. The patient was highly distractible, and exhibited sparse spontaneous speech with stuttering and perseverations. Ideomotor and ideational apraxia were prominent, the patient did not succeed in imitation, constructional tasks, and rapid alternating hand movements. Furthermore, he had severely impaired executive and memory functions, poor semantic word fluency, and naming deficits, but intact visuospatial functions. Neuroimaging revealed mild symmetrical cerebral atrophy, most prominent frontoparietal and perisylvian on MRI and symmetrical frontoparietal hypoperfusion on SPECT, which is shown in Figure 1.

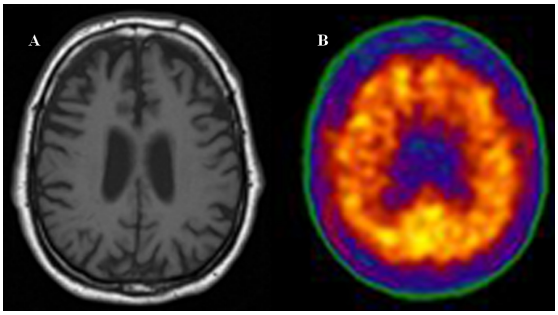

Figure 1. Mild symmetric frontoparietal atrophy on MRI (A) and symmetric parietal hypoperfusion on SPECT scan (B).

The patient's condition further declined over the subsequent years. Neurological examination at a follow-up visit one year later revealed slowed saccadic eye movements, severe symmetrical rigidity of the upper extremities, an increased tone with dystonic flexion posturing of the left leg and pathological crying. Finally,

he developed mutism and dysphagia, which did not respond to amitriptyline treatment, and he died from bronchopneumonia at the age of 63 years.

Neuropathological examination

Gross brain (1,327 g) examination showed mild generalized atrophy of the cortex with atrophy of caudate nucleus and putamen and normal pigmentation of the substantia nigra. Microscopic examination revealed moderate to severe neuronal loss, gliosis, and spongiosis in the frontal and parietal cortices, but mild changes in the temporal cortex, hippocampus, and basal ganglia. Immunohistochemistry showed TDP-43-positive dystrophic neurites, neurocytoplasmatic, and intranuclear inclusions most prominent in the second superficial layer of the frontal and parietal cortices, and to a lesser extent in the temporal cortex, hippocampus and basal ganglia, consistent with a diagnosis of FTLD-TDP type 3 (Figure 2).[12]

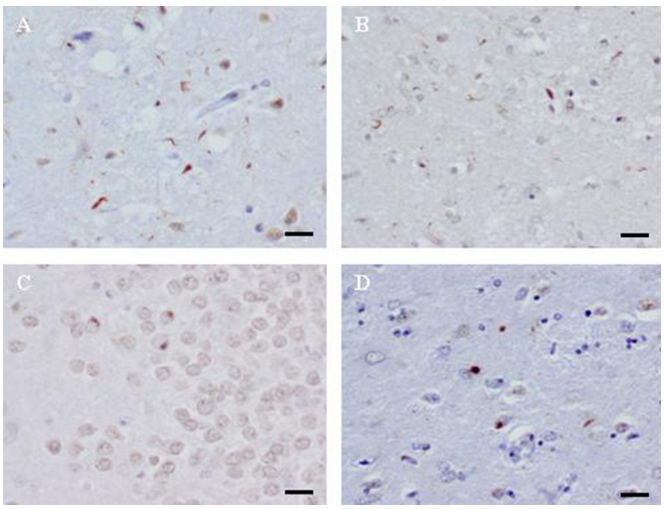

Figure 2. TDP-43 positive dystrophic neurites in the frontal cortex (A) a TDP-43 positive neuronal intranuclear inclusion in the parietal cortex (B), and TDP-43 positive neuronal cytoplasmatic inclusions in hippocampus (C) and caudate nucleus and putamen (D). Scale bar = 20 µm

Genetic analysis

After receiving autopsy results, we screened *GRN* and identified a novel c.314dup, p.Cys105fs mutation. This frameshift mutation causes premature termination of the coding sequence, which is likely to result in loss of function of Granulin.

mRNA expression analysis

GRN mRNA expression analysis in cerebellar tissue from this patient showed a 50% decrease in *GRN* mRNA levels compared to a pathologically-confirmed normal control, confirming the pathogenicity of the mutation.

DISCUSSION

We described a patient with an atypical CBS presentation with symmetrical neuroimaging findings with FTLD-TDP type 3 pathology due to a novel c.314dup, p.Cys105fs mutation in the absence of a positive family history.

The clinical presentation of our case showed strong similarities with the symmetric CBD described by Hassan et al, except for the presence of ideomotor and ideational apraxia.[14] The symmetric rigidity and the absence of alien hand phenomenon and myoclonus did not fit with the clinical diagnosis CBS in the initial phase of the disease.[1] Moreover, the symmetric pattern of frontoparietal atrophy on MRI and parietal hypoperfusion on SPECT scan was inconsistent with the typical asymmetric distribution in CBS, but was in line with the observations in symmetric CBD.[1, 14, 15] In a later stage of disease, our patient developed dystonia in the left leg, which made the clinical diagnosis CBS more likely.[1] However, we did not have the opportunity to visualize an asymmetric pattern of atrophy, as an attempt to obtain additional neuroimaging was unsuccessful.

The neuropathological findings of the present case were similar to those in the case by Spina *et al*, which showed TDP-43 pathology most prominent in parietal and frontal cortex and to a lesser extent in the temporal cortex, with involvement of basal ganglia.[5] Their patient had a more typical CBS presentation with an alien limb phenomenon and marked asymmetry of symptoms as well as neuroimaging findings, which is more often seen in CBS patients with *GRN* mutations.[5, 16, 17] The combination of parkinsonism, apraxia, behavioral changes, non-fluent speech, and memory impairment in our patient could retrospectively have indicated a *GRN* mutation, because these symptoms have been more frequently observed in FTLD patients with *GRN* mutations.[7, 9, 10]

The absence of a positive family history in the current case emphasizes the possibility of reduced penetrance of *GRN* mutations or concealed family history

due to the wide range in onset age of *GRN* mutations.[8, 11, 18, 19] We only screened for *GRN* mutations after the observation of FTLD-TDP type 3 pathology at autopsy. This case therefore underlines that genetic screening should be considered even when family history appears to be negative. The novel *GRN* frameshift mutation in this patient led to a premature stopcodon and thereby to loss of function of Granulin, which was confirmed by reduced mRNA expression levels in cerebellar tissue. Recent studies have shown reduced Granulin levels in serum in patients with *GRN* mutations.[20-24] We could not confirm this in our patient however, since his serum was not available.

Based on the clinical presentation of this patient, we did not predict FTLD-TDP pathology. Although non-fluent aphasia and parkinsonism can be seen in FTLD-TDP, an asymmetric presentation and pattern of atrophy is associated with all underlying pathologies of CBS, including CBD, PSP, FTLD and AD.[15] In addition, the extension of atrophy into the frontal cortex is typically asymmetric in patients with CBS with FTLD-TDP pathology, including those with *GRN* mutations.[15]; [6, 7, 10, 25, 26] Therefore the present case is unique by its atypical CBS presentation and symmetric atrophy pattern associated with FTLD-TDP type 3 pathology with a novel *GRN* mutation.

To conclude, we found a novel *GRN* mutation in a patient with an atypical CBS presentation with symmetric neuroimaging findings without a positive family history. *GRN* mutations are an important cause of CBS associated with FTLD-TDP pathology. Genetic screening should also be considered in CBS patients with a negative or concealed family history.

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REFERENCES

1. Boeve BF, Lang AE, Litvan I. Corticobasal degeneration and its relationship to progressive supranuclear palsy and frontotemporal dementia. Ann Neurol. 2003;54 Suppl 5:S15-9.
2. Wadia PM, Lang AE. The many faces of corticobasal degeneration. Parkinsonism Relat Disord. 2007;13 Suppl 3:S336-40.
3. Tartaglia MC, Sidhu M, Laluz V, Racine C, Rabinovici GD, Creighton K, et al. Sporadic corticobasal syndrome due to FTLD-TDP. Acta Neuropathol. 2010 Mar;119(3):365-74.
4. Boeve BF, Maraganore DM, Parisi JE, Ahlskog JE, Graff-Radford N, Caselli RJ, et al. Pathologic heterogeneity in clinically diagnosed corticobasal degeneration. Neurology. 1999 Sep 11;53(4):795-800.
5. Spina S, Murrell JR, Huey ED, Wassermann EM, Pietrini P, Grafman J, et al. Corticobasal syndrome associated with the A9D Progranulin mutation. J Neuropathol Exp Neurol. 2007 Oct;66(10):892-900.
6. van Swieten JC, Heutink P. Mutations in progranulin (GRN) within the spectrum of clinical and pathological phenotypes of frontotemporal dementia. Lancet Neurol. 2008 Oct;7(10):965-74.
7. Beck J, Rohrer JD, Campbell T, Isaacs A, Morrison KE, Goodall EF, et al. A distinct clinical, neuropsychological and radiological phenotype is associated with progranulin gene mutations in a large UK series. Brain. 2008 Mar;131(Pt 3):706-20.
8. Gass J, Cannon A, Mackenzie IR, Boeve B, Baker M, Adamson J, et al. Mutations in progranulin are a major cause of ubiquitin-positive frontotemporal lobar degeneration. Hum Mol Genet. 2006 Oct 15;15(20):2988-3001.
9. Kelley BJ, Haidar W, Boeve BF, Baker M, Graff-Radford NR, Krefft T, et al. Prominent phenotypic variability associated with mutations in Progranulin. Neurobiology of aging. 2009 May;30(5):739-51.
10. Le Ber I, Camuzat A, Hannequin D, Pasquier F, Guedj E, Rovelet-Lecrux A, et al. Phenotype variability in progranulin mutation carriers: a clinical, neuropsychological, imaging and genetic study. Brain. 2008 Mar;131(Pt 3):732-46.
11. Whitwell JL, Jack CR, Jr., Baker M, Rademakers R, Adamson J, Boeve BF, et al. Voxel-based morphometry in frontotemporal lobar degeneration with ubiquitin-positive inclusions with and without progranulin mutations. Arch Neurol. 2007 Mar;64(3):371-6.
12. Cairns NJ, Bigio EH, Mackenzie IR, Neumann M, Lee VM, Hatanpaa KJ, et al. Neuropathologic diagnostic and nosologic criteria for frontotemporal lobar degeneration: consensus of the Consortium for Frontotemporal Lobar Degeneration. Acta Neuropathol (Berl). 2007 Jul;114(1):5-22.
13. Smit ML, Giesendorf BA, Heil SG, Vet JA, Trijbels FJ, Blom HJ. Automated extraction and amplification of DNA from whole blood using a robotic workstation and an integrated thermocycler. Biotechnol Appl Biochem. 2000 Oct;32 (Pt 2):121-5.
14. Hassan A, Whitwell JL, Boeve BF, Jack CR, Jr., Parisi JE, Dickson DW, et al. Symmetric corticobasal degeneration (S-CBD). Parkinsonism Relat Disord. 2010 Mar;16(3):208-14.

15. Whitwell JL, Jack CR, Jr., Boeve BF, Parisi JE, Ahlskog JE, Drubach DA, et al. Imaging correlates of pathology in corticobasal syndrome. Neurology. 2010 Nov 23;75(21):1879-87.

16. Masellis M, Momeni P, Meschino W, Heffner R, Jr., Elder J, Sato C, et al. Novel splicing mutation in the progranulin gene causing familial corticobasal syndrome. Brain. 2006 Nov;129(Pt 11):3115-23.

17. Benussi L, Binetti G, Sina E, Gigola L, Bettecken T, Meitinger T, et al. A novel deletion in progranulin gene is associated with FTDP-17 and CBS. Neurobiol Aging. 2008 Mar;29(3):427-35.

18. Le Ber I, van der Zee J, Hannequin D, Gijselinck I, Campion D, Puel M, et al. Progranulin null mutations in both sporadic and familial frontotemporal dementia. Hum Mutat. 2007 Sep;28(9):846-55.

19. Seelaar H, Kamphorst W, Rosso SM, Azmani A, Masdjedi R, de Koning I, et al. Distinct genetic forms of frontotemporal dementia. Neurology. 2008 Oct 14;71(16):1220-6.

20. Coppola G, Karydas A, Rademakers R, Wang Q, Baker M, Hutton M, et al. Gene expression study on peripheral blood identifies progranulin mutations. Ann Neurol. 2008 Jul;64(1):92-6.

21. Finch N, Baker M, Crook R, Swanson K, Kuntz K, Surtees R, et al. Plasma progranulin levels predict progranulin mutation status in frontotemporal dementia patients and asymptomatic family members. Brain. 2009 Mar;132(Pt 3):583-91.

22. Ghidoni R, Benussi L, Glionna M, Franzoni M, Binetti G. Low plasma progranulin levels predict progranulin mutations in frontotemporal lobar degeneration. Neurology. 2008 Oct 14;71(16):1235-9.

23. Sleegers K, Brouwers N, Van Damme P, Engelborghs S, Gijselinck I, van der Zee J, et al. Serum biomarker for progranulin-associated frontotemporal lobar degeneration. Ann Neurol. 2009 May;65(5):603-9.

24. Schofield EC, Halliday GM, Kwok J, Loy C, Double KL, Hodges JR. Low serum progranulin predicts the presence of mutations: a prospective study. J Alzheimers Dis. 2010 Jan 1;22(3):981-4.

25. Whitwell JL, Jack CR, Jr., Parisi JE, Senjem ML, Knopman DS, Boeve BF, et al. Does TDP-43 type confer a distinct pattern of atrophy in frontotemporal lobar degeneration? Neurology. 2010 Dec 14;75(24):2212-20.

26. Rohrer JD, Geser F, Zhou J, Gennatas ED, Sidhu M, Trojanowski JQ, et al. TDP-43 subtypes are associated with distinct atrophy patterns in frontotemporal dementia. Neurology. 2010 Dec 14;75(24):2204-11.

CHAPTER 2.2

The clinical and pathological phenotype of *C9orf72* hexanucleotide repeat expansions

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ABSTRACT

There is increasing evidence that frontotemporal dementia and amyotrophic lateral sclerosis are part of a disease continuum. Recently, a hexanucleotide repeat expansion in *C9orf72* was identified as a major cause of both sporadic and familial frontotemporal dementia and amyotrophic lateral sclerosis. The aim of this study was to investigate clinical and neuropathological characteristics of hexanucleotide repeat expansions in *C9orf72* in a large cohort of Dutch patients with frontotemporal dementia. Repeat expansions were successfully determined in a cohort of 353 patients with sporadic or familial frontotemporal dementia with or without amyotrophic lateral sclerosis, and 522 neurologically normal controls. Immunohistochemistry was performed in a series of 10 brains from patients carrying expanded repeats using a panel of antibodies. In addition, the presence of RNA containing GGGGCC repeats in paraffin-embedded sections of post-mortem brain tissue was investigated using fluorescence *in situ* hybridization with a locked nucleic acid probe targeting the GGGGCC repeat. Hexanucleotide repeat expansions in *C9orf72* were found in 37 patients with familial (28.7%) and five with sporadic frontotemporal dementia (2.2%). The mean age at onset was 56.9 ± 8.3 years (range 39 - 76), and disease duration 7.6 ± 4.6 years (range 1 - 22). The clinical phenotype of these patients varied between the behavioral variant of frontotemporal dementia (n = 34) and primary progressive aphasia (n = 8), with concomitant amyotrophic lateral sclerosis in seven patients. Predominant temporal atrophy on neuroimaging was present in 13 of 32 patients. Pathological examination of the 10 brains from patients carrying expanded repeats revealed frontotemporal lobar degeneration with neuronal transactive response DNA binding protein-positive inclusions of variable type, size and morphology in all brains. Fluorescence *in situ* hybridization analysis of brain material from patients with the repeat expansion, a *microtubule-associated protein tau* or a *progranulin* mutation, and controls did not show RNA-positive inclusions specific for brains with the GGGGCC repeat expansion. The hexanucleotide repeat expansion in *C9orf72* is an important cause of frontotemporal dementia with and without amyotrophic lateral sclerosis, and is sometimes associated with primary progressive aphasia. Neuropathological hallmarks include neuronal and glial inclusions, and dystrophic neurites containing transactive response DNA binding protein. Future studies are needed to explain the wide variation in clinical presentation.

INTRODUCTION

Frontotemporal dementia (FTD) is the second most common type of presenile dementia and is characterized by behavioral changes, executive and language dysfunctions due to neurodegeneration of the frontal and temporal cortex.[1, 2] Amyotrophic lateral sclerosis (ALS) is the most common type of motor neuron disease, characterized by rapidly progressive paralysis due to degeneration of upper and lower motor neurons leading to death within a few years.[3] There is increasing clinical, pathological and genetic evidence for the hypothesis that FTD and ALS are part of a disease continuum. First of all, FTD patients frequently develop symptoms of motor neuron disease, and cognitive dysfunction is often seen in patients with ALS.[4] Secondly, the transactive response DNA binding protein of 43 kDa (TDP-43), an RNA binding protein, is the major pathological protein in FTD and ALS, with neuronal and glial TDP-43 positive inclusions in neocortex, basal ganglia and/or spinal cord.[5, 6] Thirdly, the two disorders have been shown to share genetic etiology, apart from the genetic defects distinctive for each. *Microtubule-associated protein tau* (*MAPT*) and *progranulin* (*GRN*) mutations are exclusively associated with FTD; the same is true for *superoxide dismutase 1* and *optineurin* mutations in ALS, but *fused in sarcoma*, *valosin-containing protein,* and *TDP* mutations are also occasionally found in patients with FTD.[7-19] Families in which affected members present with FTD, ALS, or both have shown significant linkage to chromosome 9p21.3.[20-25] Moreover, genome-wide association studies of both ALS and FTD have shown a significant association with the same chromosomal locus.[26-28] These findings indicate that this locus has a major genetic contribution to FTD and ALS. The associated risk haplotype appears to be the same for most chromosome 9p-linked families of European ancestry, suggesting a common founder.[29]

In September 2011, we and others simultaneously identified a $(GGGGCC)_n$ repeat expansion in a non-coding region of *C9orf72* on chromosome 9 in FTD and ALS.[30, 31] Pathogenic expanded repeats were found in 30 - 50% of cases with familial ALS and FTD, and in 4 - 10% of sporadic cases.[30, 31]

Quantitative messenger RNA analysis has shown that the presence of the expanded repeats leads to reduced expression of one of the transcripts of *C9orf72* encoding a

protein with an unknown function, suggesting a (partial) loss-of-function disease mechanism.[30] However, a toxic gain of function of abnormal messenger RNA has been hypothesized as well, based on the discovery of multiple nuclear RNA foci in brain tissue from patients carrying the expanded repeats using fluorescence *in situ* hybridization experiments with a probe targeting the GGGGCC repeat.[30]

As the clinical and pathological phenotype has only been studied in a few families with FTD+ALS so far, it is important to investigate the phenotypical variation of the repeat expansion in more detail in a larger cohort. In the present study, we investigated the clinical and pathological characteristics of the GGGGCC hexanucleotide repeat expansion in *C9orf72* in a large Dutch cohort of familial and sporadic FTD patients with and without ALS.

METHODS

Patients and controls

The Dutch FTD series comprises 458 FTD patients, ascertained in an on-going genetic-epidemiological study conducted in the Netherlands since 1994, including patients that were referred to Neurology departments of the Erasmus Medical Centre or the VU University Medical Centre, or that were ascertained by research physicians visiting nursing homes and psychogeriatric hospitals. The diagnosis of FTD was based on international consensus criteria,[32] and concomitant ALS was diagnosed when patients also met El Escorial criteria.[33] Pathological confirmation of frontotemporal lobar degeneration (FTLD) was obtained in 94 patients.[34]
The study was approved by the Medical Ethical Committee of the Erasmus Medical Centre and VU University Medical Centre. Following receipt of informed consent, DNA samples were obtained from each patient.
We excluded all patients with *MAPT* or *GRN* mutations (46 and 30 patients, respectively), or with tau-positive FTLD (19 patients). The remaining cohort to be screened for the repeat expansion in *C9orf72* consisted of 363 patients with FTD, including 38 patients with concomitant ALS. The mean age at onset was 58.0 ± 8.3 years (range 28 - 76). The mean age at death in patients that died during follow-up (n = 208) was 66.3 ± 9.7 years (range 35 - 89), with mean disease duration of 8.2 ± 4.4 years (range 1 - 23) (Table 1). The most common clinical presentation was the behavioral variant of FTD (bvFTD) (n = 262), followed by primary progressive

aphasia (PPA) (*n* = 101). Family history was positive for dementia (*n* = 130), ALS (*n* = 25) or Parkinson's disease (*n* = 19) in at least one first-degree relative in 133 patients from 120 families. In two families, a relative with a pure ALS presentation was also genotyped. Our control group consisted of 564 neurologically normal subjects (269 men and 295 women) from the Longitudinal Aging Study Amsterdam (LASA, http://www.lasa-vu.nl/). The mean age at clinical examination for this group was 67.8 ± 6.0 years (range 60 - 81).

Table 1. Demographic features of the Dutch FTD Cohort.

	FTD cohort (n=363)
Female (%)	178 (49.0)
Age at onset, years (range)	58.0±8.3 (28-76)
Age at death (***n***=208), years (range)	66.3±9.7 (35-89)
Disease duration (***n***=208), years (range)	8.2±4.4 (1-23)
FTD Subtype	
bvFTD (%)	262 (72.2)
PPA (%)	101 (27.8)
ALS	38 (10.5)
Family history	
Positive for dementia (%, no. of families)	130 (35.8, 117)
Positive for ALS (%, no. of families)	25 (6.9, 17)
Positive for PD (%, no. of families)	19 (5.2, 16)
Negative (%)	230 (63.4)
Neuropathological examination (*n*= 51)	
FTLD-TDP	45 (88.2)
FTLD-FUS	4 (7.8)
FTLD-ni	1 (2.0)
FTLD (subtype unknown)	1 (2.0)

FUS, fused in sarcoma; ni, no inclusions

Clinical data

Detailed clinical history and family history were obtained for all patients by interviewing their relatives and collecting data from medical records. We carried out a neurological examination of all patients and, when possible, patients underwent neuropsychological evaluation and neuroimaging (MRI or CT).

Neuropsychological evaluation consisted of tests for language (e.g. Boston Naming Test, Semantic Association Test, word fluency), memory (e.g. Rey Auditory Verbal Learning Test, Visual Association Test), attention and concentration and executive functions (e.g. Trail Making Test, Stroop color-word test, modified Wisconsin Card Sorting Test, Similarities and Proverbs of the Wechsler Adult Intelligence Scale), and visuospatial abilities (e.g. Clock drawing, Block Design of the Wechsler Adult Intelligence Scale). The presence and severity of frontal, temporal, parietal, occipital and cerebellar atrophy were review by a neurologist (J.C.v.S.) and a radiologist (M.S.). Patients with signs suggestive of ALS, such as muscle weakness, atrophy, or fasciculations, underwent EMG. The age at onset was defined as the moment partners or other relatives noticed the first symptoms attributable to the disease. Three classes of family history were distinguished: 1) autosomal dominant: patients with at least two first-degree relatives with dementia or ALS; 2) patients with only a single affected first-degree relative with dementia or ALS; and 3) patients without affected relatives or an unknown family history.

Genotyping methods

For the repeat-primed polymerase chain reaction (PCR) 50ng of genomic DNA from each patient, was mixed with FastStart Taq DNA polymerase PCR buffer (Roche Applied Science), 7-deaza-dGTP (New England Biolabs), Q-Solution (Qiagen Inc.), dimethylsulfoxide Hybri-Max (Sigma-Aldrich), MgCl2 (Roche Applied Science), reverse primer consisting of four GGGGCC repeats with an anchor tail, 6FAM™-fluorescent labeled forward primer located 128-bp telomeric to the repeat sequence, and an anchor primer corresponding to the anchor tail of the reverse primer, as described in our previous article.[31] Primer sequences are available upon request.[35, 36] Fragment length analysis was performed on an ABI 3730xl genetic analyzer (Applied Biosystems), and data analyzed using Peak Scanner software version 1.0 (Applied Biosystems). Repeat expansions produce a characteristic saw tooth pattern with a 6-bp periodicity (Supplementary figure 1). We obtained hexanucleotide repeat lengths based on the repeat-primed PCR assay by successful genotyping in 353 cases and 522 controls (94.4% of the total cohort). To note, the repeat-primed PCR assay used for these experiments does not determine the actual number of repeats in a large pathogenic expansion. This technique only allows for testing whether a given sample carries a large pathogenic expansion or not. A cut-off value of 30 repeats was used to define expanded repeats, as

previously described.[31]

Pathological examination

Brain autopsy was carried out within four hours of death according to the legal and ethical code of conduct of the Netherlands Brain Bank. Tissue blocks taken from all cortical areas, hippocampus, amygdala, basal ganglia, substantia nigra, pons, medulla oblongata, cerebellum, and cervical spinal cord were embedded in paraffin blocks, and underwent routine staining with haematoxylin-eosin, Bodian, methenamine-silver, and Congo red. Tissue blocks were taken from the right hemisphere in each case. Immunohistochemistry was performed using primary antibodies against hyperphosphorylated tau (AT8, Innogenetics; 1:40), ubiquitin (anti-ubiquitin, DAKO; 1:500, following 80˚C antigen retrieval), β-amyloid protein (anti-beta amyloid, DAKO; 1:100, following formic acid pretreatment), α-synuclein (anti-α-synuclein, Zymed Laboratories; undiluted, following formic acid pretreatment), p62 (BD Biosciences Pharmingen; 1:200, following 80˚C antigen retrieval), TDP-43 (Biotech; 1:100, following pressure cooking), TDP-43 phosphorylated at serine 409/410 (Cosmo Bio; 1:8000), fused in sarcoma (Sigma-Aldrich anti-fused in sarcoma; 1:25–1:200 with initial overnight incubation at room temperature, following pressure cooking) and C9orf72 (GeneTex; 1:200) and stained as previously described.[37] Primary antibodies were incubated overnight at 4°C. Endogenous peroxidase activity was inhibited by incubation in phosphate buffered saline-hydrogen peroxide-sodium azide solution (100ml 0.1 M phosphate buffered saline + 2 ml 30% H_2O_2 + 1 ml natriumazide) for 30 minutes. The Histostain-Plus broad-spectrum kit DAB (Zymed) was used, and slides were counterstained with Mayer's haematoxylin and mounted in Entellan. The pathological diagnosis was made by a neuropathologist (A.J.M.R.). Brain autopsy performed in 51 out of the total cohort of 363 patients revealed TDP-43 positive pathology (FTLD-TDP) in 45 patients, FTLD with fused in sarcoma positive pathology in five, FTLD with no inclusions in one, and FTLD (subtype unknown) in one.

The pattern of FTLD-TDP pathology was classified into the four following subtypes: type A is characterized by numerous short dystrophic neurites and crescentic or oval neuronal cytoplasmic inclusions, concentrated primarily in neocortical layer 2. Moderate numbers of lentiform neuronal intranuclear inclusions are also a common but inconsistent feature of this subtype; type B by moderate numbers

of neuronal cytoplasmic inclusions, throughout all cortical layers, but very few dystrophic neurites; type C by a predominance of elongated dystrophic neurites in upper cortical layers, with very few neuronal cytoplasmatic inclusions; type D by numerous short dystrophic neurites and frequent lentiform neuronal intranuclear inclusions.[34]

Fluorescence in *situ* hybridization

The hypothesis of a toxic RNA gain-of-function mechanism for FTD/ALS suggests that RNA containing expanded non-coding hexanucleotide repeats accumulates in affected cells. To test this hypothesis, we examined paraffin-embedded sections of post-mortem temporal cortex and hippocampal tissue for the presence of RNA containing GGGGCC repeats using fluorescence *in situ* hybridization. For RNA-fluorescence *in situ* hybridization, brain sections were hybridized either with an oligonucleotide probe $(GGCCCC)_3$ 5' TYE563 or a CCCCGGCCCC 5' TYE563 labeled locked nucleic acid oligonucleotide probe (both Exicon), which differs from the method used by DeJesus-Hernandez *et al.* [30] After the RNA-fluorescence *in situ* hybridization protocol the slides were incubated with Hoechst in PBS (1:15000) and washed two times for 5 min with phosphate-buffered saline, followed by one wash in de-ionized water, before mounting in Mowiol. Slides were examined using a confocal fluorescence microscope (Leica).

Statistical analysis

Fisher's Exact Test was used to test for association between the presence of *C9orf72* repeat expansion and both familial and sporadic FTD using the PLINK v1.07 toolset. Since presence of individuals from the same family could bias our association results, only one affected individual was included per family in this analysis. Independent samples *t*-tests to compare continuous variables between patients with the *C9orf72* repeat expansion and patients with *MAPT* or *GRN* mutations were performed using SPSS 17.0 for windows (SPSS). A significance level of $p < 0.05$ was used.

RESULTS

Genotyping results

A total of 353 cases with FTD and 522 controls were successfully genotyped with

the described repeat-primed PCR assay. Histograms of repeat lengths based on the repeat-primed PCR assay are shown for both cases and controls (Supplementary figure 2). The average repeat length in the control population was 9.1 ± 6.8 (range 2 - 35 repeats), and for the cases with FTD, the average repeat length was 13.9 ± 14.0 (range 1 - 64 repeats). A total of 42 cases from our cohort (11.9%) and three controls (0.6%) carried the expansion (Fisher's test *p*-value = 4.39×10^{-12}; odds ratio = 19.22, 95% confidence interval = 5.89 - 62.66 after removal of cases belonging to the same family). Thirty-seven FTD patients with the expansion had a positive family history (28.7% of genotyped FTD patients with positive family history), with an autosomal dominant mode of inheritance was in 25 patients (19 families, four of which are shown in Figure 1), including four families where reduced penetrance was observed (family 3 in Figure 1). Obligate carriers in two of the latter families died after the age of 70 without any signs of dementia or ALS. The remaining 12 patients with a positive family history had only one first-degree family member with either dementia or ALS (11 families) (Table 2). Fisher association analysis of familial FTD cases versus controls (individuals belonging to the same family were removed for this analysis) gave the following results: Fisher's test *p*-value = 1.76×10^{-20}, odds ratio = 51.47, 95% confidence interval = 15.95 - 169.90. There was a wide phenotypic variability within families with diagnoses of both FTD, ALS and FTD+ALS in 12 families. Furthermore three families included individuals with Parkinson's disease, however we cannot be certain that this is also caused by the repeat expansion, since these individuals were not genotyped.

In one family with autosomal dominant FTD+ALS the proband included in our clinical FTD cohort had a repeat length of 26 and was therefore assumed not to carry the repeat expansion. However, sequencing in family members revealed the repeat expansion in a so far unaffected person (age 42 years) and repeat length varying from eight to 29 in affected persons. Therefore we are uncertain about the pathogenicity of the repeats in this family, especially as it is not yet possible to determine exact repeat lengths. Moreover, in three of the families with the repeat expansion, there was one affected individual with a repeat length of 29.

The repeat expansion in *C9orf72* was found in five of the 224 genotyped patients with sporadic FTD (2.2%), which was not significantly different from healthy controls (Fisher's test *p*-value = 0.0569, odds ratio = 3.93, 95% confidence interval

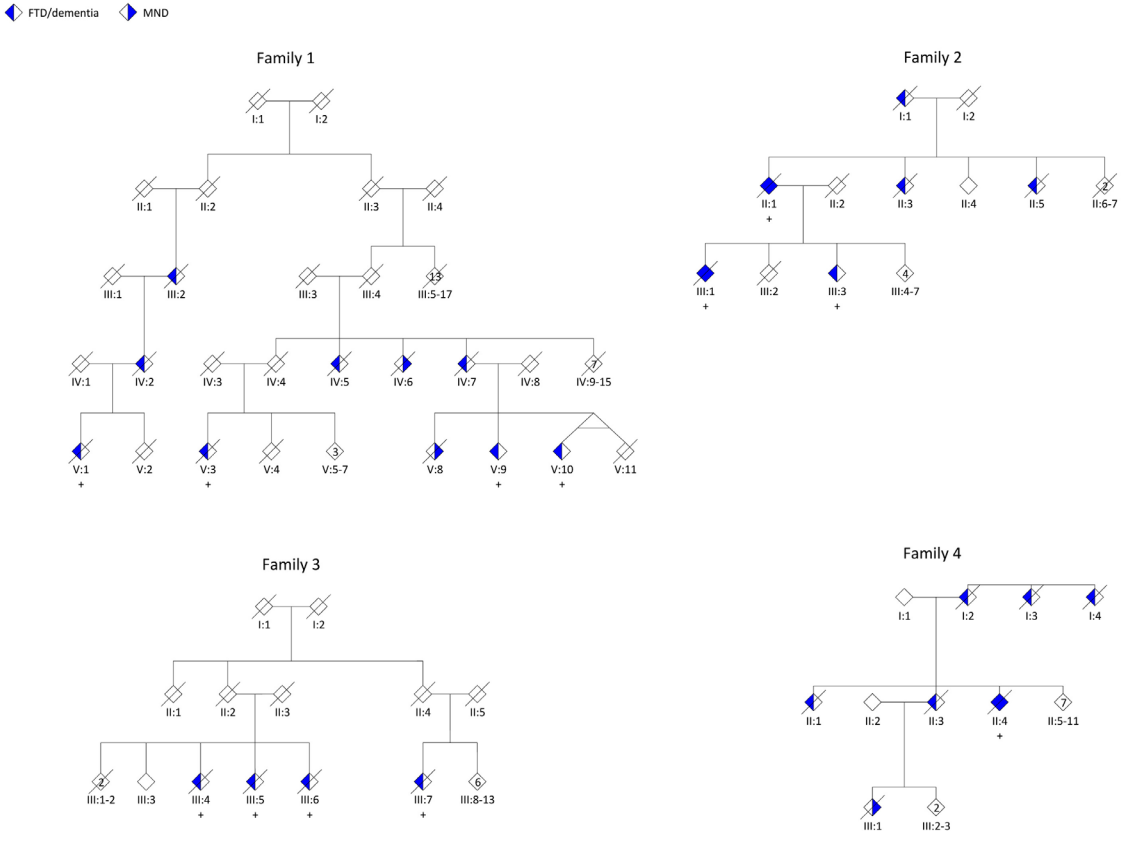

Figure 1. Pedigrees for families 1-4. Subjects that were included in the sequencing cohort are indicated with (+).

Table 2. Clinical and pathological features of patients with FTD with the repeat expansion.

	No. of affecteds (confirmed expansion)	*No. of families*	*Age at onset (range)*	*Age at death (range)*	*Disease duration (range)*	*FTD subtype (no. of patients)*	*Imaging* (no. of patients)*	*Pathology (no. of patients)*
Autosomal dominant								
Family 1	10 (3)	1	60.8 (55-67)	70.0 (64-75)	10.0 (9-12)	bvFTD (8), ALS (2)	T (1), FT (2), G (1)	na
Family 2	6 (2)	1	59.2 (39-80)	70.2 (59-84)	7.0 (1-17)	bvFTD (4), bvFTD+ALS (2)	N (1), T (1), FT (1)	FTLD-TDP (1)
Family 3	4 (3)	1	54.1 (45-62)	59.2 (52-64)	8.0 (6-9)	bvFTD (4)	N (1), T (2), G (1)	FTLD-TDP (2)
Family 4	7 (1)	1	65.0 (64-67)	69.3 (67-73)	4.0 (1-8)	bvFTD (5), ALS (1), FTD+ALS (1)	T (1)	na
Remaining autosomal dominant cases	18 (18)	15	57.1 (39-67)	65.4 (46-75)	7.2 (1-18)	bvFTD (13), PPA (1), ALS (2), bvFTD+ALS (1), PPA+ALS (1)	F (1), T (2), FT (5), N (1), G (1)	FTLD-TDP (6)
Cases with only one affected family member	12 (12)	11	54.2 (39-76)	62.7 (42-78)	8.2 (2-22)	bvFTD (6), PPA (3), bvFTD+ALS (3)	N (2), F (2), T (2), FT (1), G (1)	FTLD-TDP (2)
No family history	5 (5)	5	61.3 (55-70)	67.9 (64-72)	9.1 (6-11)	bvFTD (2), PPA (2), bvFTD+ALS (1)	T (4), F (1)	FTLD-TDP (2)

*Area of most prominent atrophy is indicated. N, no atrophy; F, frontal; T, temporal; FT, frontotemporal; G, generalized.

= 0.93 – 16.53).

Of the remaining 311 patients in the cohort without the repeat expansion, 92 had a positive family history for dementia (89 families), with ALS in six families. Moreover, 31 of the patients with FTD without the repeat expansion had concomitant ALS.

In an effort to investigate whether the expansion carriers in our cohort carry the recently identified risk haplotype on chromosome 9,[29] genotyping data from a parallel project in our lab was extracted for 20 of the 42 expansion carriers (Figure 2). Genotypes of 12 of these samples (60.0%) were concordant with the reported risk haplotype. Interestingly, all other samples shared the same core risk haplotype, differing from it only in the most distal positions. These results suggest that all Dutch *C9orf72* carriers derive from a common mutated ancestor.

Clinical features

The mean age at onset in the 42 patients with FTD with the repeat expansion was 56.9 ± 8.3 years (range 39 - 76), mean age at death (*n* = 31) was 64.7 ± 8.6 years (range 42 - 78) and mean disease duration from onset till death was 7.6 ± 4.6 years (range 1 - 22). BvFTD was the initial clinical presentation in 34 patients (apathy in 18, disinhibition in 11, obsessive-compulsive behavior in five), and PPA in eight patients (Figure 3). Concomitant ALS was present in seven patients (bulbar onset in five, limb onset in two). Furthermore, we found the repeat expansion in two relatives of FTD patients with pure limb onset ALS. Mean score at the Mini-mental state examination was 25.9 ± 3.4 (*n* = 19, range 17 - 30). Memory complaints were reported in 21 patients at clinical presentation. Six patients showed signs of Parkinsonism and apraxia was present in seven patients. Visual or auditory hallucinations were reported in two patients and delusions in none. Mean duration of follow-up from disease onset in patients with the repeat expansion was 5.2 ± 3.3 years (range 0.8 - 13.4).

Of the eight patients with PPA, two showed fluent speech, anomia, and single-word comprehension deficits at neuropsychological evaluation compatible with the diagnosis semantic dementia (SD), supported by atrophy of the anterior temporal lobes (Figure 4). Classification into one of the PPA variants was not possible in the remaining six patients due to lack of information. Four of them had fluent

SNP ID	Position	Risk allele	Freq CEU	D95.2993	D96.0248	D98.8895	D98.8369	07D01816	D96.1388	D95.3021	D96.2281	D99.3488	D99.4036	D01.2972	D99.2671	06D01622	06D05358	07D01145	07D01783	07D01836	08D01457	06D03315	05D00499
rs1822723	27468052	C	0.708	C/C	C/C	T/C	T/C	C/C	C/C	C/C	C/C	T/C	C/C	T/C	C/C	C/C	C/C	C/C	C/C	C/C	T/C	T/C	C/C
rs4879515	27472235	T	0.39	C/T	C/T	T/T	C/T	T/T	C/T	C/T	T/T	C/T	C/T	C/T	C/T	C/T	T/T	C/T	C/T	C/T	T/T	T/T	C/T
rs868856	27479251	T	0.275	C/T	C/T	C/T	T/T	T/T	C/T	C/T	C/T	T/T	C/T	C/T	C/T	C/T	C/T	C/T	C/T	C/T	C/T	C/T	C/T
rs7046653	27480967	A	0.317	G/A	G/A	G/A	A/A	A/A	G/A	G/A	G/A	A/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A
rs1977661	27492986	C	0.892	C/C	C/C	A/C	C/C	C/C	C/C	C/C	C/C	C/C	C/C	C/C	C/C	C/C	C/C	C/C	C/C	C/C	C/C	A/C	C/C
rs903603	27519316	C	0.475	T/C	T/C	T/C	C/C	C/C	T/C	T/C	C/C	C/C	T/C	T/C	T/C	T/C	C/C	T/C	T/C	T/C	C/C	T/C	T/C
rs10812610	27523984	C	0.433	A/C	A/C	A/C	C/C	C/C	A/C	A/C	C/C	C/C	A/C	A/C	A/C	A/C	C/C	A/C	A/C	A/C	C/C	A/C	A/C
rs2814707	27526397	A	0.192	G/A	G/A	G/A	G/A	A/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A
rs3849942	27533281	A	0.183	G/A	G/A	G/A	G/A	A/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A	G/A
rs10122902	27546780	G	0.833	G/G	G/G	G/G	A/G	G/G	G/G	G/G	G/G	A/G	G/G	G/G	G/G	G/G	A/G	G/G	G/G	G/G	A/G	G/G	G/G
rs10757665	27547919	T	0.733	C/T	T/T	T/T	T/T	T/T	T/T	T/T	T/T	T/T	C/T	T/T	T/T	T/T	T/T	T/T	T/T	C/T	T/T	T/T	T/T
rs1565948	27549733	G	0.467	A/G	G/G	G/G	A/G	G/G	G/G	G/G	A/G	A/G	A/G	G/G	G/G	G/G	A/G	G/G	G/G	A/G	A/G	G/G	G/G
rs774359	27551049	C	0.225	T/C	T/C	T/C	T/C	C/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C	T/C
rs2282241	27562255	G	0.533	G/G	T/G	T/G	T/G	G/G	T/G	T/G	T/G	T/G	G/G	T/G	T/G	T/G	T/G	T/G	T/G	G/G	G/G	T/G	T/G
rs1948522	27565785	C	0.767	C/C	C/C	C/C	T/C	C/C	C/C	C/C	T/C	T/C	C/C	C/C	C/C	C/C	T/C	C/C	C/C	C/C	T/C	C/C	C/C
rs1982915	27569560	G	0.5	A/G	A/A	A/G	G/G	G/G	A/G	A/G	G/G	G/G	A/G	A/G	A/G	G/G	G/G	G/G	A/G	A/G	G/G	A/G	A/G
rs2453556	27576162	G	0.325	A/G	A/A	A/G	G/G	G/G	A/G	A/A	A/G	G/G	A/G	A/G	A/G	A/G	G/G	A/G	G/G	A/G	G/G	A/G	A/G
rs702231	27578731	A	0.783	C/A	A/A	A/A	A/A	A/A	A/A	A/A	A/A	C/A	A/A	A/A	A/A	C/A	C/A	A/A	C/A	A/A	C/A	A/A	A/A
rs696826	27579657	G	0.892	A/G	G/G	G/G	G/G	G/G	G/G	G/G	G/G	A/G	G/G	G/G	G/G	G/G	A/G	G/G	G/G	G/G	A/G	G/G	G/G
rs2477518	27589746	T	0.725	C/T	T/T	T/T	C/C	C/C	C/T	T/T	T/T	C/C	T/T	C/C	C/T	C/T	C/C	C/T	C/T	C/T	C/C	C/T	T/T

Figure 2. Genotyping data for 20 expansion carriers for which genome-wide association data were available. Those genotypes that are concordant with the allele in the risk haplotype according to Mok *et al*. are shaded in blue [29]. Individuals 06D03315, 08D01457, D98.8895, D952993 and 07D01783 did not present with family history of FTD or ALS. Freq CEU = Frequency in CEU population (Ceph Europeans from Utah). SNP, single nucleotide polymorphism.

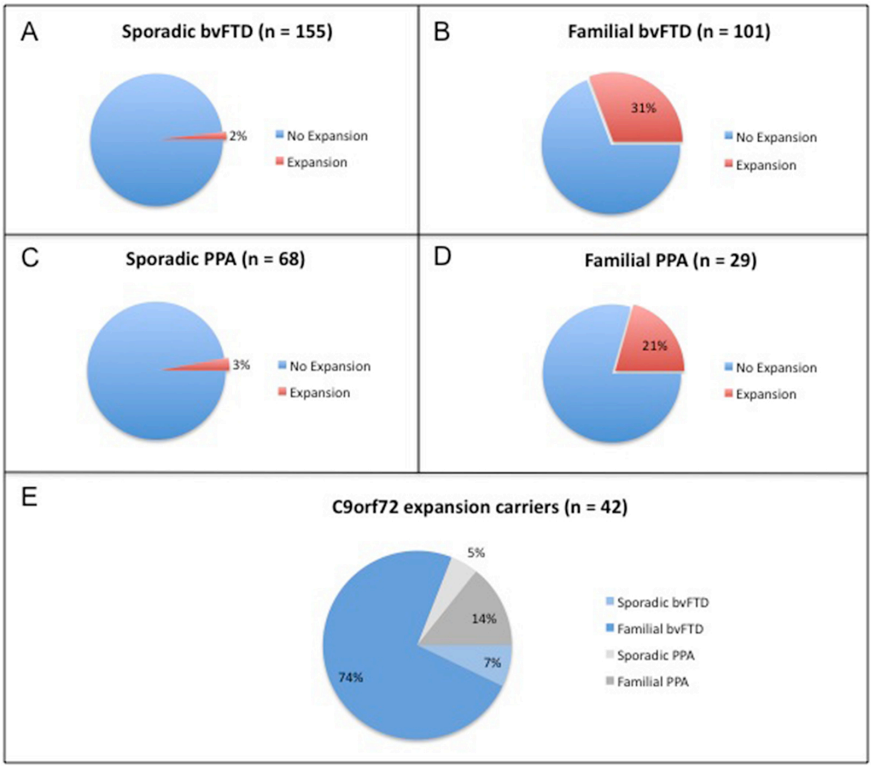

Figure 3. Clinical features of *C9orf72* expansion carriers. *C9orf72* expansion distribution depending on the clinical phenotype (bvFTD or PPA) and family history for FTD or ALS (A-D). Clinical distribution of all 42 expansion carriers (E).

speech, anomia and impaired language comprehension according to the history, but extensive neuropsychological evaluation was not available or possible at the time of out-clinic visits. The other two had non-fluent speech with anomia and comprehension deficits in one. All patients with bvFTD who underwent extensive neuropsychological evaluation had executive dysfunctions, and 10 of them had severe language deficits on initial presentation.

Neuroimaging was available for 32 patients with the repeat expansion. The pattern of cerebral atrophy was predominantly anterior temporal in 13 patients, frontal in four and frontotemporal in seven patients. In all patients with PPA, atrophy was most prominent in the temporal cortex. In four patients, the atrophy was generalized, and four patients (including a patient with pure ALS) had no atrophy.

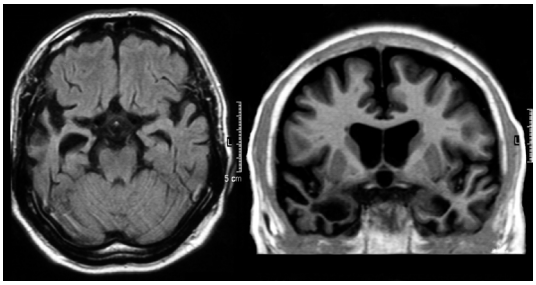

Figure 4. MRI scan of a patient with semantic dementia showing severe temporal atrophy. L, left.

Atrophy was extended into the parietal cortex in 10 patients, and into the occipital cortex in one. Mild cerebellar atrophy was found in eight patients.

Neuropathological findings

Brain autopsy was carried out by the Netherlands Brain Bank in 10 patients carrying the pathogenic repeat expansion and in the patient with a repeat length of 26 with an unaffected family member with the expansion (Table 3). The brain weight was reduced (mean 1112 g, range 886 - 1297). Macroscopy showed moderate to severe frontal and temporal atrophy in all except one brain. Hypopigmentation of the substantia nigra was found in three brains.

Routine staining showed variable neuronal loss in the frontal and/or temporal cortex in all, except for two cases with FTD+ALS. In the PPA case, atrophy and neuronal loss was most severe in the temporal cortex. Mild neuronal loss in the substantia nigra was seen in seven cases and in the caudate nucleus and putamen in two.

Immunohistochemistry with ubiquitin, p62 and TDP-43 antibodies revealed TDP-43 type B pathology in all brains.[34] Many neuronal cytoplasmatic inclusions of variable size (round, crescent, granular) and morphology (diffuse, dense) were seen, most abundant in the dentate gyrus of the hippocampus (Figure 5A), in superficial and deeper layers of the temporal, frontal and parietal cortex (Figure 5B), and with less density in the basal ganglia. Irregular-shaped aggregates were seen in many pyramidal cells of cornu ammonis 3 and 4. Many short, thin or swollen dystrophic neurites were seen in cortical areas in most cases, with the presence of long dystrophic neurites in the parietal cortex of three brains, and in the temporal cortex in only the PPA case (Figure 5C). Three brains showed

Table 3. Neuropathological findings in patients with a repeat expansion in *C9orf72*.

Case	*1 (Family 3 III:5)*	*2 (Family 3 III:7)*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*¶
Clinical presentation	bvFTD	bvFTD	bvFTD	bvFTD+ALS	bvFTD	bvFTD	PPA	bvFTD	bvFTD	bvFTD+ALS	bvFTD+ALS
Brain weight	1065	1297	1220	1096	1095	1150	1245	1146	886	958	1076
Atrophy	F, T, P, SN	F, T,	F, T, SN	Normal	F, T, P	F, T, SN	T	F, T, P	F, T	F, T, P	F
Neuronal loss											
Frontal	+	-	-	-	+	+	-	+	+	+	-
Temporal	++	++	++	+	++	++	++	++	+	+	++
Spinal cord	-	-	-	+	-	-	+	-	na	na	++
SN	+	-	+	++	+	+	-	-	-	+	++
IHC: TDP43/p62											
Frontal	++	++	++‡	+§	+	+	-	++	+	-	+
Temporal	++	++	++	+	+	++	+†	++	+	++	++
Parietal	+†‡	+†	+	++§	+	++	+†	+	+	+	+
Hippocampus	+	++	++	+	++	++	++	++	++	++	++‡
Basal ganglia	+	+	++‡	+§	+	na	+	++	-	-	+‡
Cerebellum	++	++	+	++§	+	na	-	+	++	-	++
Brainstem	+	-	na	+	+	-	-	-	+	+	-
Spinal cord	na	na	na	+	na	-	na	-	na	na	+

*p62- or TDP43-postive neuronal cytoplasmatic inclusions and/or dystrophic neurites.

†Long dystrophic neurites.

‡Neuronal intranuclear inclusions.

§Glial inclusions.

¶The repeat expansion was not confirmed in this case, but repeat length was 26, and a so far unaffected relative did carry the repeat expansion.

IHC, immunohistochemistry with p62 and/or TDP43 antibodies; F, frontal; T, temporal; P, parietal; H, hippocampus; SN, substantia nigra; -, none; +, mild, ++, moderate; +++, severe; na, not available;

a variable number of neuronal intranuclear inclusions in neocortex or basal ganglia (Figure 5D). A few irregular shaped or skein-like TDP-43- and p62-positive inclusions were found in the substantia nigra (Figure 5E) and brain stem (Figure 5F) of four brains. Small dense neuronal p62-positive inclusions and short neurites in the granular layer if the cerebellum were seen in nine out of 11 brains (Figure 5G). Some p62- and TDP-43-positive glial inclusions (oligodendroglia-like) were found in the subcortical white matter in a number of brains (Figure 5H). These glial inclusions did not stain with ubiquitin antibody. In one brain, many p62-, and TDP-43-positive glial inclusions of astrocytic nature were seen in the parietal and temporal cortex and the neostriatum (Figure 5I). TDP-pathology was not more abundant in areas of atrophy. The p62 staining of inclusions was more intense than TDP-43 staining in all cases. Immunohistochemistry with AT8 and β-amyloid antibodies showed abundant neurofibrillary tangles and β-amyloid plaques in the temporal cortex in two brains (Braak stage 2C) [38]. Corticospinal tract degeneration was found in three brains. Immunohistochemistry with C9orf72 antibody shows that C9orf72 is a largely cytoplasmatic protein in neurons. Immunostaining with the antibody against C9orf72 protein showed a granular staining of the cytoplasm into the dendritic arborizations of neurons in cornu ammonis 3 and 4, but this was observed in FTD both with and without the repeat expansion.

Of another five brains from patients with the pathogenic repeat expansion from other academic centers, the pathological diagnosis was FTLD with ubiquitin pathology. However, brain tissue was not available for extensive assessment using TDP-43 and p62 antibodies.

Fluorescence in *situ* hybridization

The RNA-fluorescence *in situ* hybridization results with the locked nucleic acid probe were inconsistent, that is, we analyzed post-mortem tissue of three patients with the expanded GGGGCC repeat, three patients with a *GRN* mutation, three patients with *MAPT* mutations, one patient with fragile X-associated tremor/ataxia syndrome (CGG_{98} repeat expansion) and three non-demented controls for the presence of RNA foci. With the locked nucleic acid probe we did find RNA-positive inclusions in brains with GGGGCC repeat expansions, but also in three cases with *MAPT* mutations and in a non-demented control. The specificity of the staining observed with the locked nucleic acid probe remains to be determined. Using the

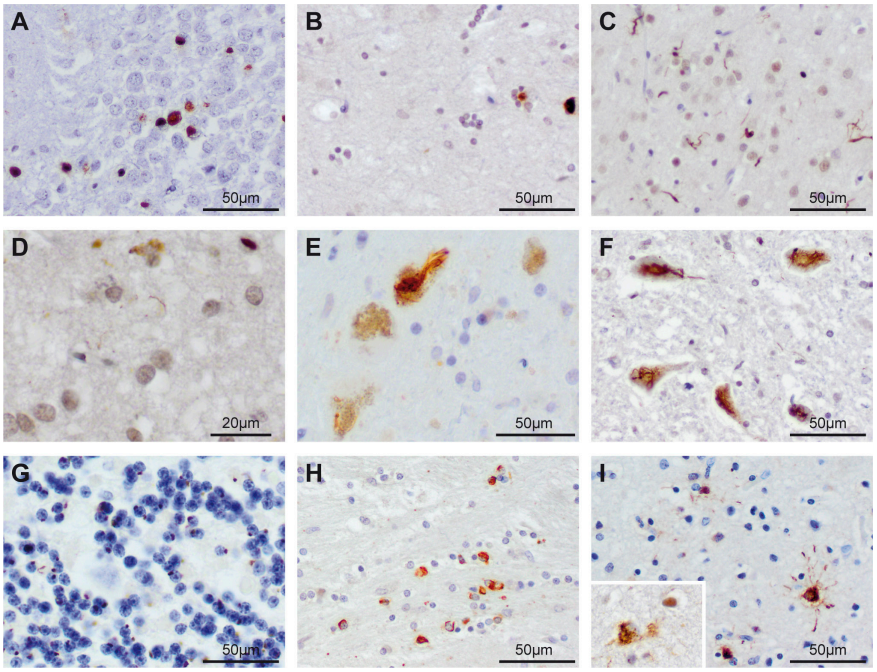

Figure 5. Immunohistochemistry with p62 and TDP-43 antibodies in brains of patients carrying the GGGGCC repeat expansion in *C9orf72*. Many dense neuronal cytoplasmatic inclusions were present in the granular cells of the dentate gyrus (A). Dense or granular cytoplasmatic inclusions of variable size, and short dystrophic neurites were visible in the deep and superficial layers of the frontal and temporal cortex (B). Long dystrophic neurites were seen in the cortical areas of a few brains (C). The temporal and parietal cortex showed a number of TDP-43 positive neuronal intranuclear inclusions (D). Some skein-like or filamentous TDP-43-positive inclusions were found in neurons of the substantia nigra (E) and lower motor neurons in the brainstem (F). Abundant small cytoplasmatic p62-positive, TDP-43-negative inclusions and short neurites were seen in the granular layer of the cerebellum (G). Abundant p62-positive cytoplasmatic glial inclusions were present in white matter of the striatum (H). Several p62-positive inclusions in the cytoplasm and dendritic processes of glial (probably astrocytic) cells in the temporal cortex of one brain from a patient with FTD+ALS (I), TDP-43 antibody also has a positive, although weaker, staining of these inclusions (inset).

oligonucleotide probe $(GGCCCC)_3$ 5' TYE563 no RNA foci could be detected in any of the samples studied.

Comparison with *MAPT* and *GRN* mutation carriers

Whereas the *MAPT* and *GRN* mutation carriers in the Dutch cohort came from 12 and six large families, respectively, the carriers of the repeat expansions in *C9orf72*

came from 35 apparently unrelated families. There was no significant difference in age at onset, age at death or disease duration between repeat expansion carriers and *MAPT* or *GRN* mutation carriers (Table 4). The *C9orf72* repeat expansions are associated with a wider phenotypic variability than *MAPT* and *GRN* mutations. In contrast to patients with *MAPT* and *GRN* mutations, concomitant ALS was a frequent finding in patients with the *C9orf72* repeat expansion. The frequent finding of predominant temporal atrophy contrasts to the imaging features in *MAPT* and *GRN* mutations, where predominant frontal atrophy was more common.

Table 4. Clinical features in FTD patients with a repeat expansion in *C9orf72* compared with patients with *MAPT* and *GRN* mutations.

	Repeat expansion in C9orf72 (n=42)	*MAPT mutation (n=46)*	*GRN mutation (n=30)*
No. of families	35	12	6
Female (%)	22 (52.4)	24 (52.2)	19 (63.3)
Age at onset, years (range)	56.9±8.3 (39-76)	52.3±6.0 (39-65)	60.6±9.3 (45-79)
Age at death, years (range)	64.7±8.6 (42-78)	61.2±7.7 (44-76)	69.4±10.2 (52-87)
Duration of illness, years (range)	7.6±4.6 (1-22)	9.2±4.5 (3-20)	7.4±2.8 (2-13)
Clinical subtype			
BvFTD (%)	34 (81..0)	45 (97.8)	28 (93.3)
PPA (%)	8 (19.0)	1 (2.2)	2 (6.7)
ALS (%)	7 (16.7)	0	0
Imaging*			
No atrophy (%)	3 (9.7)	0	0
Frontal (%)	4 (12.9)	23 (53.5)	23 (85.2)
Temporal (%)	13 (41.9)	15 (34.9)	1 (3.7)
Frontotemporal (%)	7 (22.6)	4 (9.3)	1 (3.7)
Generalized (%)	4 (12.9)	1 (2.3)	2 (7.4)

*Area of predominant atrophy.

DISCUSSION

The present study shows that the pathogenic hexanucleotide expansion in *C9orf72* is one of the most common genetic causes of familial FTD in the Netherlands, and that the repeat expansion is associated with a wide variation in clinical phenotype (bvFTD, PPA, ALS) and with predominant temporal atrophy on neuroimaging.

The percentage of the repeat expansion in the present series of familial FTD is higher than in the study by DeJesus-Hernandez *et al*. (28.7% versus 11.7%), which can be explained by the exclusion of patients with *MAPT* and *GRN* mutations in this study [30]. In this regard, if the whole population of screened FTD patients and patients with *MAPT* and *GRN* mutations or tau-positive FTLD *(n* = 448)) is accounted for, the *C9orf72* hexanucleotide expansion accounts for 17.8% of familial and 9.4% of total FTD in the Netherlands. Of the total population of FTD patients in the Netherlands 26.3% is explained by mutations in *GRN* (6.7%), *MAPT* (10.3%) and the hexanucleotide repeat expansion in *C9orf72* (9.4%). Considering only familial FTD, we can now explain up to 53.8% cases in the Netherlands (*GRN*: 13.9%, *MAPT*: 22.1% and *C9orf72*: 17.8%).

The frequency of the repeat expansion in the present series of sporadic FTD is in line with the findings in the study by DeJesus *et al.*[30] Although a larger proportion of sporadic FTD cases carry the expansion in comparison to controls, this difference was not statistically significant (p = 0.0569). A larger cohort of sporadic patients has to be screened to unveil the role of this expansion in the sporadic form of the disease.

Genetic analysis of a subset of cases carrying this expansion showed that patients from apparently unrelated families carry the same risk haplotype, indicating that there is a common ancestor for all patients with expanded alleles of *C9orf72*. As this risk haplotype was also found in the five apparently sporadic cases, this indicates that these cases are in fact, cryptically related familial cases. The negative family history for these patients could be explained by early death of affected family members, non-paternity or a lack of medical information in previous generations. Another possible explanation of the occurrence of the repeat expansion in apparently sporadic cases in this and other studies, could be reduced penetrance of the repeat expansion in *C9orf72*.[30, 31] This reduced penetrance is evident in at least two of the families, in which unaffected obligate carriers lived long enough to develop the disease, although the possibility of non-paternity cannot be ruled out. The reduced penetrance is in line with previous reports on chromosome 9p-linked FTD, in which the repeat expansion has yet to be confirmed [25]. Another explanation may be that additional genetic and/or environmental factors may determine the

wide inter- and intra-familial variation in age at onset and clinical presentation in the present and other chromosome 9p-linked families.[20, 21, 23, 24] If reliable measurement of the exact length of the expanded repeats becomes possible in the future, further studies are needed to investigate the correlation between age at onset and repeat expansion length, and the possibility of anticipation, as observed in other repeat expansion disorders.

The observed clinical heterogeneity within families, including bvFTD, PPA, ALS, and Parkinsonism in the present study, is in line with previous observations in chromosome 9p-linked families. Concomitant ALS was a frequent finding (seven patients) in patients with FTD with the repeat expansion, and this frequency may even be an underestimation, since follow-up duration from disease onset was highly variable. PPA, defined as a prominent, isolated language deficit during the initial phase,[39] also frequently occurred in the present series (eight patients) and in a Finnish series of FTD patients,[31] but not in previously reported chromosome 9p-linked families.[21, 22, 24] An association between PPA and ALS is further supported by a the high frequency of a language-dominant presentation in patients with FTD+ALS,[40] which suggests a common cortical degenerative process for the language abnormalities in PPA, and tongue and bulbar muscle weakness in ALS. Two of the present patients with PPA with the pathogenic repeat expansion, without ALS symptoms, were classified as SD, which was supported by the presence of severe anomia and single-word comprehension deficits, and by atrophy of the anterior temporal lobes on neuroimaging.[39] The occurrence of SD was unexpected, as the association of SD and ALS has only been described in a few cases in a recent report.[40, 41] Furthermore, patients with SD usually have a negative family history, which diminishes the likelihood of a genetic factor with a dominant effect in SD.[16, 42, 43] Therefore, further clinical studies of patients with the repeat expansion are needed to confirm our observation and to elucidate the genetic contribution in SD. In contrast to a study by Lillo *et al.* that reported that psychotic symptoms are a common feature in FTD+ALS, hallucinations were reported in only two patients with the repeat expansion in the present series and delusions in none.[44]

Predominant temporal atrophy on neuroimaging is a frequent (40.6%) finding in the present series of patients carrying the repeat expansion, especially in those

with PPA. This clearly contrasts with the frontal or frontotemporal pattern of atrophy in chromosome 9p-linked families, and with the absence of a specific atrophy pattern for FTD+ALS in a correlative voxel-based morphometry study.[20, 21, 23, 45] However, Coon *et al*. also found a trend towards more temporal atrophy in patients with language-dominant FTD+ALS than in those with behavioral-dominant FTD+ALS,[40] and severe and circumscribed atrophy of the anterior temporal lobes has also been described in a case report of FTD+ALS.[46] Therefore, a voxel-based morphometry study in a large series of patients carrying the pathogenic repeat expansion is required to confirm our observation of temporal involvement.

The neuropathological findings were consistent with type B FTLD-TDP pathology, with characteristic ubiquitin- and p62-pathology in the granular layer of the cerebellar cortex, in all except for two brains. This cerebellar pathology has been found in other families with FTD+ALS with the pathogenic repeat expansion,[20, 30, 31, 47] and in a series of patients with FTD+ALS or FTLD-TDP.[48] Our observation confirms its strong association with the pathogenic repeat expansion, but it is not an absolute requisite for this disorder. Whether the cerebellar pathology is pathognomic for the repeat expansion has to be investigated in future studies. The TDP-43-negative staining probably indicates that the involvement of the TDP-43 protein is more downstream in the formation of these inclusions, whereas the p62 protein as a non-specific protein reflects the degradation of ubiquitinated proteins via the ubiquitin proteasome system.[48]

In contrast to ubiquitin staining of neuronal inclusions, TDP-43-, p62-positive glial inclusions in the subcortical white matter found in several of the present brains did not stain with ubiquitin antibody, which is in accordance with the observations in other studies.[49, 50] TDP-43- and p62-positive astrocytic inclusions in a single brain from our youngest patient with FTD+ALS were ubiquitin negative as well, as also mentioned by Zhang *et al*.[49, 50] Perhaps, these lesions merely reflect that glial abnormalities are associated with a faster progression of the same disease process, instead of a different underlying pathophysiology. Gliebus *et al*. reported asymmetric TDP-pathology in a PPA case, we could not confirm this in our PPA patient, as only the right hemisphere was available for immunohistochemistry.[51]

Neuronal intranuclear inclusions are found in several of our patients with the pathogenic repeat expansion, as has previously been found in familial FTD+ALS.[37, 52] The prediction of Bigio *et al*. that these inclusions might be associated with a repeat expansion disorder has been confirmed.[52] Using a different method, we could not confirm the results from DeJesus *et al*., who demonstrated the specific presence of RNA-positive foci in the nucleus of frontal cortex neurons using a $(GGCCCC)_4$ Cy3-labeled oligonucleotide probe.[30] Nevertheless, the nuclear localization of the disease process was already suggested by the presence of TDP-43 positive intranuclear inclusions in a few of the present brains, and the absence of cytoplasmic abnormalities of C9orf72 protein in brains carrying the pathogenic repeat expansions. However, it is known from other non-coding expanded repeat disorders that repeat expansions in the transcripts may result in cellular toxicity and the formation of RNA foci of distinct morphology and size.[53] The mutant messenger RNA may interact with specific RNA-binding proteins, and sequestration of RNA-splicing factors and RNA binding proteins may lead to disruption of nuclear processes, including transcription, splicing or messenger RNA processing.[54] Another possible pathogenic mechanism underlying disease is loss-of-function, which is supported by reduced expression in one of the three transcripts of *C9orf72*, as demonstrated in the study of DeJesus *et al*.[30] However, their findings have to be confirmed in future studies. Further studies to clarify these mechanisms may hopefully provide a pharmacological target for preventing or delaying the disease.

A few limitations of this study have to be addressed. First of all, our observations on the frequency and phenotype of the repeat expansion are confined to FTD, as patients with ALS were not included in this study. Secondly, the finding of predominant temporal atrophy in a subset of patients carrying the repeat expansion was semi-quantitatively assessed, and should be confirmed by voxel-based morphometry in another cohort.

In conclusion, the hexanucleotide repeat expansion in *C9orf72* is an important genetic cause of FTD and FTD+ALS. It will be a challenge to explain the wide variation in clinical phenotype of this genetic defect, including bvFTD, ALS and PPA. In addition, it would be interesting to determine whether the severe glial involvement upon the uniform TDP-43 pathology found in some cases has

a pathophysiological significance or is just an epiphenomenon. Hopefully, revealing these underlying mechanisms of the repeat expansions will lead to the development of therapeutic interventions for this devastating disease.

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REFERENCES

1. Ratnavalli E, Brayne C, Dawson K, Hodges JR. The prevalence of frontotemporal dementia. Neurology. 2002 Jun 11;58(11):1615-21.
2. Seelaar H, Rohrer JD, Pijnenburg YA, Fox NC, van Swieten JC. Clinical, genetic and pathological heterogeneity of frontotemporal dementia: a review. J Neurol Neurosurg Psychiatry. 2011 May;82(5):476-86.
3. Rowland LP, Shneider NA. Amyotrophic lateral sclerosis. N Engl J Med. 2001 May 31;344(22):1688-700.
4. Lomen-Hoerth C, Anderson T, Miller B. The overlap of amyotrophic lateral sclerosis and frontotemporal dementia. Neurology. 2002 Oct 8;59(7):1077-9.
5. Cairns NJ, Neumann M, Bigio EH, Holm IE, Troost D, Hatanpaa KJ, et al. TDP-43 in familial and sporadic frontotemporal lobar degeneration with ubiquitin inclusions. Am J Pathol. 2007 Jul;171(1):227-40.
6. Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science. 2006 Oct 6;314(5796):130-3.
7. Baker M, Mackenzie IR, Pickering-Brown SM, Gass J, Rademakers R, Lindholm C, et al. Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. Nature. 2006 Aug 24;442(7105):916-9.
8. Cruts M, Gijselinck I, van der Zee J, Engelborghs S, Wils H, Pirici D, et al. Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. Nature. 2006 Aug 24;442(7105):920-4.
9. Gros-Louis F, Gaspar C, Rouleau GA. Genetics of familial and sporadic amyotrophic lateral sclerosis. Biochim Biophys Acta. 2006 Nov-Dec;1762(11-12):956-72.
10. Hutton M, Lendon CL, Rizzu P, Baker M, Froelich S, Houlden H, et al. Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. Nature. 1998 Jun 18;393(6686):702-5.
11. Johnson JO, Mandrioli J, Benatar M, Abramzon Y, Van Deerlin VM, Trojanowski JQ, et al. Exome sequencing reveals VCP mutations as a cause of familial ALS. Neuron. 2010 Dec 9;68(5):857-64.
12. Kwiatkowski TJ, Jr., Bosco DA, Leclerc AL, Tamrazian E, Vanderburg CR, Russ C, et al. Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. Science. 2009 Feb 27;323(5918):1205-8.
13. Majoor-Krakauer D, Ottman R, Johnson WG, Rowland LP. Familial aggregation of amyotrophic lateral sclerosis, dementia, and Parkinson's disease: evidence of shared genetic susceptibility. Neurology. 1994 Oct;44(10):1872-7.
14. Maruyama H, Morino H, Ito H, Izumi Y, Kato H, Watanabe Y, et al. Mutations of optineurin in amyotrophic lateral sclerosis. Nature. 2010 May 13;465(7295):223-6.
15. Rosen DR. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature. 1993 Jul 22;364(6435):362.

16. Seelaar H, Kamphorst W, Rosso SM, Azmani A, Masdjedi R, de Koning I, et al. Distinct genetic forms of frontotemporal dementia. Neurology. 2008 Oct 14;71(16):1220-6.

17. Sreedharan J, Blair IP, Tripathi VB, Hu X, Vance C, Rogelj B, et al. TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. Science. 2008 Mar 21;319(5870):1668-72.

18. van Swieten J, Spillantini MG. Hereditary frontotemporal dementia caused by Tau gene mutations. Brain Pathol. 2007 Jan;17(1):63-73.

19. Vance C, Rogelj B, Hortobagyi T, De Vos KJ, Nishimura AL, Sreedharan J, et al. Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. Science. 2009 Feb 27;323(5918):1208-11.

20. Boxer AL, Mackenzie IR, Boeve BF, Baker M, Seeley WW, Crook R, et al. Clinical, neuroimaging and neuropathological features of a new chromosome 9p-linked FTD-ALS family. J Neurol Neurosurg Psychiatry. 2011 Feb;82(2):196-203.

21. Le Ber I, Camuzat A, Berger E, Hannequin D, Laquerriere A, Golfier V, et al. Chromosome 9p-linked families with frontotemporal dementia associated with motor neuron disease. Neurology. 2009 May 12;72(19):1669-76.

22. Morita M, Al-Chalabi A, Andersen PM, Hosler B, Sapp P, Englund E, et al. A locus on chromosome 9p confers susceptibility to ALS and frontotemporal dementia. Neurology. 2006 Mar 28;66(6):839-44.

23. Pearson JP, Williams NM, Majounie E, Waite A, Stott J, Newsway V, et al. Familial frontotemporal dementia with amyotrophic lateral sclerosis and a shared haplotype on chromosome 9p. J Neurol. 2011 Apr;258(4):647-55.

24. Valdmanis PN, Dupre N, Bouchard JP, Camu W, Salachas F, Meininger V, et al. Three families with amyotrophic lateral sclerosis and frontotemporal dementia with evidence of linkage to chromosome 9p. Arch Neurol. 2007 Feb;64(2):240-5.

25. Vance C, Al-Chalabi A, Ruddy D, Smith BN, Hu X, Sreedharan J, et al. Familial amyotrophic lateral sclerosis with frontotemporal dementia is linked to a locus on chromosome 9p13.2-21.3. Brain. 2006 Apr;129(Pt 4):868-76.

26. Laaksovirta H, Peuralinna T, Schymick JC, Scholz SW, Lai SL, Myllykangas L, et al. Chromosome 9p21 in amyotrophic lateral sclerosis in Finland: a genome-wide association study. Lancet Neurol. 2010 Oct;9(10):978-85.

27. Van Deerlin VM, Sleiman PM, Martinez-Lage M, Chen-Plotkin A, Wang LS, Graff-Radford NR, et al. Common variants at 7p21 are associated with frontotemporal lobar degeneration with TDP-43 inclusions. Nat Genet. 2010 Mar;42(3):234-9.

28. van Es MA, Veldink JH, Saris CG, Blauw HM, van Vught PW, Birve A, et al. Genome-wide association study identifies 19p13.3 (UNC13A) and 9p21.2 as susceptibility loci for sporadic amyotrophic lateral sclerosis. Nat Genet. 2009 Oct;41(10):1083-7.

29. Mok K, Traynor BJ, Schymick J, Tienari PJ, Laaksovirta H, Peuralinna T, et al. The chromosome 9 ALS and FTD locus is probably derived from a single founder. Neurobiol Aging. 2011 Sep 16.

30. Dejesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, et al. Expanded GGGGCC Hexanucleotide Repeat in Noncoding Region of C9ORF72 Causes Chromosome

9p-Linked FTD and ALS. Neuron. 2011 Sep 21.

31. Renton AE, Majounie E, Waite A, Simon-Sanchez J, Rollinson S, Gibbs JR, et al. A Hexanucleotide Repeat Expansion in C9ORF72 Is the Cause of Chromosome 9p21-Linked ALS-FTD. Neuron. 2011 Sep 21.
32. Neary D, Snowden JS, Gustafson L, Passant U, Stuss D, Black S, et al. Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. Neurology. 1998 Dec;51(6):1546-54.
33. Brooks BR. El Escorial World Federation of Neurology criteria for the diagnosis of amyotrophic lateral sclerosis. Subcommittee on Motor Neuron Diseases/Amyotrophic Lateral Sclerosis of the World Federation of Neurology Research Group on Neuromuscular Diseases and the El Escorial "Clinical limits of amyotrophic lateral sclerosis" workshop contributors. J Neurol Sci. 1994 Jul;124 Suppl:96-107.
34. Mackenzie IR, Neumann M, Baborie A, Sampathu DM, Du Plessis D, Jaros E, et al. A harmonized classification system for FTLD-TDP pathology. Acta Neuropathol. 2011 Jul;122(1):111-3.
35. Kobayashi H, Abe K, Matsuura T, Ikeda Y, Hitomi T, Akechi Y, et al. Expansion of intronic GGCCTG hexanucleotide repeat in NOP56 causes SCA36, a type of spinocerebellar ataxia accompanied by motor neuron involvement. Am J Hum Genet. 2011 Jul 15;89(1):121-30.
36. Warner JP, Barron LH, Goudie D, Kelly K, Dow D, Fitzpatrick DR, et al. A general method for the detection of large CAG repeat expansions by fluorescent PCR. J Med Genet. 1996 Dec;33(12):1022-6.
37. Seelaar H, Schelhaas HJ, Azmani A, Kusters B, Rosso S, Majoor-Krakauer D, et al. TDP-43 pathology in familial frontotemporal dementia and motor neuron disease without Progranulin mutations. Brain. 2007 May;130(Pt 5):1375-85.
38. Thal DR, Rub U, Orantes M, Braak H. Phases of A beta-deposition in the human brain and its relevance for the development of AD. Neurology. 2002 Jun 25;58(12):1791-800.
39. Gorno-Tempini ML, Hillis AE, Weintraub S, Kertesz A, Mendez M, Cappa SF, et al. Classification of primary progressive aphasia and its variants. Neurology. 2011 Mar 15;76(11):1006-14.
40. Coon EA, Sorenson EJ, Whitwell JL, Knopman DS, Josephs KA. Predicting survival in frontotemporal dementia with motor neuron disease. Neurology. 2011 May 31;76(22):1886-93.
41. Kim SH, Seo SW, Go SM, Suh MK, Chin J, Jeong JH, et al. Semantic dementia combined with motor neuron disease. J Clin Neurosci. 2009 Dec;16(12):1683-5.
42. Goldman JS, Farmer JM, Wood EM, Johnson JK, Boxer A, Neuhaus J, et al. Comparison of family histories in FTLD subtypes and related tauopathies. Neurology. 2005 Dec 13;65(11):1817-9.
43. Hodges JR, Mitchell J, Dawson K, Spillantini MG, Xuereb JH, McMonagle P, et al. Semantic dementia: demography, familial factors and survival in a consecutive series of 100 cases. Brain. 2010 Jan;133(Pt 1):300-6.
44. Lillo P, Garcin B, Hornberger M, Bak TH, Hodges JR. Neurobehavioral features in frontotemporal dementia with amyotrophic lateral sclerosis. Arch Neurol. 2010 Jul;67(7):826-30.
45. Rohrer JD, Geser F, Zhou J, Gennatas ED, Sidhu M, Trojanowski JQ, et al. TDP-43 subtypes are associated with distinct atrophy patterns in frontotemporal dementia. Neurology. 2010 Dec 14;75(24):2204-11.

46. Kuwahara H, Tsuchiya K, Saito Y, Kobayashi Z, Miyazaki H, Izumiyama Y, et al. Frontotemporal lobar degeneration with motor neuron disease showing severe and circumscribed atrophy of anterior temporal lobes. J Neurol Sci. 2010 Oct 15;297(1-2):92-6.

47. Polvikoski TM, Murray A, Harper PS, Neal JW. Familial motor neurone disease with dementia: phenotypic variation and cerebellar pathology. J Neurol Neurosurg Psychiatry. 2003 Nov;74(11):1516-20.

48. King A, Maekawa S, Bodi I, Troakes C, Al-Sarraj S. Ubiquitinated, p62 immunopositive cerebellar cortical neuronal inclusions are evident across the spectrum of TDP-43 proteinopathies but are only rarely additionally immunopositive for phosphorylation-dependent TDP-43. Neuropathology. 2011 Jun;31(3):239-49.

49. Hiji M, Takahashi T, Fukuba H, Yamashita H, Kohriyama T, Matsumoto M. White matter lesions in the brain with frontotemporal lobar degeneration with motor neuron disease: TDP-43-immunopositive inclusions co-localize with p62, but not ubiquitin. Acta Neuropathol. 2008 Aug;116(2):183-91.

50. Zhang H, Tan CF, Mori F, Tanji K, Kakita A, Takahashi H, et al. TDP-43-immunoreactive neuronal and glial inclusions in the neostriatum in amyotrophic lateral sclerosis with and without dementia. Acta Neuropathol. 2008 Jan;115(1):115-22.

51. Gliebus G, Bigio EH, Gasho K, Mishra M, Caplan D, Mesulam MM, et al. Asymmetric TDP-43 distribution in primary progressive aphasia with progranulin mutation. Neurology. 2010 May 18;74(20):1607-10.

52. Bigio EH, Johnson NA, Rademaker AW, Fung BB, Mesulam MM, Siddique N, et al. Neuronal ubiquitinated intranuclear inclusions in familial and non-familial frontotemporal dementia of the motor neuron disease type associated with amyotrophic lateral sclerosis. J Neuropathol Exp Neurol. 2004 Aug;63(8):801-11.

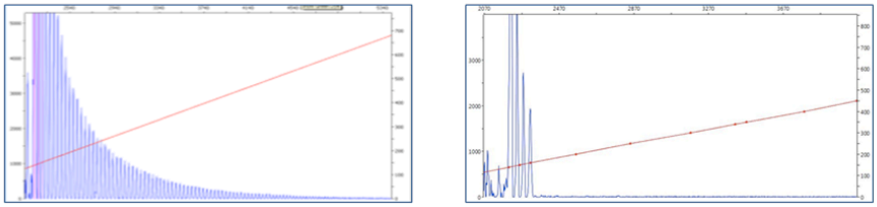
53. Wojciechowska M, Krzyzosiak WJ. Cellular toxicity of expanded RNA repeats: focus on RNA foci. Hum Mol Genet. 2011 Oct 1;20(19):3811-21.

54. Todd PK, Paulson HL. RNA-mediated neurodegeneration in repeat expansion disorders. Ann Neurol. 2010 Mar;67(3):291-300.

APPENDIX

A. Affected case

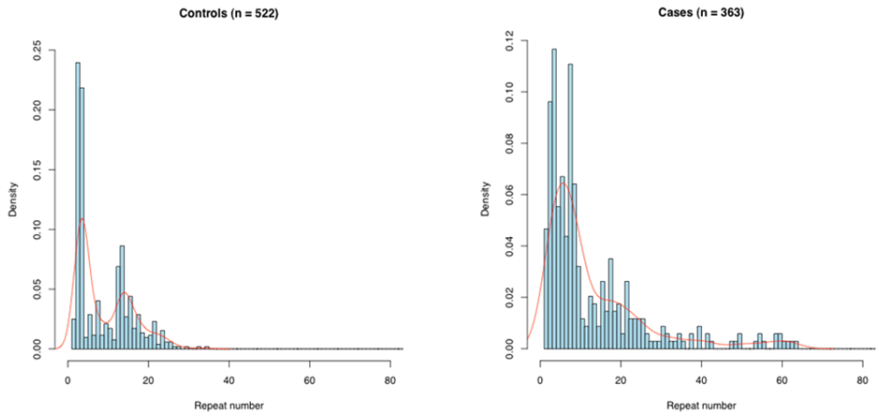
B. Unaffected control



Supplementary figure 1. Capillary-based sequence traces of the repeat-primed PCR assay are shown. Vertical axis represents fluorescence intensity. A typical saw tooth tail pattern with a 6bp periodicity is observed in the cases carrying the GGGGCC repeat expansion (panel A). Panel B shows the results obtained for a control individual not carrying the repeat expansion.

A.

B.



Supplementary figure 2. Frequency distribution of GGGGCC hexanucleotide repeat lengths in FTLD cases and control based on the repeat-primed PCR assay. A) Histogram of repeat length observed in 522 successfully genotyped Dutch controls. B) Histogram of repeat length observed in 353 successfully genotyped Dutch FTLD cases both with and without positive family history. A bimodal distribution is evident for the FTLD cases.

CHAPTER 2.3

Frequency of the *C9orf72* hexanucleotide repeat expansion in patients with amyotrophic lateral sclerosis and frontotemporal dementia: a cross-sectional study

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*These authors contributed equally to this work

Lancet Neurol. 212 Apr;11(4):323-30.

ABSTRACT

Background: We aimed to accurately estimate the frequency of a hexanucleotide repeat expansion in *C9orf72* that has been associated with a large proportion of cases of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD).

Methods: We screened 4,448 patients diagnosed with ALS (El Escorial criteria) and 1,425 patients with FTD (Lund-Manchester criteria) from 17 regions worldwide for the GGGGCC hexanucleotide expansion using a repeat-primed PCR assay. We assessed familial disease status on the basis of self-reported family history of similar neurodegenerative diseases at the time of sample collection. We compared haplotype data of 262 patients carrying the expansion with the known Finnish founder risk haplotype across the chromosomal locus. We calculated age-related penetrance using the Kaplan-Meier method with data for 603 individuals with the expansion.

Findings: In patients with sporadic ALS, we identified the repeat expansion in 236 (7.0%) of 3,377 white individuals from the USA, Europe, and Australia, two (4.1%) of 49 black individuals from the USA, and six (8.3%) of 72 Hispanic individuals from the USA. The mutation was present in 217 (39.3%) of 552 white individuals with familial ALS from Europe and the USA. 59 (6.0%) of 981 white Europeans with sporadic FTD had the mutation, as did 99 (24.8%) of 400 white Europeans with familial FTD. Data for other ethnic groups were sparse, but we identified one Asian patient with familial ALS (from 20 assessed) and two with familial FTD (from three assessed) who carried the mutation. The mutation was not carried by the three Native Americans or 360 patients from Asia or the Pacific Islands with sporadic ALS who were tested, or by 41 Asian patients with sporadic FTD. All patients with the repeat expansion had (partly or fully) the founder haplotype, suggesting a one-off expansion occurring about 1500 years ago. The pathogenic expansion was non-penetrant in individuals younger than 35 years, 50% penetrant by 58 years, and almost fully penetrant by 80 years.

Interpretation: A common Mendelian genetic lesion in *C9orf72* is implicated in many cases of sporadic and familial ALS and FTD. Testing for this pathogenic expansion should be considered in the management and genetic counselling of patients with these fatal neurodegenerative diseases.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by rapidly progressive paralysis and death from respiratory failure, typically within three years of symptom onset. The disease is inherited in about 5% of cases, following a clear Mendelian pattern, whereas most cases are classified as sporadic because they seem to arise at random.[1] Substantial progress has been made in understanding the genetic underpinnings of familial ALS.[2] By contrast, the causes of sporadic or idiopathic ALS are far less well understood. Mutations in the known familial ALS genes -*SOD1*, *FUS*, and *TDP-43*- occur only rarely in sporadic cases (each accounting for less than 1.0% of cases);[3–5] genome-wide association studies have identified few risk loci, and these have proven difficult to replicate.[6]

Frontotemporal dementia (FTD) is a degenerative disorder of the frontal and anterior temporal lobes, and is a common form of dementia affecting individuals younger than 65 years. The syndrome is characterized clinically by initial behavioral disturbances, followed by cognitive decline leading to dementia and death within a median of seven years from symptom onset. Akin to ALS and other neurodegenerative diseases, a large proportion (~60.0%) of these cases are categorized as sporadic, and the causes of this idiopathic form of disease are largely unknown.[7] A growing consensus suggests that ALS and FTD form part of a continuum of neurological disease that share a common pathological background, consisting of TAR DNA-binding protein 43 (TDP-43)-positive inclusions within the central nervous system.[8]

We recently reported that a large hexanucleotide repeat expansion located within the non-coding portion of *C9orf72* is the cause of chromosome 9-linked ALS and FTD.[9,10] This genetic lesion accounted for a large proportion (~40.0%) of familial cases of ALS and FTD. The same mutation was present in nearly a quarter of apparently sporadic cases of ALS and FTD in the genetically homogeneous Finnish population, and in 4.1% of sporadic cases of ALS and 3.0% of sporadic cases of FTD from the USA. However, these estimates were based on relatively small cohorts drawn from a limited number of institutions.

These findings prompted us to aim to estimate the frequency of this *C9orf72*

hexanucleotide repeat expansion more accurately, in a large cohort of European and US patients with sporadic ALS and sporadic FTD. We also examined the occurrence of this mutation in diverse non-white populations around the world.

METHODS

Participants and study design

In this cross-sectional study, we screened 4,448 patients diagnosed with ALS and 1,425 patients diagnosed with FTD from 17 distinct regions worldwide. The Appendix shows ethnic origin and clinical features of the patients. 3,860 patients had sporadic ALS, 1,022 had sporadic FTD,
588 had familial ALS, and 403 had familial FTD. Data for 401 Finnish patients with ALS, 233 other Europeans with familial ALS, 75 Finnish patients with FTD, 340 Dutch patients with FTD, and 420 English patients with FTD have been published previously.[10–12] All these cohorts were analyzed to provide a comprehensive assessment of the global frequency of the expansion.

Patients with ALS were diagnosed according to the El Escorial diagnostic criteria[13], and patients with FTD were diagnosed according to the Lund-Manchester criteria.[14] We classified patients' disease as familial in nature on the basis of a diagnosis of ALS or FTD in any other family member (irrespective of relationship), as reported at the time of sample collection. We based ethnic and racial classification on self-reports from patients at the time of sample collection. Case numbers listed for European countries and Australia and the Middle East refer to self-reported white individuals from that region. Italian are from a population-based cohort that had been collected through the Piemonte ALS Registry, an ongoing population-based epidemiological study of ALS based in north-western Italy.[15] The remaining cohorts were recruited through medical centers and from repositories in various countries.

We also screened 2,585 neurologically healthy control individuals from Australia (n = 213), Finland (n = 478), Germany (n = 309), the Human Gene Diversity Panel (n = 300), mainland Italy (n = 354), Sardinia (n = 87), and the USA (n = 844) for the presence of the pathogenic repeat expansion. 1,167 of these individuals have been reported elsewhere.[10] None of the control individuals had been diagnosed with ALS, FTD, dementia, or any other neurodegenerative disease. Ethics committees

of the respective institutions approved the study, and written informed consent was obtained from all patients and control individuals.

Procedures

We used our previously described[10] repeat-primed PCR assay to screen patients and control individuals for the presence of the chromosome 9p21 GGGGCC hexanucleotide repeat expansion (see Appendix for technical details). The assay allows samples to be categorized into those that carry a pathogenic repeat expansion (> 30 repeats) and those that carry only wild-type alleles (< 20 repeats).

For haplotype analysis, we analyzed genome-wide single-nucleotide polymorphism (SNP) data from 262 patients who carried the repeat expansion. We previously reported the identification in the Finnish population of a 42-SNP founder haplotype across the 232 kb block of chromosome 9p21 where the pathogenic hexanucleotide expansion was ultimately established.[16,17] In this study, we used a custom perl software script to compare unphased sample genotype data with the 42-SNP founder risk haplotype.[16]

We estimated mutation ages for all populations separately with the DMLE+ version 2.3 Bayesian linkage disequilibrium gene mapping package.[18] Mutation ages were iterated for 10,000 burn-in iterations and a further 10,000 iterations of the maximum-likelihood model. To obtain generalizable estimates of age of the repeat per population, we used median values of binned estimates passing the α threshold of 0.05 per iteration.

Statistical analysis

We calculated 95% confidence intervals (Cis) for proportions with the Clopper-Pearson exact method. We estimated penetrance of the GGGGCC hexanucleotide repeat expansion in relation to the patients' age on the basis of data available for 603 mutant-gene carriers with the Kaplan-Meier method using the survival package within R statistical software (version 2.9.0), but substituting patient age at symptom onset for survival time.[19] We assessed differences between groups with the χ^2 test for discrete variables such as sex, family history, and site of onset.

Role of the funding sources
The sponsors of the study had no role in study design, data collection, analysis, interpretation, writing of the report, or in the decision to submit the paper for publication. All authors had full access to all of the data in the study and had final responsibility for the decision to submit for publication.

RESULTS

Table 1 and the Appendix show the frequency of the *C9orf72* hexanucleotide repeat expansion in patients diagnosed with sporadic ALS and sporadic FTD from different geographical regions. Data for 289 patients with sporadic ALS and 605 with sporadic FTD have been reported elsewhere.[10-12] The pathogenic expansion was identified in 236 (7.0%) of 3,337 white patients from the USA, Europe, the Middle East, and Australia, two (4.1%) of 49 black patients from USA, and six (8.3%) of 72 Hispanic patients from the USA who were diagnosed with sporadic ALS. The rate of the pathogenic expansion was lower in sporadic FTD: 59 (6.0%) of 981 white patients from Europe carried the mutation. By contrast, the GGGGCC repeat expansion was not present in patients of Native American, Asian, or Pacific Islander origin who had sporadic disease (Table 1), although this might reflect the smaller size of the cohorts screened in these populations.

In addition to the sporadic cases, we screened 588 familial cases of ALS and 403 familial cases of FTD for the presence of the *C9orf72* repeat expansion (Table 2 and Appendix). Of these, 345 patients with familial ALS and 230 with familial FTD have been reported elsewhere.[10-12] Overall, 221 (37.6%) of 588 patients with familial ALS and 101 (25.1%) of 403 patients with familial FTD carried the genetic lesion, reinforcing our previous findings that this mutation was responsible for an unparalleled proportion of cases of these diseases.[10] We identified one Japanese individual diagnosed with familial ALS, who carried the hexanucleotide repeat expansion. We also showed that one patient with familial FTD from Lund, Sweden, carried the expansion, suggesting that the chromosome 9p21 genetic lesion might be responsible for the geographical cluster of patients with FTD patients noted in that region.[20]

Of 2,585 neurologically healthy control samples screened for the *C9orf72* repeat

Table 1. Frequency of the pathogenic GGGGCC hexanucleotide repeat expansion of *C9orf72* in patients diagnosed with sporadic ALS or sporadic FTD classified by region.

	Sporadic ALS			*Sporadic FTD*		
Origin	*n*	*Carriers*	*% (95% CI)*	*n*	*Carriers*	*% (95% CI)*
Europe*						
Finnish	289	61	21.1 (16.5-26.3)	48	9	18.8 (8.9-32.6)
Swedish	-	-	-	6	0	0 (0.0-45.9)
English	916	62	6.8 (5.2-8.6)	543	31	5.7 (3.9-8.0)
German	421	22	5.2 (3.3-7.8)	-	-	-
Dutch	-	-	-	224	5	2.2 (0.7-5.1)
French	-	-	-	150	14	9.3 (5.2-15.2)
Italian	465	19	4.1 (2.5-6.3)	-	-	-
Sardinian	129	10	7.8 (3.8-13.8)	10	0	0 (0.0-30.8)
Moldovan	3	0	0 (0.0-70.8)	-	-	-
Total (Europe)	2.223	174	7.8 (6.7-9.0)	981	59	6.0 (4.6-7.7)
USA						
White	890	48	5.4 (4.0-7.1)	-	-	-
Hispanic	72	6	8.3 (3.1-17.3)	-	-	-
African American	49	2	4.1 (0.5-14.0)	-	-	-
Native American	3	0	0 (0.0-70.8)	-	-	-
Total (USA)	1.014	56	5.5 (4.2-7.1)	-	-	-
Rest of the world						
Middle Eastern*	1	0	0 (0.0-97.5)	-	-	-
Indian	31	0	0 (0.0-11.2)	31	0	0 (0.0-11.2)
Asian	238	0	0 (0.0-1.5)	10	0	0 (0.0-30.8)
Pacific Islander/Guam	90	0	0 (0.0-4.0)	-	-	-
Australian*	263	14	5.3 (2.9-8.8)	-	-	-
Overall	3.860	244	6.3 (5.6-7.1)	1.022	59	5.8 (4.4-7.4)

Data for Finnish (289 with ALS and 48 with FTD), English (333 with FTD), and Dutch (224 with FTD) patients were previously published,[10–12] but are included here to establish global frequencies.
*All self-reported as white.

expansion, five (0.2%) were carriers: two were previously reported elderly individuals from Finland,[10] and the other three were individuals younger than 40 years from Germany and the USA (Appendix).

Table 2. Frequency of the pathogenic GGGGCC hexanucleotide repeat expansion of *C9orf72* in patients diagnosed with familial ALS and familial FTD classified by region.

	Familial ALS			*Familial FTD*		
Origin	*n*	*Carriers*	*% (95% CI)*	*n*	*Carriers*	*% (95% CI)*
Europe*						
Finnish	112	52	46.4 (37.0-56.1)	27	13	48.1 (28.7-68.0)
Swedish	-	-	-	1	1	100 (2.5-100.0)
English	98	45	45.9% (35.8-56.3)	170	28	16.5 (11.2-22.9)
Irish	1	1	100 (2.5-100.0)	-	-	
German	69	15	21.7 (12.7-33.3)	29	4	13.8 (3.9-31.7)
Dutch	-	-	-	116	30	25.9 (18.2-34.8)
French	-	-	-	50	22	44.0 (30.0-58.7)
Italian	90	34	37.8 (27.8-48.6)	-	-	-
Sardinian	19	11	57.9 (33.5-79.7)	7	1	14.3 (0.4-57.9)
Total (Europe)	389	158	40.6 (35.7-45.7)	400	99	24.8 (20.6-29.3)
USA*	163	59	36.2 (28.8-44.1)	-	-	-
Rest of the world						
Middle Eastern*	2	0	0 (0.0-84.2)	-	-	-
Israeli*	14	3	21.4 (4.7-50.8)	-	-	-
Asian	20	1	5.0 (0.1-24.9)	3	2	66.7 (9.4-99.2)
Overall	588	221	37.6 (33.7-41.6)	403	101	25.1 (20.9-29.6)

Data for Finnish (112 with ALS and 27 with FTD), English (87 with FTD), German (41 with ALS), Italian (29 with ALS), US (163 with ALS), and Dutch (116 with FTD) patients were previously published,[10–12] but are included here to establish global frequencies.

*All self-reported as white.

Within Europe, the highest mutation frequency was noted in the Finnish population (21.1% of patients with sporadic ALS and 18.8% of patients with sporadic FTD).[10] About 6% of patients with sporadic ALS from Germany and England carried the expansion, whereas Italian patients with ALS had a lower rate (4.1%). 7.8% of patients with sporadic ALS from the genetically isolated island population of Sardinia had the mutation and the Dutch population had the lowest detected rate observed in European countries (2.2% of sporadic cases of FTD). White populations from Australia and the USA had an intermediate rate, with about 5.0% of patients with sporadic ALS carrying the pathogenic repeat expansion, perhaps because of the population and immigration histories of these continents.

Haplotype analysis suggested that every patient carrying the pathogenic GGGGCC repeat expansion also shared the Finnish founder risk haplotype, at least in part (Figure 1). Furthermore, patients with sporadic and familial disease carried the same founder risk haplotype. These findings suggest that the pathogenic hexanucleotide repeat expansion in *C9orf72* might have occurred on one occasion in human history and subsequently disseminated throughout these populations. Analysis of haplotype sharing between these cases estimated the age of the *C9orf72* repeat expansion to be about 1,500 years old (representing a median of 100.5 generations (interquartile range = 57.6 - 127.6), assuming a generation is 15 years old).

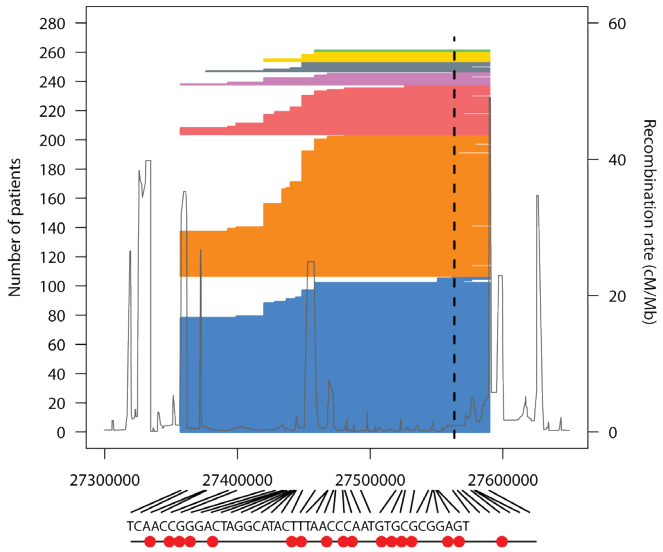

Figure 1. Finnish risk haplotypes across the chromosome 9p21 region in 262 patients with ALS and the *C9orf72* mutations. The previously identified Finnish risk haplotype is shown below the graph (27,357,278 - 27,589,746 bp; NCBI build 36; 42 single-nucleotide polymorphisms (SNPs)).[16] Underneath the haplotype is a binary representation of the same data, with red circles at SNP positions where the haplotype has the less common allele at that site. In the graph, individual patients are shown as horizontal lines showing the extent to which they share the risk haplotype. Blue = Finnish (*n* = 107), orange = US (*n* = 97), red = Italian (*n* = 34), violet = Australian cases (*n* = 9), grey = German (*n* = 7), yellow = Israeli (*n* = 7), green = Japanese patient (*n* = 1). The vertical black dashed line shows the location of the *C9orf72* hexanucleotide repeat expansion. Recombination rates (centimorgans per megabase (cM/Mb)) from phase 2 Centre d'Etude du Polymorphisme Humain (CEPH) samples of HapMap are shown with a grey line.

In analysis of age-related penetrance (Figure 2), the pathogenic expansion was non-penetrant in carriers younger than 35 years of age, increasing to 50% penetrance by 58 years, and to almost full penetrance by 80 years. We noted no difference in disease penetrance according to familial status, ALS or FTD diagnosis, sex, or age of symptom onset in patients with ALS or FTD (Appendix).

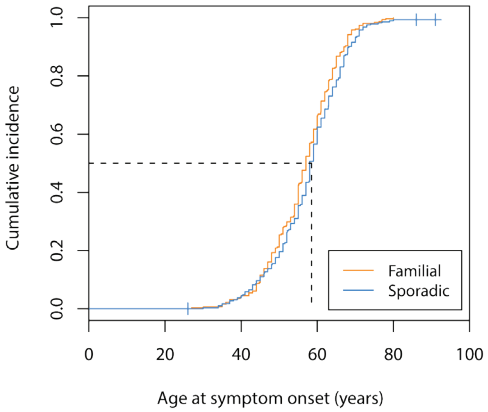

Figure 2. Age-related penetrance of the GGGGCC hexanucleotide repeat expansion *C9orf72*. Kaplan-Meier analysis of 603 mutant-gene carriers (212 patients with familial ALS, 234 with sporadic ALS, 99 with familial FTD, 53 with sporadic FTD and five neurologically healthy controls). Age-related penetrance (ie, the proportion of mutant-gene carriers with manifestations of the disease by a given age) rose steadily, from 10% in patients younger than 45 years, to almost 100% by the age of 80 years. The dotted lines show the age at which 50% of the cohort developed symptoms. Vertical blue lines show censored events.

Table 3 shows clinical details of patients carrying the hexanucleotide repeat expansion. Patients with ALS with the pathogenic repeat expansion were more likely to be female ($p = 0.0008$), have a family history of disease ($p < 0.0001$), and to have bulbar-onset disease ($p = 0.0011$) than were patients who did not carry the expansion. Patients with FTD carrying the repeat expansion were also more likely to have a family history of disease ($p < 0.0001$), and to present with behavioral variant FTD ($p < 0.0001$).

DISCUSSION

Our data show that the *C9orf72* hexanucleotide repeat expansion is the most frequent cause of sporadic ALS and sporadic FTD identified thus far, accounting

Table 3. Demographic and clinical features of patients classified by diagnosis and by carrier status for the GGGGCC hexanucleotide repeat expansion in *C9orf72*.

	ALS		*FTD*	
	*With expansion (n=465)**	*Without expansion (n=3,983)†*	*With expansion (n=160)‡*	*Without expansion (n=1,265)§*
Mean age at onset	56.8 (27.0-80.0; 9.1)	58.7 (4.0-93.0; 12.8)	57.5 (30.0-76.3; 8.3)	60.0 (23.0-87.0; 8.8)
Sex, male	232 (50.1)	2,251 (58.4)	87 (54.4)	683 (55.4)
Positive family history	221 (47.5)	367 (9.2)	101 (63.1)	302 (23.9)
Presentation				
Bulbar	139 (33.1)	933 (26.0)	-	-
Limb	281 (66.9)	2,655 (74.0)	-	-
Behavioral variant	-	-	106 (85.5)	685 (65.6)
Progressive non-fluent aphasia	-	-	11 (8.9)	165 (15.8)
Semantic dementia	-	-	7 (5.6)	195 (18.6)

Data are mean (range; SD) or n (%). *Data not available for age at onset for 19 patients and site of onset for 45 patients. †Data not available for age at onset for 305 patients, sex for 130 patients, and site of onset for 395 patients. ‡Data not available for age at onset for eight patients and site of onset for 36 patients. §Data not available for age at onset for 71 patients, sex for32 patients, and site of onset for 220 patients.

for 5.0 - 7.0% of cases in white Europeans, Americans, and Australians in our large cohort. These frequency rates were slightly higher than were estimates from smaller cohorts obtained from at one institution.[9] Before the identification of the genetic lesion underlying chromosome 9-linked ALS and FTD, mutations in the *SOD1* gene were the most common known genetic cause of sporadic ALS (accounting for 0.7% of cases in a population-based cohort),[3] whereas mutations in *GRN* were the most common known cause of sporadic FTD (3.0 - 4.0% in clinic referral series).[21] The high frequency of the pathogenic expansion in our patient cohort is consistent with previous genome-wide association studies that identified the association signal on chromosome 9p21 as the only replicable locus in the sporadic form of ALS and FTD.[16,22–24] Our findings confirm the importance of genetics in the pathogenesis of the idiopathic form of these fatal neurodegenerative diseases.

Our haplotype data suggest that the pathogenic GGGGCC hexanucleotide repeat expansion in *C9orf72* arose from a one-off mutational event[16,17] that occurred

about 1,500 years ago. The geographical distribution of the mutation suggests that the mutation appeared in northern Europe and spread from there. Alternatively, the high frequencies in Finland and other isolated populations could be explained by the history of these communities. Finland and Sardinia are comparatively isolated areas, and have genetically homogeneous populations that originated from a small number of founders.[25] Genetic drift has had a large influence on allele frequencies in these populations and could explain the high occurrence of the mutation in these geographical isolates.

Recognizing that all cases carrying the *C9orf72* repeat expansion share a common ancestor has important implications for the interpretation of global frequency data for this mutation. Although the hexanucleotide repeat expansion is common in white Europeans, it is also present in black and Hispanic populations in the USA and individuals from Israel. This finding probably reflects the scale and nature of past human migration and inter-marriage between ethnic groups. Similarly, the relative absence of the pathogenic hexanucleotide repeat in India, Asia, and the Pacific Islands might be explained by the greater physical distances of these regions from Europe, and the consequent lack of admixture between these populations. Notably, the one Japanese patient who we identified as a carrier of the *C9orf72* expansion carried the Finnish risk haplotype, reinforcing the notion that the expansion occurred on one occasion in the past.

The sharing of a common risk haplotype in the *C9orf72* region of chromosome 9p21 in patients with sporadic and familial ALS suggests that these apparently sporadic cases are actually cryptically related familial cases. This scenario might have occurred for several reasons, including unfamiliarity with the pedigree on the part of the patient or neurologist or because previous generations might have died at a young age before onset of neurological symptoms. The median age at onset in patients with the expansion was 57 years, and life expectancy in the USA began to exceed this point only in the early 1940s.[26] Furthermore, the incomplete penetrance of the mutation, in which not all individuals carrying the expansion manifest a clinical phenotype, might be a contributing factor in apparently sporadic disease. Indeed, we have reported symptom onset in the ninth decade of life in patients carrying the expansion and also encountered two elderly, neurologically healthy individuals with the expansion. Thus, the penetrance of this mutation seems to

be complete only at a late stage of life, which is an observation that is particular relevance for genetic counselling of healthy individuals carrying the expansion. The molecular biological substrate underlying this variability in age at onset is unclear: it might be driven by differences in expansion lengths between patients, by age-related methylation across the locus, or by genetic factors elsewhere in the genome.

We compared our results with those of previous studies that reported the frequency of the *C9orf72* hexanucleotide repeat expansion in the pathogenesis of ALS and FTD (panel). Data were available from seven studies (Appendix). Our study screened one of the largest cohort of cases with ALS and FTD assessed to date, and also provides an initial report of the frequency of the pathogenic repeat expansion in non-white patients, a detailed examination of the haplotype across the locus, and an initial estimate of age-related disease penetrance in a large group of individuals carrying the expansion.

Panel: **Research in context**

Systematic review

We searched Medline up to December, 2011, without language restrictions for relevant publications and selected studies that reported the GGGGCC hexanucleotide repeat expansion in *C9orf72* in pathogenesis of amyotrophic lateral sclerosis (ALS) or frontotemporal dementia (FTD). On the basis of these criteria, seven studies were identified for further assessment (Appendix). The number of patients screened for the pathogenic repeat expansion and the phenotype and ethnic origin reported by these studies are summarized in the Appendix.

Interpretation

We report the frequency of the *C9orf72* repeat expansion in a large cohort of patients with sporadic ALS and sporadic FTD. We also screened a large number of non-white patients for the expansion, and present frequency data for the mutation in these populations. We confirmed that the *C9orf72* repeat expansion explains a substantial proportion of sporadic ALS (~7.0%) and sporadic FTD (~6.0%) cases in white populations. We also noted that patients with sporadic and familial disease carrying the expansion share a founder risk haplotype, suggesting that these patients have a common ancestor and that the original mutational event that led to the repeat expansion occurred only once in the past. We provide initial estimates of age-related penetrance, showing that 50% of carriers manifest disease by 58 years of age, and that the mutation is fully penetrant by 80 years of age.

Our data have implications for the clinical care of patients diagnosed with ALS and FTD. The clinical standard care is to offer genetic testing to patients reporting a family history of ALS or FTD,[27] and to reassure patients classified as having sporadic disease that their relatives are not at increased risk of neurodegeneration. On the basis of an analysis of 191 Irish patients with ALS, Byrne and colleagues[28] suggested that genetic testing for the *C9orf72* repeat expansion is unnecessary in affected individuals without a family history of disease or substantial cognitive impairment. By contrast, we believe that genetic testing is a valuable technique for accurate diagnosis of the two disorders and in the decision-making process for patients and their families. The discrepancy between these two views might stem from differences in how sporadic and familial disease were defined in the two studies. Accumulating of sufficient data is an important step towards answering this key question for management of patients. In view of the large number of patients who carry the repeat expansion, investigators and clinicians should at least consider a focused debate on this issue.

Our paper has some limitations. First, the number of patients from some geographical regions was small and the mutational frequencies may change for those ethnic groups as additional cases are screened. Nevertheless, our data on more than 5,000 patients with ALS or FTD provide a reasonable estimation of *C9orf72* global frequency. Second, although we have examined the chromosome 9p21 haplotype in a large and diverse cohort of individuals carrying the pathogenic expansion, additional testing of carriers might reveal other haplotypes, thereby indicating that the expansion arose on more than one occasion. Nevertheless, our data suggest that most expansion carriers share a common ancestor.[16,17] Third, we generated age-related penetrance estimates on the basis of data from retrospective cohorts, which potentially leads to over-estimation of penetrance. Additional prospective studies examining family kindreds are needed to confirm these estimates. Finally, case classification as familial or sporadic was done on the basis of clinical questioning at sample collection. The level of scrutiny might have varied between centers and countries, but re-collection of this information was not feasible.

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CONFLICTS OF INTEREST

Jeffrey Rothstein is Director of the Packard Center for ALS Research at Hopkins,

while Pentti Tienari, Peter Heutink, Huw Morris, Stuart Pickering-Brown and Bryan Traynor have a patent pending on the discovery of the hexanucleotide repeat expansion of the *C9orf72* gene. None of the other authors has any conflicts of interest.

REFERENCES

1. Logroscino G, Traynor BJ, Hardiman O, et al. Incidence of amyotrophic lateral sclerosis in Europe. *J Neurol Neurosurg Psychiatry* 2010; 81: 385-90.
2. Valdmanis PN, Daoud H, Dion PA, Rouleau GA. Recent advances in the genetics of amyotrophic lateral sclerosis. *Curr Neurol Neurosci Rep* 2009; 9: 198-205.
3. Chiò A, Traynor BJ, Lombardo F, et al. Prevalence of SOD1 mutations in the Italian ALS population. *Neurology* 2008; 70: 533-37.
4. Guerreiro RJ, Schymick JC, Crews C, Singleton A, Hardy J, Traynor BJ. TDP-43 is not a common cause of sporadic amyotrophic lateral sclerosis. *PLoS One* 2008; 3: e2450.
5. Lai SL, Abramzon Y, Schymick JC, et al. FUS mutations in sporadic amyotrophic lateral sclerosis. *Neurobiol Aging* 2011; 32: 550 e1-4.
6. Dion PA, Daoud H, Rouleau GA. Genetics of motor neuron disorders: new insights into pathogenic mechanisms. *Nat Rev Genet* 2009; 10: 769-82.
7. Ratnavalli E, Brayne C, Dawson K, Hodges JR. The prevalence of frontotemporal dementia. *Neurology* 2002; 58: 1615-21.
8. Neumann M, Sampathu DM, Kwong LK, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* 2006; 314: 130-3.
9. Dejesus-Hernandez M, Mackenzie IR, Boeve BF, et al. Expanded GGGGCC Hexanucleotide Repeat in Noncoding Region of C9ORF72 Causes Chromosome 9p-Linked FTD and ALS. *Neuron* 2011; 72: 245-56.
10. Renton AE, Majounie E, Waite A, et al. A Hexanucleotide Repeat Expansion in C9ORF72 Is the Cause of Chromosome 9p21-Linked ALS-FTD. *Neuron* 2011; 72: 257-68.
11. Simón-Sánchez J, Dopper EGP, Cohn-Hokke PE, et al. The clinical and pathological phenotype of *C9orf72* hexanucleotide repeat expansions. Brain 2012. Epub ahead of print. doi: 10.1093/brain/awr353
12. Snowden JS, Rollinson S, Thompson JC, et al. Distinct clinical and pathological characteristics of frontotemporal dementia associated with *C9ORF72* mutations. *Brain* 2012; published online Feb 2. DOI:10.1093/brain/awr355.
13. Brooks BR. El Escorial World Federation of Neurology criteria for the diagnosis of amyotrophic lateral sclerosis. Subcommittee on Motor Neuron Diseases/Amyotrophic Lateral Sclerosis of the World Federation of Neurology Research Group on Neuromuscular Diseases and the El Escorial "Clinical limits of amyotrophic lateral sclerosis" workshop contributors. *J Neurol Sci* 1994; 124 Suppl: 96-107.
14. Clinical and neuropathological criteria for frontotemporal dementia. The Lund and Manchester Groups. *J Neurol Neurosurg Psychiatry* 1994; 57: 416-8.
15. Traynor BJ, Nalls M, Lai SL, et al. Kinesin-associated protein 3 (KIFAP3) has no effect on survival in a population-based cohort of ALS patients. *Proc Natl Acad Sci USA* 2010; 107: 12335-8.
16. Laaksovirta H, Peuralinna T, Schymick JC, et al. Chromosome 9p21 in amyotrophic lateral sclerosis in Finland: a genome-wide association study. *Lancet Neurol* 2010; 9: 978-85.

17. Mok K, Traynor B, Schymick J, et al. The chromosome 9 ALS and FTD locus is probably derived from a single founder. *Neurobiol Aging* 2011; published online August 5. DOI: 10.1016/j.neurobiolaging.2011.08.005.

18. Reeve JP, Rannala B. DMLE+: Bayesian linkage disequilibrium gene mapping. *Bioinformatics* 2002; 18: 894-5.

19. Bender BU, Eng C, Olschewski M, et al. VHL c.505 T>C mutation confers a high age-related penetrance bu no increased overall mortality. *J Med Genet* 2001; 38: 508-14.

20. Passant U, Gustafson L, Brun A. Spectrum of frontal lobe dementia in a Swedish family. *Dementia* 1993; 4: 160-2.

21. Le Ber I, van der Zee J, Hannequin D, et al. Progranulin null mutations in both sporadic and familial frontotemporal dementia. *Hum Mutat* 2007; 28: 846-55.

22. Van es MA, Veldink JH, Saris CG, et al. Genome-wide association study identifies 19p13.3 (UNC13A) and 9p21.2 as susceptibility loci for sporadic amyotrophic lateral sclerosis. *Nature Genet* 2009; 41: 1083-7.

23. Shatunov A, Mok K, Newhouse S, et al. Chromosome 9p21 in sporadic amyotrophic lateral sclerosis in the UK and seven other countries: a genome-wide association study. *Lancet Neurol* 2010; 9: 986-94.

24. Van Deerlin VM, Sleiman PM, Martinez-Lage M, et al. Common variants at 7p21 are associated with frontotemporal lobar degeneration with TDP-43 inclusions. *Nat Genet* 2010; 42:234-9.

25. Kristiansson K, Naukkarinen J, Peltonen L. Isolated populations and complex disease gene identification. *Genome Biol* 2008; 9:109.

26. US Census Bureau. Statistical Abstract of the United States: 2012. 131st ed. Washington, DC, USA; 2011.

27. The EFNS Task Force on Diagnosis and Management of Amyotrophic Lateral Sclerosis. EFNS guidelines on the Clinical Management of Amyotrophic Lateral Sclerosis (MALS) – revised report of an EFNS task force. *Eur J Neurol* 2011 doi:10.1111/j.1468-1331.2011.03501.x.

28. Byrne S, Elamin M, Bede P, et al. Cognitive and clinical characteristics of patients with amyotrophic lateral sclerosis carrying a C9orf72 repeat expansion: a population-based cohort study. *Lancet Neurol* 2012 DOI:10.1016/S1474-4422(12)70014-5.

APPENDIX

The Chromosome 9-ALS/FTD Consortium

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SUPPLEMENTARY METHODS

Repeat-primed PCR

Briefly, 100ng of genomic DNA were used as template in a final volume of 28ul containing 14ul of FastStart PCR Master Mix (Roche Applied Science, Indianapolis, IN, USA), and a final concentration of 0.18mM 7-deaza-dGTP (New England Biolabs Inc., Ipswich, MA, USA), 1x Q-Solution (Qiagen Inc., Valencia, CA, USA), 7% DMSO (Qiagen), 0.9mM MgCl2 (Qiagen), 0.7uM reverse primer consisting of ~four GGGGCC repeats with an anchor tail (TACGCATCCCAGTTTGAGACGGGGGCCGGGGCCGGGGCCGGGG), 1.4uM 6FAM-fluorescent labeled forward primer located 280bp telomeric to the repeat sequence (AGTCGCTAGAGGCGAAAGC), and 1.4uM anchor primer corresponding to the anchor tail of the reverse primer (TACGCATCCCAGTTTGAGACG).[1,2] A touchdown PCR cycling program was used where the annealing temperature was gradually lowered from 70°C to 56°C in 2°C increments with a 3-minute extension time for each cycle. Fragment length analysis was performed on an ABI 3730xl genetic analyzer (Applied Biosystems Inc., Foster City, CA, USA), and data analyzed using GeneScan software (version 4, ABI). Repeat expansions produce a characteristic sawtooth pattern with a 6-bp periodicity when fragment lengths are analyzed on a capillary-based sequencer.[3]

SUPPLEMENTARY TABLES

Supplementary table 1. Demographics and clinical features of patients screened for the GGGGCC hexanucleotide repeat expansion of *C9orfF72* classified by diagnosis and familial status. (A) Demographic and clinical features of patients diagnosed with sporadic ALS screened for the GGGGCC hexanucleotide repeat expansion in *C9orf72*.

				Site of symptom onset	
Origin	*N*	*Age at onset (range)*	*Male (%)*	*Bulbar-onset (%)*	*Spinal-onset (%)*
Europe					
Finnish	289	58·2 (30·0-85·0)	149 (51·6%)	74 (31·2%)	163 (68·8%)
English	916	60·1 (16·0-86·0)	534 (58·9%)	237 (29·9%)	555 (70·1%)
German	421	57·3 (16·0 - 85·0)	263 (62·5%)	87 (20·7%)	334 (79·3%)
Italian	465	61·7 (20·5 - 87·3)	258 (55·5%)	127 (27·3%)	338 (72·7%)
Sardinian	129	59·7 (27·0 - 82·0)	80 (62·0%)	27 (20·9%)	102 (79·1%)
Moldovan	3	MD	3 (100·0%)	MD	MD
USA					
White	890	56·5 (19·0 - 93·0)	514 (57·8%)	179 (21·2%)	666 (78·8%)
Hispanic	72	58·8 (15·0 - 83·0)	44 (61·1%)	37 (52·9%)	33 (47·1%)
African American	49	55·2 (14·0 - 82·0)	20 (40·8%)	17 (34·7%)	32 (65·3%)
Native American	3	61·0 (56·0 - 66·0)	1 (33·3%)	0 (0·0%)	3 (100·0%)
Rest of the world					
Middle Eastern	1	73·0	1 (100·0%)	0 (0·0%)	1 (100·0%)
Indian	31	MD	MD	MD	MD
Asian	238	59·2 (24·0 - 89·0)	148 (62·2%)	61 (28·6%)	152 (71·4%)
Pacific Islander/ Guam	90	53·7 (43·0 - 70·0)	3 (100·0%)	2 (66·7%)	1 (33·3%)
Australian	263	64·0 (25·0 - 90·0)	171 (65·0%)	78 (29·7%)	185 (70·3%)

MD = missing data; data was not available for age at onset (*n* = 12 Finnish patients; *n* = 134 English; *n* = 3 Moldovan; *n* = 1 US white; *n* = 31 Indian; *n* = 3 Asians; *n* = 87 Pacific Islanders), for gender (*n* = 10 English patients; *n* = 31 Indian; *n* = 87 Pacific Islanders), and for site of onset (*n* = 52 Finnish patients; *n* = 124 English; *n* = 3 Moldovan, *n* = 34 US white; *n* = 2 US Hispanic; *n* = 31 Indian; *n* = 25 Asians; *n* = 87 Pacific Islanders).

Supplementary table 1 (B). Demographic and clinical features of patients diagnosed with familial ALS screened for the GGGGCC hexanucleotide repeat expansion in *C9orf72*.

				Site of symptom onset	
Origin	*N*	*Age at onset (range)*	*Male (%)*	*Bulbar-onset (%)*	*Spinal-onset (%)*
Europe					
Finnish	112	52.8 (18.0 - 81.0)	49 (43.8)	19 (20.7)	73 (79.3)
English	98	56.5 (27.0 - 88.0)	47 (48.5)	25 (30.1)	58 (69.9)
Irish	1	45	1 (100.0)	0 (0.0)	1 (100.0)
German	69	52.0 (23.0 - 90.0)	35 (50.7)	11 (37.9)	18 (62.1)
Italian	90	58.9 (18.0 - 79.0)	49 (54.4)	32 (35.6)	58 (64.4)
Sardinian	19	58.7 (33.0 - 78.0)	10 (52.6)	7 (36.8)	12 (63.2)
USA					
White	163	54.8 (15.0 - 80.0)	87 (53.3)	40 (25.2)	119 (74.8)
Rest of the world					
Middle Eastern	2	32.5 (29.0 - 36.0)	1 (50.0)	0 (0.0)	1 (100.0)
Israeli	14	49.4 (22.0 - 74.0)	5 (35.7)	2 (14.3)	12 (85.7)
Asian	20	47.3 (4.0 - 77.0)	11 (57.9)	4 (22.2)	14 (77.8)

Data was not available for age at onset (*n* = 17 Finnish patients; *n* = 10 English; *n* = 1 US White; *n* = 1 Asian), for gender (*n* = 1 English patient; *n* = 1 Asian patient), and for site of onset (*n* = 20 Finnish patients; *n* = 15 English; *n* = 40 Germans; *n* = 4 US White; *n* = 1 Middle Eastern; *n* = 2 Asians).

Supplementary table 1 (C). Demographic and clinical features of patients diagnosed with sporadic FTD screened for the GGGGCC hexanucleotide repeat expansion in *C9orf72*.

		Site of symptom onset				
Origin	*N*	*Age at onset (range)*	*Male (%)*	*Behavioral variant FTD (%)*	*PNFA (%)*	*Semantic dementia (%)*
Europe						
Finnish	48	58.0 (38.0 - 73.0)	19 (39.6)	30 (62.5)	14 (29.2)	4 (8.3)
Swedisch	6	60.2 (32.0 - 75.0)	4 (66.7)	5 (100.0)	0 (0.0)	0 (0.0)
English	543	60.8 (23.0 - 87.0)	306 (56.5)	284 (57.0)	102 (20.5)	112 (22.5)
Dutch	224	57.6 (28.6 - 76.0)	111 (49.6)	156 (69.6)	19 (8.5)	49 (21.9)
French	150	62.4 (40.0 - 79.0)	92 (61.3)	93 (97.8)	1 (1.1)	1 (1.1)
Sardinian	10	68.4 (59.0 - 79.0)	6 (60.0)	7 (70.0)	3 (30.0)	0 (0.0)
Rest of the world						
Indian	31	MD	MD	MD	MD	MD
Asian	10	57.8 (44.0 - 80.0)	3 (30.0)	9 (81.8)	1 (9.2)	0 (0.0)

PNFA, progressive non-fluent aphasia; MD = missing data; Data was not available for age at onset (*n* = 16 English patients; *n* = 14 French; *n* = 31 Indians; *n* = 2 Sardinians), for gender (*n* = 1 English patients; *n* = 31 Indians), and for type of FTD at onset (*n* = 1 Swedish patient; *n* = 45 English patients; *n* = 55 French; *n* = 31 Indian patients).

Supplementary table 1 (D). Demographic and clinical features of patients diagnosed with familial FTD screened for the GGGGCC hexanucleotide repeat expansion in *C9orf72.*

		Type of FTD at presentation				
Origin	*N*	*Age at onset (range)*	*Male (%)*	*Behavioral variant FTD (%)*	*PNFA (%)*	*Semantic dementia (%)*
Europe						
Finnish	27	59.0 (46.0 - 79.0)	15 (55.6)	18 (66.7)	6 (22.2)	3 (11.1)
Swedish	1	68	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)
English	170	58.1 (39.0 - 83.0)	95 (55.9)	50 (69.4)	11 (15.3)	11 (15.3)
German	29	MD	MD	MD	MD	MD
Dutch	116	58.6 (36.2 - 76.3)	61 (52.6)	87 (75.0)	13 (11.2)	16 (13.9)
French	50	59.0 (30.0 - 75.0)	33 (66.0)	24 (100.0)	0 (0.0)	0 (0.0)
Sardinian	7	63.3 (49.0 - 74.0)	5 (71.4)	6 (85.7)	1 (14.3)	0 (0.0)
Global:						
Asian	3	51.7 (45.0 - 56.0)	1 (33.3)	3 (100.0)	0 (0.0)	0 (0.0)

PNFA, progressive non-fluent aphasia; MD = missing data; Data was not available for age at onset (*n* = 6 English patients; *n* = 29 Germans; *n* = 10 French), for gender (*n* = 29 Germans), and for type of FTD at onset (*n* = 98 English patients; *n* = 29 Germans; *n* = 26 French).

Supplementary table 2. Demographics and clinical features of neurologically healthy individuals carrying the GGGGCC hexanucleotide repeat expansion in *C9orf72*.

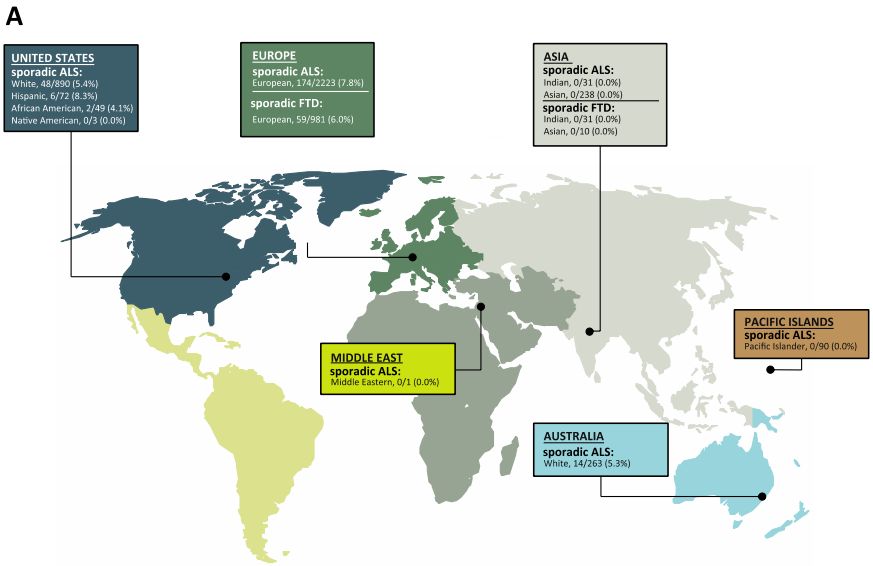
	Age at reported collection	*Gender*	*Race*	*Ethnicity*	*Country of origin*	*Comment*
Vantaa1	85-90 years	M	W	Non-Hispanic	Finland	Deceased without personal history of neurological disease
Vantaa2	90-95 years	F	W	Non-Hispanic	Finland	Deceased without personal history of neurological disease
German1	35-40 years	F	W	Non-Hispanic	German	No reported history of neurological disease at time of sample collection
German2	25-30 years	F	W	Non-Hispanic	German	No reported history of neurological disease at time of sample collection
ND15567	36	F	W	Non-Hispanic	US	No reported personal or family history of neurological diseases

Additional details are available for ND15567 at www.coriell.org; To protect privacy, the sample IDs of the other samples has been modified, and their age at collection has been provided as a 5 year range.

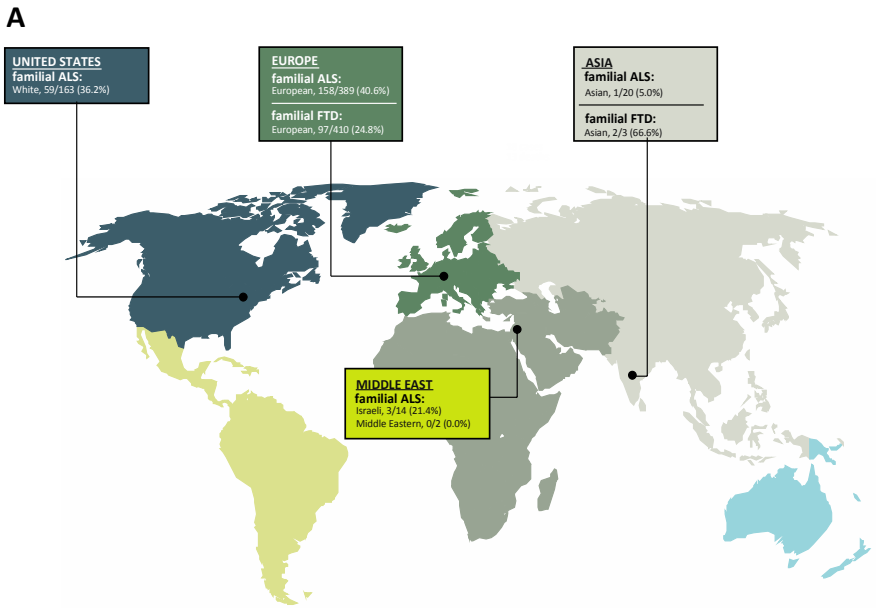
Supplementary table 3. Systematic review of relevant publications that reported the GGGGCC hexanucleotide repeat expansion of the *C9orf72* gene relevant to the pathogenesis of ALS or FTD.

Study	*Reference#*	*N*	*Phenotype*	*Race*
Renton et al	3	112	Familial ALS	Finnish
Renton et al	3	290	Sporadic ALS	Finnish
Renton et al	3	27	Familial FTD	Finnish
Renton et al	3	48	Sporadic FTD	Finnish
Renton et al	3	268	Familial ALS	Outbred European-ancestry
DeJesus-Hernandez et al	4	34	Familial ALS	US
DeJesus-Hernandez et al	4	195	Sporadic ALS	US
DeJesus-Hernandez et al	4	171	Familial FTD	US
DeJesus-Hernandez et al	4	203	Sporadic FTD	US
Byrne et al	5	49	Familial ALS	Irish
Byrne et al	5	386	Sporadic ALS	Irish
Simon-Sanchez et al	6	353	FTD	Dutch
Snowden et al	7	398	FTD	English
Stewart et al	8	231	ALS	US
Gijselinck et al	9	328	FTD	Belgian
Gijselinck et al	9	137	ALS	Belgian

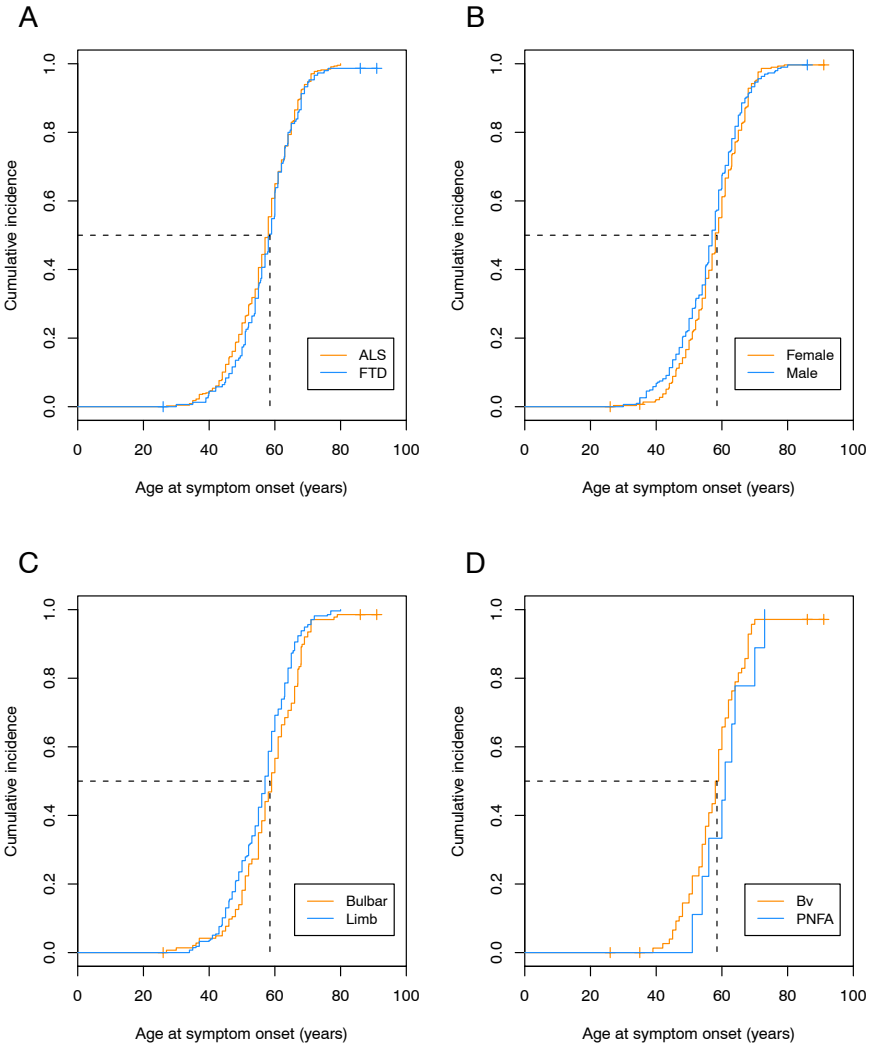
SUPPLEMENTARY FIGURES



Supplementary figure 1. Geographical distribution of the pathogenic GGGGCC hexanucleotide repeat expansion in *C9orf72* in patients diagnosed with sporadic ALS and FTD.



Supplementary figure 2. Geographical distribution of the pathogenic GGGGCC hexanucleotide repeat expansion of the *C9orf72* gene in patients diagnosed with familial ALS and FTD.



Supplementary figure 3. Age-related penetrance of the GGGGCC hexanucleotide repeat expansion in the *C9orf72* gene. (A) Age-related penetrance of patients presenting with ALS (*n* = 441) and FTD (*n* = 157). (B) Age related penetrance of male (*n* = 296) and female (*n* = 307) ALS and FTD patients. (C) Age-related penetrance of ALS patients presenting with bulbar-onset (*n* = 140) and limb-onset (*n* = 276) disease. (D) Age-related penetrance of FTD patients presenting with behavioral type (*n* = 73) and progressive non-fluent aphasia (PNFA, *n* = 9). Crosses represent censored-events (*n* = 5 neurologically normal individuals carrying the expansion). The dotted lines represent the age at which 50·0% of the cohort developed symptoms.

SUPPLEMENTARY REFERENCES

1. Kobayashi H, Abe K, Matsuura T, et al. Expansion of Intronic GGCCTG Hexanucleotide Repeat in NOP56 Causes SCA36, a Type of Spinocerebellar Ataxia Accompanied by Motor Neuron Involvement. *Am J Hum Genet* 2011; **89**: 121-30.
2. Warner JP, Barron LH, Goudie D, et al. A general method for the detection of large CAG repeat expansions by fluorescent PCR. *J Med Genet* 1996; **33**: 1022-6.
3. Renton AE, Majounie E, Waite A, et al. A Hexanucleotide Repeat Expansion in *C9ORF72* Is the Cause of Chromosome 9p21-Linked ALS-FTD. *Neuron* 2011; **72**: 257-68.
4. Dejesus-Hernandez M, Mackenzie IR, Boeve BF, et al. Expanded GGGGCC Hexanucleotide Repeat in Noncoding Region of C9ORF72 Causes Chromosome 9p-Linked FTD and ALS. *Neuron* 2011; **72**: 245-56.
5. Byrne S, Elamin M, Bede P, et al. Cognitive and clinical characteristics of patients with amyotrophic lateral sclerosis carrying a C9orf72 repeat expansion: a population-based cohort study. *Lancet Neurol* 2012 DOI:10.1016/S1474-4422(12)70014-5.
6. Simón-Sánchez J, Dopper EG, Cohn-Hokke PE, et al. The clinical and pathological phenotype of C9orf72 hexanucleotide repeat expansions. *Brain* 2012 Feb 1. [Epub ahead of print]
7. Snowden JS, Rollinson S, Thompson JC, et al. Distinct clinical and pathological characteristics of frontotemporal dementia associated with C9ORF72 mutations. *Brain* 2012 Feb 2. [Epub ahead of print]
8. Stewart H, Rutherford NJ, Briemberg H, et al. Clinical and pathological features of amyotrophic lateral sclerosis caused by mutation in the C9ORF72 gene on chromosome 9p. *Acta Neuropathol* 2012 Jan 7. [Epub ahead of print]
9. Gijselinck I, Van Langenhove T, van der Zee J, et al. A C9orf72 promoter repeat expansion in a Flanders-Belgian cohort with disorders of the frontotemporal lobar degeneration-amyotrophic lateral sclerosis spectrum: a gene identification study. *Lancet Neurol* 2012; **11**: 54-65.

CHAPTER 3.1

Structural and functional brain connectivity in presymptomatic familial frontotemporal dementia

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ABSTRACT

Objective: We aimed to investigate whether cognitive deficits and structural and functional connectivity changes can be detected before symptom onset in a large cohort of carriers of *microtubule-associated protein tau* (*MAPT*) or *progranulin* (*GRN*) mutations.

Methods: In this case-control study 75 healthy individuals (aged 20 - 70 years) with 50% risk of frontotemporal dementia (FTD) underwent DNA screening, neuropsychological assessment, structural and functional MRI. We used voxel-based morphometry and tract-based spatial statistics for voxel-wise analyses of grey matter volume and diffusion tensor imaging measures. Using resting-state fMRI scans, we assessed whole-brain functional connectivity to frontoinsular, anterior midcingulate and posterior cingulate cortices.

Results: Carriers (n = 39) and non-carriers (n = 36) had similar neuropsychological performance, except for lower Letter Digit Substitution Test scores in carriers. Worse performance on Stroop III, Rivermead Behavioral Memory Test and Happé cartoons correlated with higher age in carriers, but not controls. Reduced fractional anisotropy in the right uncinate fasciculus was found in carriers compared with controls. Reductions in functional connectivity between anterior midcingulate cortex and frontoinsula and several other brain regions were found in carriers compared with controls and correlated with higher age in carriers, but not controls. We found no significant differences or age correlations in posterior cingulate cortex connectivity. No differences in regional grey matter volume were found, except for a small cluster of higher volume in the precentral gyrus in carriers.

Conclusions: This study demonstrates that alterations in structural and functional connectivity develop before the first symptoms of FTD arise. These findings suggest that diffusion tensor imaging and resting-state fMRI may have the potential to become sensitive biomarkers for early FTD in future clinical trials.

INTRODUCTION

Frontotemporal dementia (FTD) is characterized by behavioral and language disorders, executive dysfunction, and impaired social cognition.[1-3] The frontoinsula, anterior cingulate (ACC) and anterior midcingulate cortex (aMCC) are among the first affected brain regions in FTD.[4, 5] However, in early disease atrophy may be subtle or difficult to detect.[6, 7] With current research focusing on potential disease-modifying treatments, the identification of appropriate biomarkers to detect early FTD and track disease progression is crucial.[8, 9] *Microtubule-associated protein tau* (*MAPT*), *progranulin* (*GRN*), and *chromosome 9 open reading frame 72* (*C9orf72*) mutations are the major genetic causes of autosomal dominant FTD.[10-12] Asymptomatic carriers of these mutations provide the ideal study population to investigate the first alterations in FTD.[13]

Several diffusion tensor imaging (DTI) studies in patients with FTD have reported reduced white matter (WM) integrity, most prominent in the frontotemporal cortex,[14-20] corresponding to WM pathology.[21] A small study in presymptomatic *GRN* carriers indicated reduced integrity of the left uncinate and inferior fronto-occipital fasciculus.[22]

Resting-state fMRI studies in FTD demonstrated reduced functional connectivity within the salience network (SN), which is involved in emotional processing, and is anchored by the frontoinsula, ACC, and aMCC, spatially corresponding to the specific atrophy pattern in FTD.[7, 23-25] Conflicting results were found in two small series of presymptomatic *MAPT* and *GRN* carriers.[6, 7]

In the current study of a large cohort of presymptomatic *MAPT* and *GRN* carriers, we investigated whether cognitive and structural or functional imaging changes occur before symptom onset.

METHODS

Subjects

From December 2009 through March 2011 we recruited participants for this case-control study from a pool of 160 healthy first-degree relatives (aged

20 - 70 years) of patients with FTD due to *GRN* or *MAPT* mutations. We defined subjects as asymptomatic when participant and spouse denied cognitive or behavioral disturbances. We contacted the first 105 subjects, of whom 86 were willing to participate. Reasons for nonparticipation were nonrelated illness, lack of motivation, claustrophobia, and disinterest in disease confrontation. We excluded subjects with MRI contraindications (n = 4), history of drug abuse (n = 2), or neurological or psychiatric disorders (n = 2). DNA of the remaining 78 participants was screened for *GRN* and *MAPT* mutations, as previously described.[12] Family members without a mutation constituted the control group. Researchers remained blinded to the genetic status of participants. Participants underwent neuropsychological assessment, structural MRI, DTI, and resting-state fMRI. We excluded three individuals from analyses because of a cerebellar cyst (n = 1) or technical failure during scanning (n = 2), and one additional participant from DTI analyses due to motion artefacts. For all measures, we performed both between-group comparisons and age correlation analyses, to investigate whether alterations arise as carriers approach their estimated onset age.

Standard protocol approvals, registrations, and patient consents

All participants provided written informed consent. The local ethics committee approved the study.

Neuropsychological assessment

We screened all subjects with the Mini-Mental state examination (MMSE),[e1] Beck Depression Inventory (BDI),[e2] and State-Trait Anxiety Inventory (STAI).[e3] The neuropsychological test battery included the Dutch version of the Rey Auditory Verbal Learning Test (RAVLT),[e4] stories of the Rivermead Behavioral Memory Test (RBMT),[e5] Visual Association Test (VAT),[e6] Wechsler Adult Intelligence Scale III subtests digit span, proverbs, similarities, and block design[e7-8], Trailmaking Test (TMT),[e9] Stroop color-word test,[e10] categorical and letter fluency,[e11] modified Wisconsin Card Sorting Test (WCST),[e12] Letter Digit Substitution Test (LDST),[e13] Boston Naming Test (BNT),[e14] Semantic Association Test (SAT),[e15] ScreeLing,[e16] clock drawing (Royall),[e17] cube copying,[e18] and social cognition tests: Ekman faces[e19], Happé Cartoons,[e20] short Faux pas.[e21]

Image acquisition and analyses

We acquired whole brain anatomical, DTI, and resting-state fMRI scans on a Philips 3.0-T Achieva MRI scanner (Philips Medical Systems, Best, The Netherlands) using an eight-channel SENSE head coil (see Appendix). We used FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk) for all imaging analyses.[26]

We used FSL-VBM, a voxel-based morphometry style analysis giving individual images of grey matter concentration, for T1-weighted scans (see Appendix).[e22-29] We performed permutation-based testing using 5,000 permutations, applying a two-sample *t*-test model with confound regressors for age and sex, to compare regional grey matter and to investigate correlations with age (see www.fmrib.ox.ac.uk/fsl/randomise/index). We set the statistical threshold at $p < 0.05$, corrected for multiple comparisons using threshold-free cluster enhancement (TFCE).[e28]

For voxel-wise comparisons of multiple DTI measures, including fractional anisotropy (FA) and mean (MD), axial (DA) and radial (DR) diffusivity we used tract-based spatial statistics (see Appendix).[e22, e26-27, e30] We used permutation testing with 5,000 permutations, applying a two-sample *t*-test, with age and sex as confound regressors, for between-group and age correlation analyses. We thresholded the resulting statistical maps at $p < 0.05$, corrected for multiple comparisons using TFCE.[e28] Subsequently, we reran analyses using templates of the uncinate fasciculi and the forceps minor[e31], tracts most consistently affected in FTD,[17-20, 27] as prethreshold masks to investigate these WM tracts specifically.

For resting-state fMRI data we used a seed-based approach in the FMRI Expert Analysis Tool. After standard preprocessing (see Appendix)[e22, e24-25] we carried out within-subject analyses to determine whole-brain regional connectivity with three bilateral seed regions of interest: left and right frontoinsula (36-voxel clusters around Montreal Neurological Institutie (MNI) coordinates x = ± 38, y = 26, z = -10) and aMCC (4 mm spheres around x = ± 5; y = 19; z = 28) to define the SN and left and right posterior cingulate cortex (PCC) (4 mm spheres around x = ± 2; y = -51; z = 27) to define the default mode network (DMN).[24, 28, 29] We extracted mean time-series for each seed for each subject in native space by applying the inverse transform from MNI space to fMRI. For each individual, we carried out time-series statistical analyses per seed with local autocorrelation correction using the

Table 1. Demographic features and neuropsychological performance.

(Maximum scores)	*Total study group (n=75) - age range 22.2-68.6 years*		
	Controls (n=36)	*Mutation carriers (n=39)*	*p-value*
Age, years	50.3 (11.2)	51.0 (10.0)	0.770
Female	50%	59%	0.435
GRN mutation†	-	72%	-
Level of education‡	5.1 (1.1)	5.6 (1.0)	**0.047**
MMSE score (30)	29.0 (1.3)	29.2 (1.4)	0.572
BDI (63)	3.7 (4.2)	3.4 (4.6)	0.740
STAI DY1 (80)	32.9 (6.5)	34.7 (9.9)	0.378
STAI DY2 (80)	32.5 (6.9)	34.1 (10.7)	0.463
Attention and executive functions			
Digit span (30)	14.8 (3.6)	15.7 (3.5)	0.800
Proverbs (8)	6.1 (1.7)	6.6 (1.3)	0.307
Similarities (33)	24.5 (4.8)	25.9 (4.8)	0.784
TMT A§	31.7 (14.4)	30.8 (11.7)	0.731
TMT B§	67.9 (29.8)	70.8 (40.2)	0.211
Stroop I§	46.5 (7.9)	45.1 (8.6)	0.735
Stroop II§	58.0 (10.8)	59.7 (12.2)	0.227
Stroop III§	92.3 (22.7)	94.8 (24.9)	0.279
Categorical fluency¶	41.6 (8.1)	41.4 (10.4)	0.501
Letter fluency¶	32.7 (9.9)	36.3 (12.3)	0.554
WCST concepts (6)	5.5 (0.8)	5.7 (0.8)	0.445
LDST¶	34.6 (7.2)	33.2 (6.7)	**0.024**
Memory			
Orientation (10)	9.9 (0.4)	9.8 (0.6)	0.235
RAVLT total (75)	42.0 (9.8)	46.4 (10.3)	0.158
RAVLT recall (15)	8.4 (3.3)	9.3 (3.5)	0.482
RAVLT recognition (30)	28.5 (2.1)	29.1 (1.5)	0.455
RBMT immediate (42)	19.3 (5.5)	20.8 (6.1)	0.487
RBMT delayed (42)	15.8 (6.1)	17.3 (6.1)	0.620
VAT (12)	11.8 (0.6)	11.4 (1.2)	0.157
Language			
BNT (60)	53.4 (4.3)	54.6 (4.1)	0.626

SAT verbal (30)	27.9 (1.3)	27.5 (1.8)	0.319
SAT nonverbal (30)	28.8 (1.5)	29.0 (1.0)	0.537
ScreeLing (72)	70.7 (1.5)	71.4 (0.7)	0.083
Visuospatial			
Royall clock (14)	12.5 (1.4)	12.3 (1.5)	0.470
Cube copying (8)	7.8 (0.5)	7.9 (0.5)	0.650
Block design (68)	35.8 (13.9)	34.8 (14.9)	0.433
Social cognition			
Ekman faces (60)	45.6 (6.7)	47.0 (5.8)	0.561
Happé Cartoons (36)	23.1 (5.6)	25.7 (5.1)	0.074
Faux pas (10)	9.0 (1.1)	9.1 (1.1)	0.739

Values denote mean (SD) or percentage of subjects.

Scores on SAT nonverbal were missing in three individuals, and scores on Block design in two.

†Remaining mutation carriers have a *MAPT* mutation

‡Level of education was determined on a Dutch seven-point scale ranging from one (less than elementary school) to seven (university or technical college)[30]

§Time in seconds

¶Number of correct responses in one minute

General Linear Model, which included time series for WM, cerebrospinal fluid and the global signal, and six motion parameters as confound regressors. We acquired parameter estimates for each regressor. Contrasts of interest were the left and right seed separately and the left and right seeds together, for all regions of interest (frontoinsula, aMCC, and PCC). We transformed subject-level contrast images and corresponding images of variance to MNI space using the combined transformation matrix, for group analysis. For group analyses we used a two sample *t*-test, with age, sex and voxel-wise grey matter volume included as additional regressors to investigate between-group differences and age correlations. We thresholded *Z*-statistic images using clusters determined by an initial cluster-forming threshold $Z > 2.3$ and a corrected cluster significance threshold of $p < 0.05$.[e29] Age correlation analyses were Bonferroni-corrected for multiple testing.

Statistical analyses

We performed statistical analyses in SPSS 17.0 for Windows (SPSS, Chicago, IL). We analyzed demographic features using independent samples *t*-tests and Pearson χ^2 tests, and neuropsychological data using analyses of covariance controlling for age, sex, and education. We applied a significance level of $p < 0.05$ (two-tailed)

across all analyses.

RESULTS

Demographic features

Genetic screening revealed 39 mutation carriers (*GRN* mutation in 28, *MAPT* in 11) and 36 noncarriers. Age, sex, and MMSE, BDI, and STAI scores of carriers and controls were similar, but carriers had a slightly higher education level[30] compared with controls (Table 1). The current age of *MAPT* carriers (43.7 ± 11.5 years) was 6.2 ± 10.2 years younger than the mean onset age in their families; the current age of *GRN* carriers (53.9 ± 7.9 years) was 7.7 ± 7.4 years younger than their mean onset ages ($p = 0.619$).

Neuropsychological assessment

Carriers and controls performed similarly on all cognitive tests, except for worse performance on the LDST in carriers (Table 1). Worse performances on Stroop III, RBMT and Happé Cartoons significantly correlated with higher age in carriers, but not controls (Supplementary table 1, Figure 1A). Conversely, scores on proverbs increased with higher age in carriers, with a trend for a similar age-effect in controls. Performances on TMT A, and RAVLT total and recall decreased with higher age in both groups, whereas Block design and faux pas scores decreased only in controls. The age effect in Faux pas performance is probably a result of ceiling effects, due to the limited range in test scores.

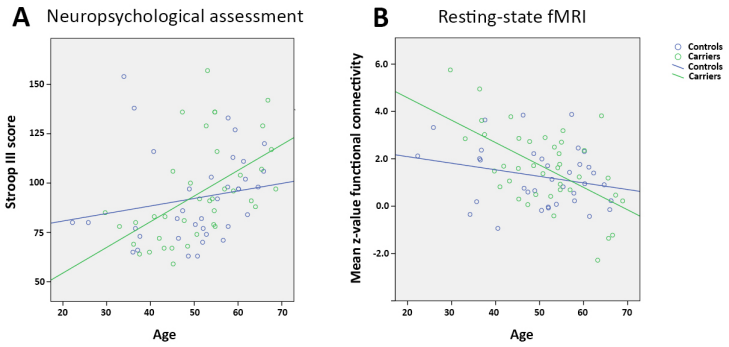

Figure 1. Relationship between age and performance on Stroop III (higher scores represent worse performance) (A), and mean bilateral frontoinsula connectivity with aMCC cluster from correlation analysis (Supplementary table 3) (B).

Structural imaging – Voxel-based morphometry

VBM analysis revealed no significant differences in regional grey matter volume between controls and carriers, except for a small cluster of higher grey matter volume in the right precentral gyrus in carriers. A decline in grey matter volume with higher age was found in widespread regions in both groups, whereas there were no areas showing an increase in grey matter volume in either group.

Structural connectivity – Tract-based spatial statistics

TBSS analyses revealed no significant differences between carriers and controls. Restricting the analyses to templates of the uncinate fasciculi and the forceps minor, however, we found significantly reduced FA in the right uncinate fasciculus in carriers compared with controls (Figure 2). Both carriers and controls showed a negative age correlation with FA and a positive age correlation with MD and DR in widespread regions, but only carriers showed a significant correlation between higher age and DA increase (Supplementary table 2).

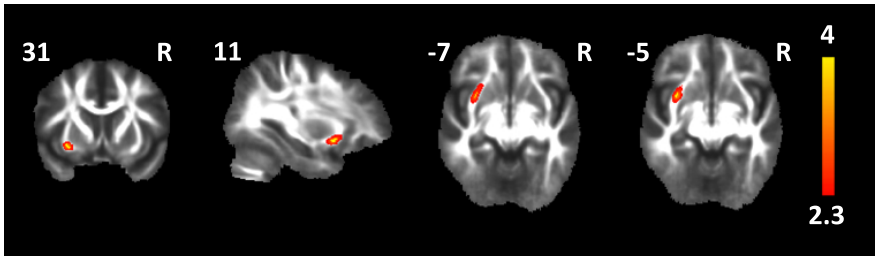

Figure 2. Alterations in DTI measures in mutation carriers. Maps illustrate significantly decreased FA in mutation carriers compared with controls, with a template of the uncinate fasciculus as prethreshold mask. Thresholded statistic images were thickened using tbss_fill in FSL for better visibility. Color bar represents *p*-values.

Separate analysis of *GRN* carriers (n = 27) versus non-carriers (n = 28) revealed no significant differences. *MAPT* carriers (n = 11) showed significantly decreased FA and increased DR in bilateral uncinate fasciculi and reduced FA in the forceps minor compared with controls (n = 8) (Figure 3).

Functional connectivity – Frontoinsula seed

Carriers showed significantly reduced functional connectivity between frontoinsula seeds and left temporal and right occipital regions compared with controls (Figure

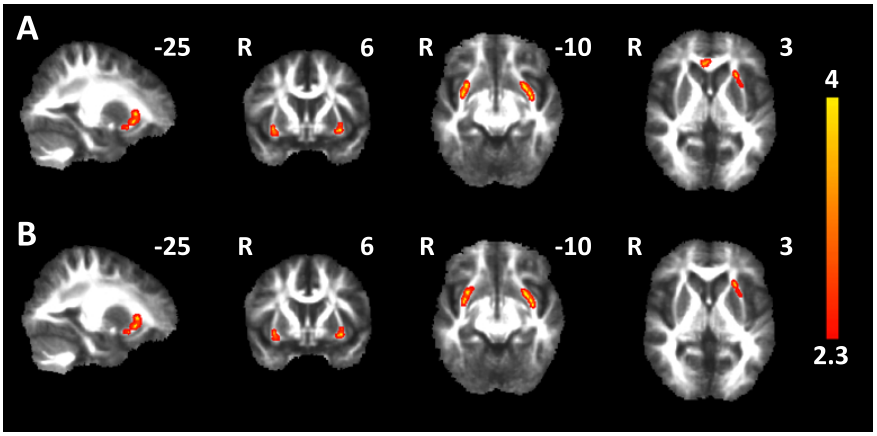

Figure 3. Alterations in DTI measures in *MAPT* mutation carriers. Maps illustrate significant decreases in FA (A) and DR (B) in *MAPT* mutation carriers compared with controls from their families with templates of the uncinate fasciculi and forceps minor as prethreshold masks. Thresholded statistic images were thickened using tbss_fill in FSL for better visibility. Color bar represents *p*-values.

4A, Supplementary table 3). Moreover, lower functional connectivity between frontoinsula seeds and aMCC and several other cortical regions correlated with higher age in carriers, but not in controls (Figure 1B and 4B, Supplementary table 3).

Functional connectivity – aMCC seed

Carriers showed significantly lower functional connectivity between the left aMCC seed and left insula and surrounding structures and between the right aMCC seed and parietal regions compared with controls (Figure 4C, Supplementary table 3). Connectivity between the aMCC seeds and several, mainly posterior, brain regions showed both positive and negative age correlations in carriers (Supplementary table 3).

Functional connectivity – PCC seed

PCC connectivity did not differ between carriers and controls, except for increased out-of-network connectivity of the right PCC seed with the cerebellum and decreased connectivity of the left PCC seed with the cerebellum in carriers (Supplementary table 3). PCC connectivity was not correlated with age.

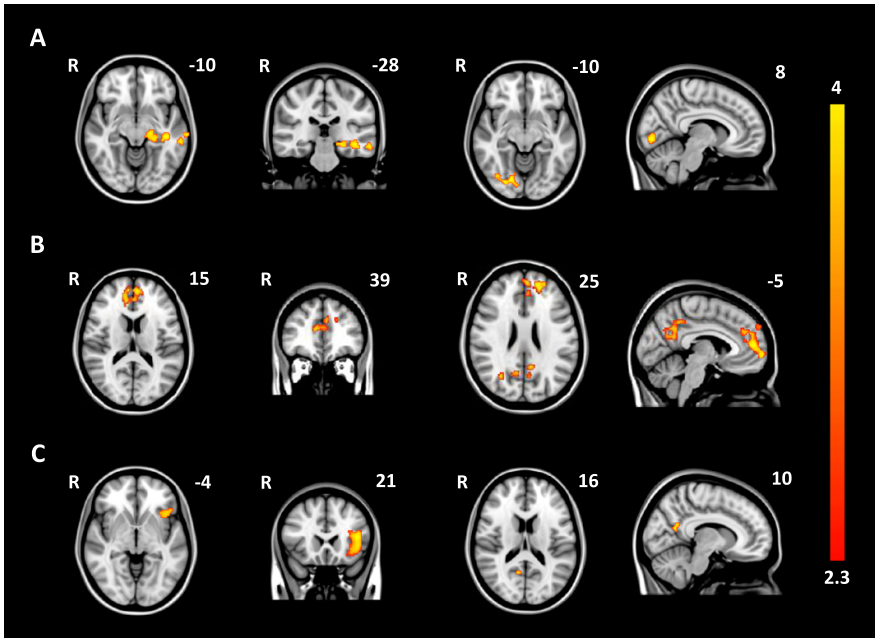

Figure 4. Alterations in frontoinsula and aMCC connectivity in mutation carriers. Maps illustrate clusters of significantly decreased frontoinsula connectivity in mutation carriers compared with controls (A), decreasing bilateral frontoinsula connectivity in mutation carriers (B) with higher age, and significantly decreased aMCC connectivity in mutation carriers compared with controls (C). Color bar represents *Z*-scores

Functional connectivity – *GRN* and *MAPT* subgroup analyses

GRN carriers showed lower functional connectivity between the frontoinsula seeds and frontal medial cortex, paracingulate gyrus, occipital pole and lateral occipital cortex compared with controls. Using the aMCC seeds, *GRN* carriers showed a largely similar pattern of decreased connectivity as the total carrier group, although increased aMCC connectivity with several other cortical regions was also found. In contrast to the total carriers group, reduced connectivity between the PCC seeds and paracingulate, middle and inferior frontal gyri, frontal orbital cortex, and frontal pole was found in *GRN* carriers compared with controls.

MAPT carriers showed no significant differences in connectivity of the frontoinsula, aMCC, and PCC seeds compared with controls.

DISCUSSION

This study demonstrates that alterations in structural and functional brain connectivity emerge in FTD mutation carriers before the first symptoms of dementia arise. Our findings have important implications for future clinical trials, suggesting that DTI and resting-state fMRI could possibly be developed into quantitative biomarkers for detecting FTD presymptomatically.

Our finding of a decline in performances on Stroop III, and Happé cartoons with increasing age in carriers, but not controls, suggests that impairment on these tasks is associated with disease activity prior to symptom onset. This is further supported by previous observations that social cognition and executive deficits are the earliest neuropsychological characteristics of FTD.[1, 2, 31] The executive dysfunction in carriers decades before the estimated disease onset in a previous small study, suggesting a developmental origin of these deficits,[32] could not be confirmed in the current and another[22] study, as we found no between-group differences, except for lower performance on the Letter Digit Substitution Test in carriers. Decreasing performances on TMT A and RAVLT in carriers and controls probably reflect normal aging effects, whereas increasing scores on proverbs with higher age are probably explained by greater familiarity with proverbs among older participants. Previous studies have shown an age-related decline in Block design performance in healthy individuals, as observed in the current control group.[e7, e32, e33] The lack of such age-related decline in the mutation carriers is in line with the hypothesis that visuospatial functions are preserved or even enhanced in FTD.[25]

The FA reduction within the uncinate fasciculus in carriers compared with controls support the hypothesis that WM damage is an early feature in the FTD disease process, which is in line with a previous study in presymptomatic *GRN* carriers.[22] Reduced integrity of the uncinate fasciculus is a consistent finding in FTD patients[17-20, 27] and correlates with disinhibition,[27] which is an early feature of FTD.[1, 3] The lack of significant differences in MD, DA, and DR may be explained by the lower sensitivity of these parameters compared with FA in early dementia.[33]

The more widespread WM changes in *MAPT* carriers, with involvement of bilateral uncinate fasciculi and forceps minor, compared with the lack of DTI changes in

the *GRN* analysis may suggest a mutation-specific distribution of WM damage, as the time till estimated disease onset was similar in *MAPT* and *GRN* carriers. One previous study investigated WM changes in FTD patients with *MAPT* and *GRN* mutations using VBM, and revealed more widespread WM changes and a faster rate of whole-brain atrophy in *GRN* than *MAPT* carriers.[36] This may suggest that the onset of WM degeneration is earlier in *MAPT* carriers, but that the progression is faster in *GRN* carriers. However, the current results should be interpreted with caution, due to the relatively small *MAPT* group.

The finding of lower connectivity between aMCC and frontoinsula in carriers compared with controls and declining frontoinsula connectivity with aMCC with higher age in carriers but not in controls corroborates findings of SN impairment in FTD patients, since these regions are central nodes of this network.[24, 25] The SN plays an important role in social-emotional processing, and failing connectivity between its central nodes may relate to the impaired social behavior typically seen in early FTD.[24] The hypothesis that decreasing frontoinsula connectivity with higher age in carriers probably reflects the disease process is further supported by previous observations of reducing frontoinsula connectivity within the SN with advancing disease severity in symptomatic FTD.[25] The lower connectivity of FI and aMCC with posterior regions is remarkable, but corresponds to the more generalized pattern of atrophy in *GRN* mutations,[36, 37] and may therefore relate to the high proportion of *GRN* carriers, which is further supported by similar findings in the separate *GRN* analysis.

The increased connectivity within the DMN found in symptomatic FTD probably arises around the time of symptom onset, as this was not found in the current and a previous study in presymptomatic FTD.[6, 25] The reduced DMN connectivity in *MAPT* carriers in a recent study may be mutation-specific,[7] but we could not confirm these findings. However, larger studies of *MAPT* carriers are needed to further elucidate the specific alterations within this group.

The relationship between structural and functional connectivity is still subject of discussion. Several studies have indicated a general correspondence between the two, but functional connectivity has also been observed between regions without direct structural connections.[40] Although functional connectivity changes

appeared to be more widespread than structural alterations in the current carriers, a direct comparison between these measures remains difficult due to large differences in analytical methods. More studies combining these MRI techniques are needed to further elucidate this relationship.

The observed connectivity changes in the presymptomatic stage of FTD highlight the potential of MRI to detect disease-related changes. The starting point of decrease in functional connectivity may represent an ideal moment to begin future therapeutic interventions. DTI and resting-state fMRI may have the potential to become diagnostic tools in future clinical trials. The wide availability of MRI scanners, lack of exposure to radioactivity and independence of task performance are major advantages of these techniques for implementation in clinical trials. Correlation of connectivity changes with age indicates that these tools might also be useful in tracking disease progression. Longitudinal studies are needed to explore these possibilities. Moreover, additional work is required to investigate how pharmacological interventions impact functional connectivity.

Strengths of our study included the large number of participating at-risk individuals, the extensive neuropsychological battery, and the combination of functional and structural imaging. The major limitation was the limited number of *MAPT* carriers and larger studies are needed to further investigate early changes in this subgroup. Furthermore, it would be interesting to investigate connectivity alterations in carriers of the recently discovered *C9orf72* repeat expansion, and compare these with *MAPT* and *GRN* carriers. Finally, cognitive and connectivity changes with higher age are based on cross-sectional data and should be confirmed in follow-up studies.

This study provides evidence that brain pathophysiology is disrupted in FTD years before symptom onset and these alterations in presymptomatic FTD can be measured with DTI and resting-state fMRI. Structural and functional connectivity may have the potential to be developed into sensitive biomarkers in clinical trials in presymptomatic individuals. These measures may also be sensitive to disease progression, a hypothesis we hope to pursue in follow-up studies.

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REFERENCES

1. Neary D, Snowden JS, Gustafson L, et al. Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. Neurology 1998;51:1546-1554.
2. Snowden JS, Gibbons ZC, Blackshaw A, et al. Social cognition in frontotemporal dementia and Huntington's disease. Neuropsychologia 2003;41:688-701.
3. Rascovsky K, Hodges JR, Knopman D, et al. Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. Brain 2011;134:2456-2477.
4. Boccardi M, Sabattoli F, Laakso MP, et al. Frontotemporal dementia as a neural system disease. Neurobiol Aging 2005;26:37-44.
5. Seeley WW, Crawford R, Rascovsky K, et al. Frontal paralimbic network atrophy in very mild behavioral variant frontotemporal dementia. Arch Neurol 2008;65:249-255.
6. Borroni B, Alberici A, Cercignani M, et al. Granulin mutation drives brain damage and reorganization from preclinical to symptomatic FTLD. Neurobiol Aging 2012;33:2506-2520.
7. Whitwell JL, Josephs KA, Avula R, et al. Altered functional connectivity in asymptomatic MAPT subjects: a comparison to bvFTD. Neurology 2011;77:866-874.
8. Rogers BS, Lippa CF. A clinical approach to early-onset inheritable dementia. Am J Alzheimers Dis Other Demen 2012;27:154-161.
9. Cenik B, Sephton CF, Dewey CM, et al. Suberoylanilide hydroxamic acid (vorinostat) up-regulates progranulin transcription: rational therapeutic approach to frontotemporal dementia. J Biol Chem 2011;286:16101-16108.
10. Dejesus-Hernandez M, Mackenzie IR, Boeve BF, et al. Expanded GGGGCC Hexanucleotide Repeat in Noncoding Region of C9ORF72 Causes Chromosome 9p-Linked FTD and ALS. Neuron 2011;72:245-256.
11. Renton AE, Majounie E, Waite A, et al. A Hexanucleotide Repeat Expansion in C9ORF72 Is the Cause of Chromosome 9p21-Linked ALS-FTD. Neuron 2011;72:257-268.
12. Seelaar H, Kamphorst W, Rosso SM, et al. Distinct genetic forms of frontotemporal dementia. Neurology 2008;71:1220-1226.
13. Rohrer JD. Structural brain imaging in frontotemporal dementia. Biochim Biophys Acta 2012;1822:325-332.
14. Borroni B, Brambati SM, Agosti C, et al. Evidence of white matter changes on diffusion tensor imaging in frontotemporal dementia. Arch Neurol 2007;64:246-251.
15. Yoshiura T, Mihara F, Koga H, et al. Cerebral white matter degeneration in frontotemporal dementia detected by diffusion-weighted magnetic resonance imaging. Acad Radiol 2006;13:1373-1378.
16. McMillan CT, Brun C, Siddiqui S, et al. White matter imaging contributes to the multimodal diagnosis of frontotemporal lobar degeneration. Neurology 2012;78:1761-1768.
17. Agosta F, Scola E, Canu E, et al. White Matter Damage in Frontotemporal Lobar Degeneration Spectrum. Cereb Cortex 2012;22:2705-2714.
18. Matsuo K, Mizuno T, Yamada K, et al. Cerebral white matter damage in frontotemporal dementia

assessed by diffusion tensor tractography. Neuroradiology 2008;50:605-611.

19. Whitwell JL, Avula R, Senjem ML, et al. Gray and white matter water diffusion in the syndromic variants of frontotemporal dementia. Neurology 2010;74:1279-1287.
20. Zhang Y, Schuff N, Du AT, et al. White matter damage in frontotemporal dementia and Alzheimers disease measured by diffusion MRI. Brain 2009;132:2579-2592.
21. Larsson EM, Englund E, Sjobeck M, Latt J, Brockstedt S. MRI with diffusion tensor imaging post-mortem at 3.0 T in a patient with frontotemporal dementia. Dement Geriatr Cogn Disord 2004;17:316-319.
22. Borroni B, Alberici A, Premi E, et al. Brain magnetic resonance imaging structural changes in a pedigree of asymptomatic progranulin mutation carriers. Rejuvenation Res 2008;11:585-595.
23. Seeley WW, Crawford RK, Zhou J, Miller BL, Greicius MD. Neurodegenerative Diseases Target Large-Scale Human Brain Networks. Neuron 2009;62:42-52.
24. Seeley WW, Menon V, Schatzberg AF, et al. Dissociable intrinsic connectivity networks for salience processing and executive control. J Neurosci 2007;27:2349-2356.
25. Zhou J, Greicius MD, Gennatas ED, et al. Divergent network connectivity changes in behavioural variant frontotemporal dementia and Alzheimer's disease. Brain 2010;133:1352-1367.
26. Smith SM, Jenkinson M, Woolrich MW, et al. Advances in functional and structural MR image analysis and implementation as FSL. Neuroimage 2004;23 Suppl 1:S208-219.
27. Hornberger M, Geng J, Hodges JR. Convergent grey and white matter evidence of orbitofrontal cortex changes related to disinhibition in behavioural variant frontotemporal dementia. Brain 2011;134:2502-2512.
28. Greicius MD, Krasnow B, Reiss AL, Menon V. Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. Proc Natl Acad Sci U S A 2003;100:253-258.
29. Margulies DS, Kelly AM, Uddin LQ, Biswal BB, Castellanos FX, Milham MP. Mapping the functional connectivity of anterior cingulate cortex. Neuroimage 2007;37:579-588.
30. Verhage F. [Intelligence and age: Research on Dutch people aged twelve to seventy-seven years old]. Assen: van Gorcum 1964.
31. Torralva T, Roca M, Gleichgerrcht E, Bekinschtein T, Manes F. A neuropsychological battery to detect specific executive and social cognitive impairments in early frontotemporal dementia. Brain 2009;132:1299-1309.
32. Geschwind DH, Robidoux J, Alarcon M, et al. Dementia and neurodevelopmental predisposition: cognitive dysfunction in presymptomatic subjects precedes dementia by decades in frontotemporal dementia. Ann Neurol 2001;50:741-746.
33. Haller S, Nguyen D, Rodriguez C, et al. Individual prediction of cognitive decline in mild cognitive impairment using support vector machine-based analysis of diffusion tensor imaging data. J Alzheimers Dis 2010;22:315-327.
34. Song SK, Sun SW, Ju WK, Lin SJ, Cross AH, Neufeld AH. Diffusion tensor imaging detects and differentiates axon and myelin degeneration in mouse optic nerve after retinal ischemia. Neuroimage 2003;20:1714-1722.
35. Song SK, Sun SW, Ramsbottom MJ, Chang C, Russell J, Cross AH. Dysmyelination revealed

through MRI as increased radial (but unchanged axial) diffusion of water. Neuroimage 2002;17:1429-1436.

36. Rohrer JD, Ridgway GR, Modat M, et al. Distinct profiles of brain atrophy in frontotemporal lobar degeneration caused by progranulin and tau mutations. Neuroimage 2010;53:1070-1076.

37. Whitwell JL, Jack CR, Jr., Boeve BF, et al. Voxel-based morphometry patterns of atrophy in FTLD with mutations in MAPT or PGRN. Neurology 2009;72:813-820.

38. Rombouts SA, van Swieten JC, Pijnenburg YA, Goekoop R, Barkhof F, Scheltens P. Loss of frontal fMRI activation in early frontotemporal dementia compared with early AD. Neurology 2003;60:1904-1908.

39. Schmahmann JD, Sherman JC. The cerebellar cognitive affective syndrome. Brain 1998;121 (Pt 4):561-579.

40. Damoiseaux JS, Greicius MD. Greater than the sum of its parts: a review of studies combining structural connectivity and resting-state functional connectivity. Brain Struct Funct 2009;213:525-533.

APPENDIX

SUPPLEMENTARY METHODS

Image acquisition

T1-weighted images were acquired using the following scanning parameters: repetition time (TR) = 9.8ms, echo time (TE) = 4.6ms, flip angle = 8°, 140 slices, voxel size = 0.88x0.88x1.20mm. DTI was performed by means of single-shot echo planar images with gradients applied along 60 non-collinear directions (TR = 8250ms, TE = 80ms, flip angle = 90°, 70 axial slices, voxel size = 2x2x2mm, total scan time = 9 minutes). For resting-state fMRI T2*-weighted images were acquired using whole brain multislice gradient echo planar imaging: 200 volumes, TR = 2200ms, TE = 30ms, flip angle = 80°, 38 axial slices, voxel size = 2.75 x 2.75 x 2.72mm + 10% interslice gap, total scan time = 8 minutes. Participants were instructed to lie still with their eyes closed and not to fall asleep. Finally, for registration purposes a high-resolution echo planar imaging scan (TR = 2200ms, TE = 30ms, flip angle = 80°, 84 slices, voxel size = 1.96 x 1.96 x 2.00mm) was acquired.

Imaging analysis

For VBM analyses T1-weighted images were brain-extracted,[e22] tissue-type segmented,[e23] aligned to MNI-152 (T1 standard brain averaged over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada) standard space,[e24-25] and nonlinearly registered to each other.[e26-27] A study-specific template was created and native grey matter images were non-linearly re-registered to this template. The registered partial volume images were modulated to correct for local expansion or contraction by dividing by the Jacobian of the warp field. These images were smoothed with an isotropic Gaussian kernel with a sigma of 3 mm.[e28-29]

DTI scans were corrected for movement and eddy currents correction by aligning images to the b0 volume using FMRIB Diffusion Toolbox. The diffusion tensor model was fitted at each voxel using DTIFIT to create fractional anisotropy (FA) images. These image were subsequently brain-extracted.[e22] Voxelwise statistical analysis of the FA data was carried out using tract-based spatial statistics.[e30] All FA images were nonlinearly aligned to MNI-152 standard space, using the most representative “target” image.[e26-27] The mean FA image was created from these

individual FA images, which was thinned to create a mean FA skeleton, representing the centres of all white matter tracts common to the group. Individual FA data were then projected onto this skeleton, resulting in skeletonised FA data for each subject, which were fed into voxelwise statistics. Applying spatial transformation parameters that were estimated in the FA analysis similar analyses were run on mean (MD), axial (DA) and radial (DR) diffusivity images.

The following pre-processing steps were applied to resting-state fMRI scans: motion correction using MCFLIRT,[e24] removal of non-brain structures,[e22] spatial smoothing using a Gaussian kernel of 6 mm full-width at half-maximum, grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor, and high pass temporal filtering (0.01Hz cut-off). The fMRI volumes were registered to the individual's high resolution echo planar images, which were registered to the corresponding T1-weighted images, which in turn were registered to MNI-152 standard space images and subsequently registration matrices were combined to transfer fMRI results to MNI space.[e24-25]

SUPPLEMENTARY TABLES

Supplementary table 1. Correlation between neuropsychological performance and age in controls and mutation carriers.

Neuropsychological test	*Controls*			*Mutation carriers*		
	Adjusted R^2	β	*p-value*	*Adjusted R^2*	β	*p-value*
Attention and executive functions						
Digit span	-0.027	0.044	0.798	-0.005	-0.145	0.377
Proverbs	0.067	0.306	0.069	0.121	0.380	**0.017**
Similarities	-0.021	-0.090	0.602	-0.016	-0.101	0.539
TMT A*	0.085	0.334	**0.047**	0.280	0.547	**<0.001**
TMT B*	0.014	0.205	0.230	0.040	0.256	0.115
Stroop I*	0.024	0.227	0.182	-0.001	0.160	0.330
Stroop II*	0.025	0.231	0.176	0.036	0.247	0.129
Stroop III*	0.007	0.188	0.271	0.255	0.524	**0.001**
Categorical fluency	-0.028	0.039	0.820	0.069	-0.305	0.059
Letter fluency	-0.029	0.017	0.920	-0.027	0.006	0.972
WCST concepts	-0.028	0.040	0.817	0.061	-0.292	0.071
LDST	0.044	-0.268	0.114	0.073	-0.312	0.053
Memory						
Orientation	0.018	0.215	0.207	-0.016	-0.101	0.540
RAVLT total	0.100	-0.354	**0.034**	0.187	-0.457	**0.003**
RAVLT recall	0.128	-0.391	**0.018**	0.110	-0.365	**0.022**
RAVLT recognition	-0.015	-0.119	0.490	-0.012	-0.119	0.470
RBMT immediate	-0.005	-0.155	0.366	0.118	-0.376	**0.018**
RBMT delayed	0.031	-0.242	0.155	0.115	-0.372	**0.020**
VAT	0.036	-0.251	0.139	0.004	-0.173	0.292
Language						
BNT	-0.028	-0.031	0.856	-0.026	-0.027	0.872
SAT verbal	0.047	-0.273	0.108	0.051	-0.276	0.089
SAT nonverbal	-0.022	-0.088	0.615	0.039	-0.256	0.126
ScreeLing	-0.029	-0.014	0.935	-0.025	0.044	0.790
Visuospatial						
Royall clock	-0.002	-0.163	0.342	-0.026	-0.023	0.889

Cube copying	-0.023	-0.079	0.649	0.070	0.308	0.057
Block design	0.113	-0.372	**0.026**	-0.004	-0.154	0.362
Social cognition						
Ekman faces	0.049	-0.276	0.103	0.060	-0.292	0.072
Happé Cartoons	0.044	-0.266	0.116	0.104	-0.358	**0.025**
Faux pas	0.104	-0.360	**0.031**	0.048	-0.271	0.095

*Higher test scores represent worse performance.

Supplementary table 2. Clusters of significant correlation between diffusion parameters and age.

Parameter		*Region*	*Size (voxels)*	*x, y, z*	*Minimum p-value*
FA	*Negative age correlation in controls*	Forceps minor	24167	-9, 48, -15	0.001
		Uncinate fasciculus L			
		Inferior fronto-occipital fasciculus L+R			
		Anterior thalamic radiation L+R			
		Forceps major			
		Superior longitudinal fasciculus L+R			
		Body corpus callosum			
		Splenium corpus callosum			
		Cingulum L+R			
		Inferior longitudinal fasciculus L+R			
		Corticospinal tract L+R			
		Superior longitudinal fasciculus R	827	33, 11, 2	0.034
		Inferior fronto-occipital fasciculus R			
		Inferior longitudinal fasciculus R			
		Uncinate fasciculus R			
		Inferior longitudinal fasciculus L	637	-108	0.043
		Inferior fronto-occipital fasciculus L			
		Forceps major			
		Inferior fronto-occipital fasciculus L	427	-36, -38, 3	0.039
		Inferior longitudinal fasciculus L			
		Superior longitudinal fasciculus L			

		Inferior longitudinal fasciculus L	163	-34, -73, 19	0.046
		Inferior fronto-occipital fasciculus L			
		Superior longitudinal fasciculus L	107	-42, -53, 7	0.046
		Inferior longitudinal fasciculus R	57	54, -22, -15	0.047
		Inferior longitudinal fasciculus R	47	28, -18, -6	0.047
		Inferior fronto-occipital fasciculus L	31	-48	0.048
		Superior longitudinal fasciculus L	11	-41, -41, 28	0.048
		Inferior longitudinal fasciculus R	3	43, -22, -16	0.050
	Negative age correlation in carriers	Forceps minor	49417	-9, 54, -13	<0.001
		Superior longitudinal fasciculus L+R			
		Inferior longitudinal fasciculus L+R			
		Cingulum L+R			
		Corticospinal tract L+R			
		Uncinate fasciculus L+R			
		Inferior fronto-occipital fasciculus L+R			
		Anterior thalamic radiation L+R			
		Forceps major			
		Fornix			
		Body corpus callosum			
		Splenium corpus callosum			
MD	*Positive age correlation in controls*	Forceps minor	2032	11, 22, 20	0.024
		Body corpus callosum			
		Anterior thalamic radiation L			
		Inferior fronto-occipital fasciculus L			
	Positive age correlation in carriers	Forceps minor	43853	12, 34, 4	<0.001
		Superior longitudinal fasciculus L+R			
		Inferior longitudinal fasciculus L+R			
		Cingulum L+R			
		Corticospinal tract L+R			
		Uncinate fasciculus L+R			
		Inferior fronto-occipital fasciculus L+R			

		Anterior thalamic radiation L+R			
		Forceps major			
		Fornix			
		Body corpus callosum			
		Splenium corpus callosum			
		Superior longitudinal fasciculus L	30	-41, -58, 17	0.048
DA	*Positive age correlation in carriers*	Superior corona radiate L+R	16507	25, 6, 29	0.002
		Superior longitudinal fasciculus L+R			
		Corticospinal tract L+R			
		Forceps minor			
		Body corpus callosum			
		Splenium corpus callosum			
		Fornix			
		Cingulum L+R			
		Anterior thalamic radiation L+R			
		Inferior fronto-occipital fasciculus L+R			
		Inferior longitudinal fasciculus R			
		Uncinate fasciculus R			
DR	*Positive age correlation in controls*	Forceps minor	12951	4, 26, 13	0.004
		Uncinate fasciculus L			
		Inferior fronto-occipital fasciculus L+R			
		Cingulum L			
		Anterior thalamic radiation L+R			
		Superior longitudinal fasciculus L+R			
		Body corpus callosum			
		Splenium Corpus callosum			
		Corticospinal tract R			
		Superior longitudinal fasciculus R	1904	53, 2, 16	0.030
		Inferior fronto-occipital fasciculus R			
		Anterior thalamic radiation R			

		Inferior fronto-occipital fasciculus R	8	50, 27, 10	0.050
	Positive age correlation in carriers	Forceps minor	54685	-28, 27, -14	<0.001
		Superior longitudinal fasciculus L+R			
		Inferior longitudinal fasciculus L+R			
		Cingulum L+R			
		Corticospinal tract L+R			
		Uncinate fasciculus L+R			
		Inferior fronto-occipital fasciculus L+R			
		Anterior thalamic radiation L+R			
		Forceps major			
		Fornix			
		Body corpus callosum			
		Splenium corpus callosum			

Supplementary table 3. Clusters of significant differences in functional FI, aMCC, and PCC connectivity between mutation carriers and controls and clusters of significant correlation between FI, aMCC, and PCC connectivity and age in mutation carriers.

		Region	*Size (voxels)*	*x, y, z*	*Z-max*
Right FI seed	*Carriers < controls*	Lingual gyrus R	715	12, -82, -6	3.84
		Occipital fusiform gyrus R			
		Lateral occipital cortex R			
Bilateral FI seeds	*Carriers < controls*	Middle temporal gyrus L	546	-106	3.77
		Temporal fusiform cortex L			
		Parahippocampal gyrus L			
		Hippocampus L			
	Negative age correlation in carriers	Frontal pole	1005	-18, 48, 28	3.78
		Paracingulate gyrus			
		Anterior midcingulate gyrus			
		Lateral occipital cortex R	742	28, -64, 30	4.43
		Precuneus			
		Superior parietal lobe R			
		Posterior cingulate cortex			

Left aMCC seed	*Carriers < controls*	Inferior frontal gyrus L	683	-42, 26, 12	4.0
		Insula L			
		Frontal orbital cortex L			
		Frontal operculum cortex L			
		Middle frontal gyrus L			
	Positive age correlation in carriers	Lateral occipital cortex L	1889	-16, -68, 46	4.14
		Postcentral gyrus L			
		Supramarginal gyrus L			
Right aMCC seed	*Carriers < controls*	Superior parietal lobe R	553	28, -24, 32	3.45
		Precuneus			
		Angular gyrus R			
		Lateral occipital cortex R			
		Supramarginal gyrus R			
	Negative age correlation in *carriers*	Lateral occipital cortex L	1287	-14, -74, 50	4.47
		Postcentral gyrus L			
		Superior parietal lobe L			
		Temporal fusiform cortex L	1110	-86	4.17
		Lingual gyrus L			
		Occipital fusiform cortex L			
		Putamen L			
Bilateral aMCC seeds	*Negative age correlation in controls*	Putamen L	832	-58	4.0
		Brainstem			
		Lingual gyrus L			
	Negative age correlation in carriers	Superior frontal gyrus R	1305	22, -2, 68	4.94
		Precentral gyrus			
		Supplementary motor cortex			
Left PPC seed	*Carriers < controls*	Cerebellum	449	-78	3.7
Right PCC seed	*Carriers > controls*	Cerebellum	491	-78	3.75

L, left; R, right

SUPPLEMENTARY REFERENCES

e1. Folstein MF, Folstein SE, McHugh PR. “Mini-mental state”. A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189-198.

e2. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. Arch Gen Psychiatry 1961;4:561-571.

e3. Spielberger CD, Gorssuch RL, Lushene PR, Vagg PR, Jacobs GA. Manual for the State-Trait Anxiety Inventory (Form Y). Palo Alto, CA: Consulting psychologists press 1983.

e4. Rey A. L’examen clinique en psychologie. Paris, France: Presses Universitaires de France 1958.

e5. Wilson BA, Cockburn J, Baddeley A. The Rivermead Behavioural Memory Test. Bury St Edmunds, UK: Thames Valley Test Company 1985.

e6. Lindeboom J, Schmand B, Tulner L, Walstra G, Jonker C. Visual association test to detect early dementia of the Alzheimer type. J Neurol Neurosurg Psychiatry 2002;73:126-133.

e7. Wechsler D. WAIS-III administration and scoring manual. San Antonio, TX: The Psychological Corporation 1997.

e8. Wechsler D. WAIS-III Nederlandse Bewerking. Technische handleiding. Lisse: Harcourt Test Publishers 2005.

e9. Army Individual Test Battery. Manual of directions and scoring. Washington, DC: War Department, Adjutant General’s office 1994.

e10. Stroop JR. Studies of interference in serial verbal reactions. Journal of Experimental Psychology 1935;18:643-662.

e11. Thurstone LL, Thurstone TG. Primary mental abilities. Chicago: Science Research Associates 1962.

e12. Nelson HE. A modified card sorting test sensitive to frontal lobe defects. Cortex 1976;12:313-324.

e13. Jolles J, Houx PJ, van Boxtel MPJ, Ponds RWHM. Maastricht Aging Study: determinants of cognitive aging. Maastricht, The Netherlands: Neuropsych publishers 1995.

e14. Kaplan E, Goodglass H, Weintraub S. The Boston Naming Test. Philadelphia: Lea & Febiger 1978.

e15. Visch-Brink E, Stronks D, Denes G. SAT: Semantische Associatie Test. Lisse: Swets & Zeitlinger 2005.

e16. Doesborgh SJ, van de Sandt-Koenderman WM, Dippel DW, van Harskamp F, Koudstaal PJ, Visch-Brink EG. Linguistic deficits in the acute phase of stroke. J Neurol 2003;250:977-982.

e17. Royall DR, Cordes JA, Polk M. CLOX: an executive clock drawing task. J Neurol Neurosurg Psychiatry 1998;64:588-594.

e18. Maeshima S, Osawa A, Maeshima E, et al. Usefulness of a cube-copying test in outpatients with dementia. Brain Inj 2004;18:889-898.

e19. Ekman P, Friesen WV. Pictures of facial affect. Palo Alto, CA: Consulting psychologists press 1976.

e20. Happe F, Brownell H, Winner E. Acquired ‘theory of mind’ impairments following stroke. Cognition 1999;70:211-240.

e21. Stone VE, Baron-Cohen S, Calder A, Keane J, Young A. Acquired theory of mind impairments in

individuals with bilateral amygdala lesions. Neuropsychologia 2003;41:209-220.

e22. Smith SM. Fast robust automated brain extraction. Hum Brain Mapp 2002;17:143-155.

e23. Zhang Y, Brady M, Smith S. Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. IEEE Trans Med Imaging 2001;20:45-57.

e24. Jenkinson M, Bannister P, Brady M, Smith S. Improved optimization for the robust and accurate linear registration and motion correction of brain images. Neuroimage 2002;17:825-841.

e25. Jenkinson M, Smith S. A global optimisation method for robust affine registration of brain images. Med Image Anal 2001;5:143-156.

e26. Andersson JLR, Jenkinson M, Smith S. Non-linear optimisation. FMRIB technical report TR07JA1 from www.fmrib.ox.ac.uk/analysis/techrep 2007.

e27. Andersson JLR, Jenkinson M, Smith S. Non-linear registration, aka Spatial normalisation FMRIB technical report TR07JA2 from www.fmrib.ox.ac.uk/analysis/techrep 2007.

e28. Smith SM, Nichols TE. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. Neuroimage 2009;44:83-98.

e29. Worsley KJ. Statistical analysis of activation images. Ch 14, in Functional MRI: An Introduction to Methods, eds P Jezzard, PM Matthews and SM Smith OUP, 2001 2001.

e30. Smith SM, Jenkinson M, Johansen-Berg H, et al. Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. Neuroimage 2006;31:1487-1505.

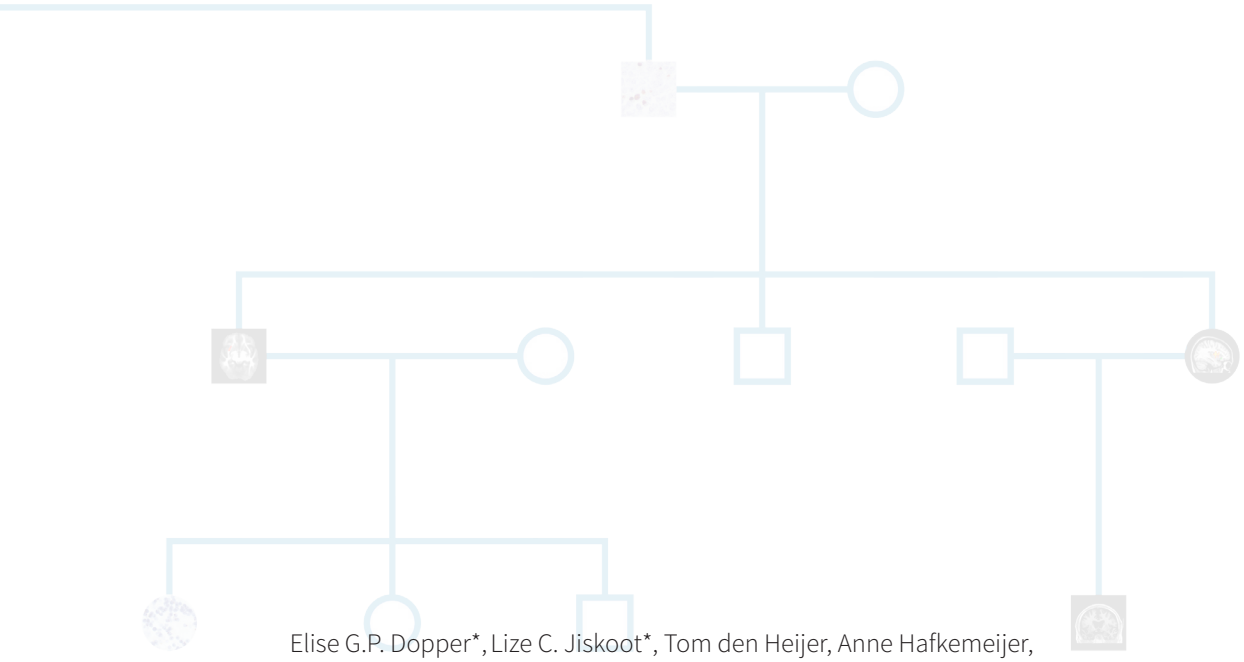
e31. Mori S, Wakana S, Nagae-Poetscher L, van Zijl P. MRI atlas of human white matter. Elsevier 2005.

e32. Troyes AK, Cullum CM, Smernoff EN, Kozora E. Age effects on Block Design : qualitative performance features and extended-time effects. Neuropsychology 1994;8:95-99

e33. Kaufman AS. Assessing Adolescent and Adult Intelligence. Allyn & Bacon, Boston 1990.

CHAPTER 3.2

Longitudinal brain changes in presymptomatic familial frontotemporal dementia



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Submitted.

ABSTRACT

Objective: Frontotemporal dementia (FTD) can be caused by mutations in *microtubule-associated protein tau* (*MAPT*), *progranulin* (*GRN*), or *C9orf72*. In light of upcoming trials with disease-modifying agents, sensitive biomarkers to detect early FTD and track disease progression are crucial. Here we investigated neuropsychological and imaging alterations over two years in a cohort of presymptomatic mutation carriers.

Methods: Healthy carriers of *MAPT* or *GRN* mutations and related non-carriers underwent neuropsychological assessment and MRI at baseline and two years later in this longitudinal case-control study. Grey matter volume, white matter integrity, and functional connectivity were analyzed using voxel-based morphometry, tract-based spatial statistics, and seed-based analyses, respectively.

Results: Longitudinal analyses revealed a significant decline in executive and social cognition tasks, stronger right insular atrophy, and stronger white matter impairment in the uncinate fasciculus over time in carriers within five years before estimated symptom onset compared to controls. Two mutation carriers converted to clinical FTD during follow-up, and showed the strongest white matter impairment already at baseline and the most severe grey matter atrophy over time. Carriers differed from controls regarding longitudinal alterations in frontoinsula and posterior cingulate functional connectivity.

Conclusions: We demonstrated longitudinal changes in neuropsychological test performance, grey matter volume, white matter integrity, and functional connectivity in presymptomatic FTD. This study suggests that DTI might provide a baseline predictor for disease conversion, and that the first DTI changes might serve as a sensitive biomarker for future therapeutic trials.

INTRODUCTION

Frontotemporal dementia (FTD) is a neurodegenerative disorder, characterized by behavioral and language problems.[1] Autosomal dominant forms are caused by mutations in the *microtubule-associated protein tau* (*MAPT*), *progranulin* (*GRN*), and *C9orf72* genes.[2-4] Improved insight into the disease process enables the development of disease-modifying agents.[5] The presymptomatic stage offers a unique time window to evaluate such agents at a time of minimal brain damage.[6] Therefore, sensitive biomarkers for presymptomatic FTD are crucial.

Various MRI techniques are increasingly recognized as potential biomarkers in the earliest stage of FTD. First, a recent international cross-sectional study of familial FTD showed grey matter (GM) atrophy approximately ten years before expected symptom onset.[6] Second, frontotemporal white matter (WM) impairment is consistently detected in clinical and presymptomatic FTD using diffusion tensor imaging (DTI),[7-13] and was shown to be more widespread than GM atrophy.[7, 8, 12] Third, reduced functional connectivity within the salience network was found using resting-state fMRI in FTD[14-20] and presymptomatic mutation carriers.[9] Therefore, these imaging techniques might provide sensitive biomarkers for future clinical trials. However, longitudinal studies of these MRI techniques in presymptomatic FTD are lacking to date.

In the current study we examine longitudinal alterations in neuropsychological performance, GM volume, WM integrity, and functional connectivity in presymptomatic *MAPT* or *GRN* mutation carriers. The objectives of the study are twofold: (1) to determine the predictive value of baseline measures for conversion to clinical FTD, and (2) to investigate longitudinal alterations in mutation carriers compared to controls and their relationship with expected symptom onset.

METHODS

Subjects

Healthy first-degree relatives of patients with FTD due to a *MAPT* or *GRN* mutation underwent extensive neuropsychological assessment (see Appendix) and MRI at baseline (May 2011-March 2011) and two-year follow-up (June 2012-April 2013).

DNA of all subjects was screened for *MAPT* and *GRN* mutations at baseline,[4] resulting in a group of presymptomatic mutation carriers and a control group. We had longitudinal neuropsychological data for 77, T1-weighted images for 74, DTI for 64, and resting-state fMRI for 70 participants (see Appendix). For all measures we investigated differences between carriers and controls in change over time, group differences in association with expected onset age, and we evaluated single-subject data. Expected onset age (AAO) was defined as the mean AAO of all affected persons per family (Figure 1). Participants were categorized into four groups: less than five ($n = 32$), five to ten ($n = 17$), ten to fifteen ($n = 14$), or more than fifteen ($n = 15$) years younger than their expected AAO. Conversion to symptomatic FTD was defined as the presence of behavioral or language symptoms as reported by informants in combination with cognitive deficits in at least two cognitive domains at neuropsychological assessment.

Standard protocol approvals, registrations, and patient consents

All participants provided written informed consent. Approval for the study was obtained from the local ethics committee.

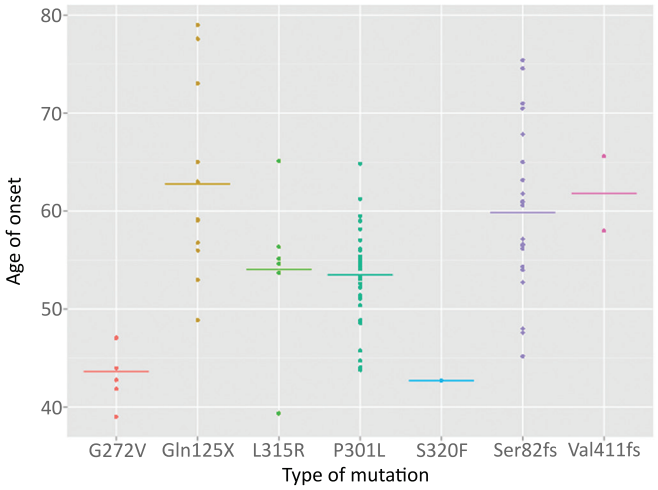

Figure 1. Age of symptom onset in *MAPT* and *GRN* mutation carriers. Age of symptom onset for affected family members of the current study participants per family. *MAPT* mutations: G272V, L315R, P301L, S320F; *GRN* mutations: Gln125X, Ser82fs, Val411fs.

Image acquisition and analyses

We acquired baseline and follow-up whole-brain anatomical, DTI, and resting-state fMRI scans.[9] We used FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk) for all imaging analyses.[e1] T1-weighted scans were preprocessed using FSL-VBM.[9] Individual maps of DTI measures fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (DA), and radial diffusivity (DR) were acquired using tract-based spatial statistics.[9] Follow-up GM volume images and DTI maps were subtracted from their baseline maps, yielding images of change over time for each participant. Longitudinal comparisons between carriers and controls were performed by means of permutation-based testing using 5,000 permutations, applying a two-sample *t*-test with age and gender as covariates (statistical threshold $p < 0.05$, corrected for multiple comparisons using family-wise error corrected threshold-free cluster enhancement).[e2] Subsequently, we extracted GM volume per subject for the bilateral insula, anterior (ACC) and posterior cingulate cortex (PCC), according to the Harvard-Oxford cortical structural atlas, and mean individual DTI values for the bilateral uncinate fasciculus and forceps minor and major using the JHU DTI-based WM atlas to investigate group differences in association with expected AAO, and to explore individual data.[e3-9] For resting-state fMRI analyses, we used a seed-based approach in FMRI expert analysis tool (FEAT) using seeds in the anterior midcingulate cortex (aMCC), frontoinsula and PCC.[9] For group comparisons of functional connectivity change over time, we used a full paired *t*-test design with age, gender, and voxel-wise GM volume as covariates. *Z*-statistic images were thresholded using clusters determined by an initial cluster-forming threshold $Z > 2.3$ and a corrected cluster significance threshold of $p < 0.05$.[e10] Subsequently, we investigated group differences in association with estimated AAO, and at the individual level.

Statistical analyses

We analyzed demographic features using independent samples *t*-tests and Pearson χ^2 tests in SPSS 21.0 for Windows (SPSS, Chicago, IL), applying a significance level of $p < 0.05$. Raw baseline and follow-up neuropsychological test results were analyzed using analyses of covariance, with age, gender, and level of education[e11] as covariates. Repeated measures analyses of covariance, with similar covariates, were used for the longitudinal neuropsychological assessments. Group differences with regard to estimated AAO were analyzed using independent samples *t*-tests.

RESULTS

Demographic features

Mutation carriers (n = 40) and controls (n = 37) did not differ in age, gender, interval between baseline and follow-up visits, level of education, and scores on Mini-Mental State Examination and Beck Depression Inventory (Table 1). The mean baseline age was 53.6 years for *GRN*, and 43.5 years for *MAPT* carriers, which was respectively 7.2 ± 7.5 and 6.7 ± 10.0 years younger than their expected AAO. Nine mutation carriers (six *GRN,* three *MAPT*) were already older (range 0.4 - 14.8 years) than their expected AAO at baseline.

Neuropsychological assessment

Supplementary table 1 shows raw baseline and follow-up neuropsychological results. At both baseline (p = 0.047) and follow-up (p = 0.007), carriers performed worse on the LDST than controls. According to their relatives, one *MAPT* and one *GRN* carrier converted into symptomatic FTD during follow-up. The *MAPT* carrier presented with disinhibition, and the *GRN* carrier with apathy and loss of initiative. Their follow-up neuropsychological assessments confirmed the diagnosis of behavioral variant of FTD. The *MAPT* converter declined significantly on tests for divided attention, emotion recognition (all ≥ 2 standard deviations (SD) below the group mean), executive function, theory-of-mind, fluency, and abstract reasoning (all ≥ 1 SD below mean). The *GRN* converter showed a significant decline concerning tests for divided attention, executive function, emotion recognition (all ≥ 2 SD below mean), fluency, abstract reasoning, and perceptual organization (all ≥ 1 SD below mean).

Longitudinal group differences between carriers and controls were found for Happé cartoons total (p = 0.047) and non-theory of mind (p = 0.025). Carriers < 5 years to their expected AAO showed a significant decline over time on WCST concepts (p = 0.044), Ekman faces 'fear' (p = 0.019) and Happé non-theory of mind (p = 0.022), whereas controls significantly improved. This remained significant for Ekman 'fear' (p = 0.045) after excluding the two converters. The other subgroups showed no significant longitudinal differences between carriers and controls.

Table 1. Demographic features

	Neuropsychological data available			*T1-weighted MRI scans available*			*DTI scans available*			*Resting-state fMRI scans available*		
	Controls (n=37)	*Carriers (n=40)*	*p-value*	*Controls (n=34)*	*Carriers (n=40)*	*p-value*	*Controls (n=31)*	*Carriers (n=33)*	*p-value*	*Controls (n=32)*	*Carriers (n=38)*	*p-value*
Age t1, y	50.0±11.5	50.6±10.3	0.812	50.7±11.3	50.3±9.8	0.858	51.4±10.4	49.7±9.9	0.506	51.3±10.3	50.4±10.0	0.710
Interval t1-t2, y	2.4±0.2	2.4±0.3	0.928	2.3±0.1	2.2±0.1	0.390	2.3±0.1	2.2±0.1	0.499	2.3±0.1	2.2±0.1	0.400
Years from expected symptom onset t1, y	8.1±9.4	6.8±8.2	0.534	7.5±8.9	7.3±8.1	0.900	7.1±8.8	8.0±7.6	0.654	7.3±8.8	7.0±8.2	0.884
Females	54%	58%	0.761	50%	60%	0.388	52%	58%	0.632	53%	61%	0.533
GRN mutation†	-	70%	-	-	70%	-	-	73%	-	-	68%	-
Level of education‡	5.2±1.1	5.6±1.0	0.082	5.2±1.1	5.6±1.0	0.078	5.2±1.0	5.6±1.1	0.152	5.2±1.0	5.6±1.0	0.146
MMSE score t2	29.2±1.3	28.8±1.8	0.282	29.2±1.3	28.8±1.8§	0.355	29.1±1.4	28.9±1.8	0.529	29.2±1.3	28.8±1.8	0.354
MMSE Δt1-t2	-0.1±1.3	0.3±1.8	0.153	-0.2±1.3	0.5±1.6§	0.068	-0.1±1.4	0.4±1.5	0.147	-0.1±1.4	0.5±1.6	0.096
BDI score t2	3.7±4.0	3.1±4.0	0.476	3.4±3.5	3.1±4.1§	0.735	3.5±3.6	3.0±4.1	0.641	3.4±3.6	3.2±4.1	0.817
BDI Δt1-t2	0.1±3.6	0.3±3.1	0.776	0.4±3.4	0.3±3.1§	0.918	-0.3±2.3	0.6±2.9	0.192	0.0±2.8	0.3±3.1	0.746

Values denote mean ± SD or percentage of subjects. †Remaining mutation carriers have a *microtubule-associated protein tau* (*MAPT*) mutation. ‡Level of education was determined on a Dutch 7-point scale ranging from 1 (less than elementary school) to 7 (university or technical college).[e11] §MMSE and BDI scores are missing in one subject.

Voxel-based morphometry

Mutation carriers < 5 years prior to their expected AAO showed a significantly stronger longitudinal decline in right insula volume ($p = 0.025$), and a significantly smaller right insular volume at follow-up ($p = 0.025$) than age-matched controls. Unexpectedly, carriers < 15 years prior to expected AAO had larger ACC volumes than controls at baseline and follow-up ($p = 0.06$ and $p = 0.023$, respectively), and PCC volume was larger in carriers 5 - 10 and < 15 years younger than their estimated AAO than controls ($p = 0.039$ and $p = 0.032$) at baseline. There were no significant longitudinal differences between the total group of carriers and controls.

The two converted carriers, together with one asymptomatic carrier, showed the lowest right insula volume at baseline (≥ 2 SD below mean), and one also had the lowest ACC volume (> 3 SD below mean) (Figure 2A). Moreover, both converters showed the strongest GM volume decline in the right insula, ACC, and PCC during follow-up (≥ 2 SD above mean), and the lowest volumes in these areas at follow-up (≥ 2 SD below mean). Without the two converters, the stronger longitudinal right insular atrophy was still significant ($p = 0.033$), with a trend for smaller right insula volume at follow-up ($p = 0.066$).

Structural connectivity

DR within the left uncinate fasciculus increased significantly more in carriers < 5 years of their estimated AAO than controls ($p = 0.039$), whereas the total group of carriers showed no longitudinal difference compared to controls. Carriers < 5 years prior to their estimated AAO also showed significantly lower FA ($p = 0.043$) and higher DR ($p = 0.040$) in the right uncinate fasciculus at baseline and significantly higher DA ($p = 0.036$) and MD ($p = 0.035$) in the forceps minor at follow-up.

The two converters showed the lowest FA measures at baseline in the bilateral uncinate fasciculi and the forceps minor; this was most apparent for the right uncinate fasciculus (> 3 SD below mean) (Figure 2B). At follow-up, these differences were even more pronounced (> 3 SD below mean for all abovementioned tracts) for one of the converters (follow-up DTI missing for the other converter due to premature termination of the scanning session). The other DTI measures showed similar individual findings for the right uncinate fasciculus and forceps minor, albeit less clear-cut than for FA. Without the two converters, the abovementioned

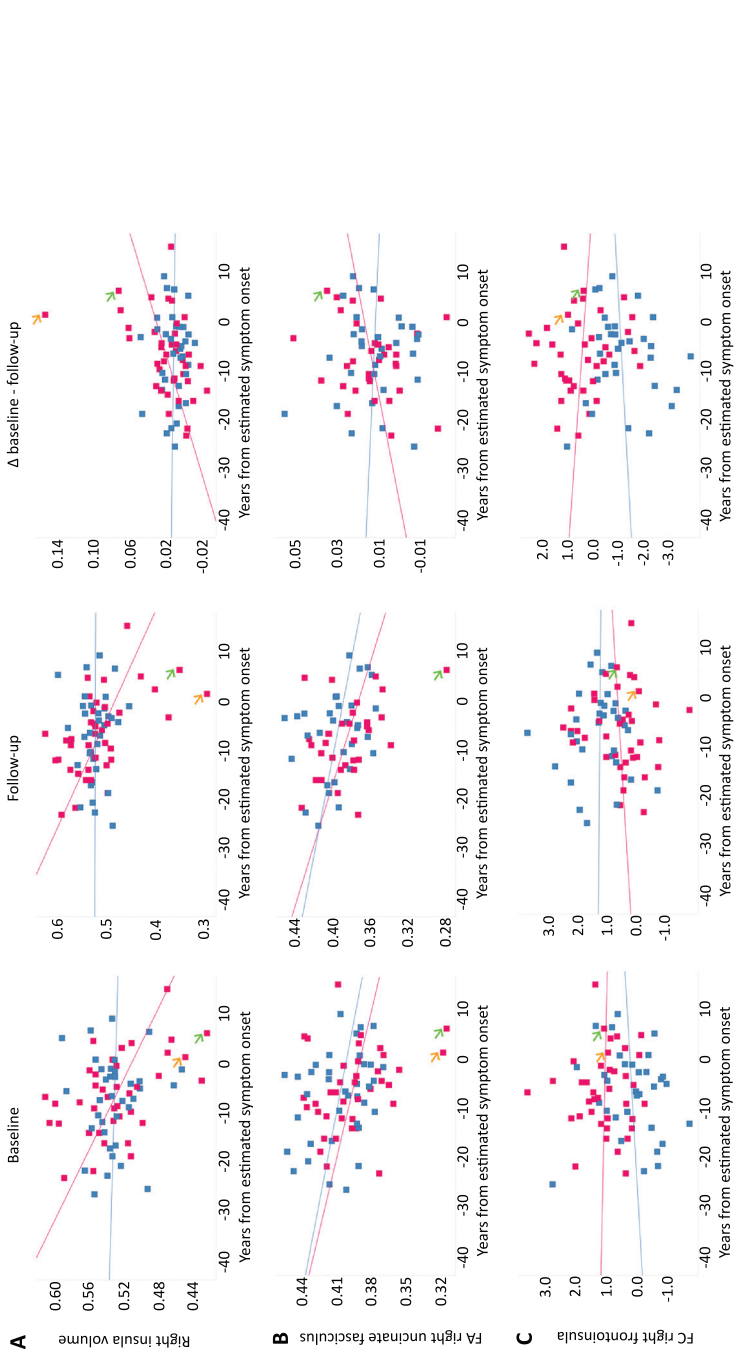

Figure 2. Individual values of GM volume in the right insula (A), fractional anisotropy in the right uncinate fasciculus (B), and functional connectivity between the right frontoinsula and right dorsal insula and surrounding regions (C) at baseline and follow-up and change over time in these values during the follow-up period. Pink squares represent mutation carriers, blue squares are controls. The yellow arrows points to the converted subject with a *MAPT* mutation (no follow-up DTI scan available due to premature termination of the MRI session) and the green arrow to the converter with a *GRN* mutation.

group differences were no longer significant, although there were still trends for stronger longitudinal DR increase in the left uncinate fasciculus and higher DA (p = 0.075) and MD (p = 0.092) in the forceps minor at follow-up (p = 0.080).

Functional connectivity

The total group of carriers showed a stronger longitudinal decrease in functional right frontoinsula connectivity with the right dorsal insula and surrounding structures compared with controls (Figure 3A, Table 2). This difference was significant for all carriers < 15 years before their estimated AAO (< 5 years p = 0.003; 5 - 10 years p = 0.015, 10 - 15 years p = 0.018). Conversely, carriers showed significantly less decrease in frontoinsula connectivity with left subcortical structures compared with controls, which also was significant for all carriers < 15 years prior to estimated AAO (< 5 years p = 0.004; 5 - 10 years p = 0.025, 10 - 15 years p = 0.04). We found no group differences in longitudinal aMCC seed connectivity. PCC connectivity with the right parietal cortex and frontoinsula connectivity with left subcortical structures showed a significantly stronger longitudinal decline in controls than carriers, (Figure 3B, Table 2), which was most significant for subjects < 5 years before their estimated AAO (p < 0.001).

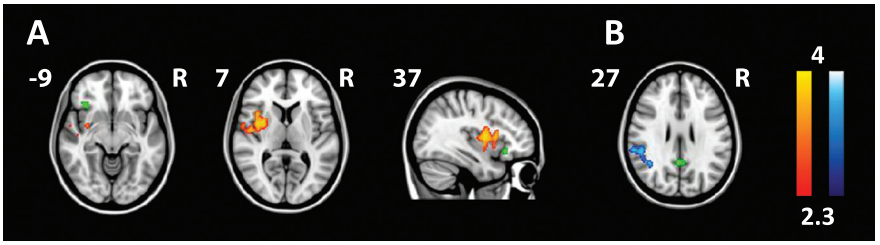

Figure 3. Longitudinal differences in functional connectivity between mutation carriers and controls. Maps illustrate clusters of significantly stronger decline in right frontoinsula (seed in green) connectivity (red-yellow) over time in mutation carriers compared to controls (A) and, significantly stronger decline in bilateral PCC (seeds in green) connectivity (blue-light blue) over time in controls compared to mutation carriers (B). Color bar represents Z-scores.
The abovementioned differences were not driven by the values of the two converters, as their functional connectivity values cannot be distinguished from those of other subjects at any time point (Figure 2C), and excluding them resulted in identical findings.

MAPT and *GRN* subgroups

Separate analyses for *MAPT* and *GRN* mutation carriers revealed no significant

Table 2. Clusters of significant differences between mutation carriers and controls in change in functional frontoinsula, aMCC, and PCC connectivity over two years of time.

	Decrease in functional connectivity over time	*Region*	Size (voxels)	*x, y, z*	*Z-max*
Left FI seed		None			
Right FI seed	*Carriers > controls*	Insula R	739	32 ,2, 12	4.06
		Heschl's gyrus R			
		Planum tempolare R			
		Putamen R			
		Frontal operculum cortex R			
		Central opercular cortex R			
Bilateral FI seeds	*Controls > carriers*	Pallidum L	634	-12, 0, -12	3.7
		Putamen L			
		Thalamus R			
		Hippocampus L			
Left aMCC seed		None			
Right aMCC seed		None			
Bilateral aMCC seeds		None			
Left PPC seed		None			
Right PCC seed		None			
Bilateral PCC seeds	*Controls > carriers*	Supramarginal gyrus R	561	50, -38, 32	3.78
		Parietal operculum cortex R			
		Angular gyrus R			

L, left; R, right.

differences compared to controls in grey matter volume, white matter integrity or functional connectivity.

DISCUSSION

In this first longitudinal neuropsychological and MRI study in presymptomatic FTD we found 1) a decline in complex executive functioning and social cognition in carriers < 5 years before their estimated AAO; 2) a stronger right insular GM loss over time in this same group, most pronounced in the two mutation carriers who converted to clinical FTD during follow-up; 3) a stronger longitudinal WM integrity reduction of the left uncinate fasciculus, and right uncinate fasciculus impairment at baseline in carriers < 5 years prior to AAO, most severe in the two converters already at baseline; 4) longitudinal frontoinsula and PCC functional connectivity differences in the total group.

In line with previous research showing neuropsychometric changes before clinical disease onset,[6, 9, 21-25] the current presymptomatic carriers showed a significant decline over time in social cognition and complex executive functions. Impairments in these domains are consistent findings in symptomatic FTD, and are thought to underlie core diagnostic behavioral features.[26, 27] Our finding of the most marked changes < 5 years before clinical onset corresponds to recent results from a large international study,[6] and underlines the notion that detection of social and executive deficits is particularly useful for diagnosis during the earliest disease stages,[28] and that these tasks should be incorporated in the standard neuropsychological battery. This is the first study investigating social cognitive dysfunction in presymptomatic FTD. Evidence of early deficits in emotion recognition has also been found in preclinical Huntington's disease (HD), showing disproportionate impairment in recognition of negative emotions.[29] Consistent with the present results, poorer recognition of fearful faces was found in HD gene carriers, which has been associated with right insular atrophy, confirming the role of the insula in emotion recognition.[30, 31]

The finding of longitudinal atrophic changes in the right insula in carriers < 5 years before AAO is in line with disease-specific atrophy in presymptomatic Alzheimer's disease and HD.[32, 33] The insula plays an important role in cognition, emotion

regulation and behavior, and serves as a major hub within the salience network, which is affected in early FTD.[14-20] Moreover, the insula was recently found as the first atrophied region in asymptomatic FTD carriers ten years before symptom onset.[6] Interestingly, we found the strongest decline in insula volume in the two converters, indicating that after very subtle insular atrophy occurring years before symptom onset, atrophy strongly accelerates shortly before clinical disease manifestation, in line with findings in presymptomatic HD.[33]
The finding of the strongest ACC and PCC atrophy in the converters is in line with the study by Rohrer *et al*,[6] showing that cingulate atrophy starts around symptom onset. The ACC has consistently been found to be affected early in FTD, whereas PCC atrophy has mainly been associated with *GRN* and not *MAPT* mutations.[34] However, our study shows very early PCC atrophy in both subgroups.

Our observation of longitudinal WM integrity loss in the left uncinate fasciculus in carriers < 5 years before expected onset corresponds to previous findings that uncinate fasciculus impairment most accurately differentiates FTD patients from controls.[7] This tract connects the orbitofrontal cortex and anterior temporal lobe, and is involved in several aspects of memory, language, and social-emotional functioning.[35] The finding of the lowest FA values in bilateral uncinate fasciculi and forceps minor at baseline in the two converters is of particular interest. As none of the other carriers showed similar values, and subgroup differences were no longer significant without the converters, these changes probably shortly precede the onset of symptoms. This implies that DTI could provide an individual baseline predictor of symptom onset.

Our baseline study revealed widespread WM impairment in *MAPT* carriers, and no significant differences in *GRN* carriers, leading us to suggest possible mutation-specific damage.[9] However, based on the lack of differences in the current subgroup analyses, and the individual findings, we now assume that our baseline results in the smaller *MAPT* subgroup were largely driven by the converted carrier. Future studies are needed to define the mutation-specific distribution of DTI changes.

The present DTI results are similar to findings of presymptomatic WM changes in Alzheimer's disease and HD,[33, 36, 37] although DTI changes in AD and HD were not

found to have predictive value regarding disease conversion, as in our study. Axonal damage secondary to neuronal loss has been suggested to explain DTI changes in FTD. However, strongly reduced WM integrity at baseline in our converters, in absence of apparent GM atrophy suggests that WM is primarily involved in the pathological process. Accordingly, WM impairment has consistently been shown to exceed the distribution of GM atrophy in FTD.[7, 8, 12] Therefore, it can be assumed that DTI alterations directly reflect pathological changes within the WM, consistent with previous pathological studies.[7, 38, 39]

Our finding of reduced longitudinal functional connectivity between the right frontoinsula and temporal regions corroborates previous findings of salience network impairment in FTD.[14-20] This finding was significant for carriers < 15 years before symptom onset, underlining that this network is affected at a very early stage. Reduced salience network connectivity is highly consistent across sporadic and genetic FTD and has been shown to correlate with disease severity.[15, 17] However, the fact that functional connectivity values of the two converters were not distinct from the other carriers may indicate that the decline in salience network connectivity starts long before symptom onset, but does not clearly accelerate when approaching symptom onset. Increased connectivity between the PCC and right parietal lobe corresponds to the finding of enhanced default mode network connectivity in FTD patients, although this finding was less consistent across studies.[14, 16-19]

The question whether the FTD disease process has a gradual progression, or a more explosive nature of neurodegeneration, is still subject to debate. The current resting-state fMRI results favor the former, since differences were already found fifteen years before expected symptom onset. However, the strong decline in WM integrity and GM volume in the two converters is suggestive of a more explosive disease acceleration when approaching symptom onset. This information is important for future clinical trials, in which VBM and DTI could be used to measure effects of therapy at the individual level in the earliest phase of FTD. Moreover, DTI seems to provide a predictor of conversion into symptomatic disease. Therefore, the time at which detectable DTI changes appear, might be the ideal time point to start a therapy in presymptomatic carriers in order to halt further neurodegeneration and thereby prevent clinical disease manifestation.

Strengths of the current study include the longitudinal design and the multimodal approach. An important methodological issue is the use of mean onset age within a family as expected AAO. One might question whether this provides more predictive value with regards to symptom onset than current age, as AAO largely varies within families: some children were even affected before their parents, and several of the current healthy carriers are already older than expected AAO. A major drawback of this study is the low number of converters, hampering sample-size estimation for therapeutic trials. Our ongoing follow-up study will probably enable this and allow us to draw firmer conclusions about the current findings. Finally, the current subgroups were too small to define mutation-specific presymptomatic changes. It will be interesting to investigate this in larger future studies, also including *C9orf72* carriers.

To conclude, in this study we found presymptomatic brain changes using VBM, DTI, and resting-state fMRI. Our data suggest that DTI might provide a sensitive predictor for conversion into symptomatic FTD, and that VBM and DTI can detect disease progression in the presymptomatic disease stage at the individual level, thereby providing powerful biomarkers for therapy-effects in future clinical trials.

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REFERENCES

1. Seelaar H, Rohrer JD, Pijnenburg YA, Fox NC, van Swieten JC. Clinical, genetic and pathological heterogeneity of frontotemporal dementia: a review. J Neurol Neurosurg Psychiatry 2011;82:476-486.
2. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron 2011;72:245-256.
3. Renton AE, Majounie E, Waite A, et al. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron 2011;72:257-268.
4. Seelaar H, Kamphorst W, Rosso SM, et al. Distinct genetic forms of frontotemporal dementia. Neurology 2008;71:1220-1226.
5. Tsai RM, Boxer AL. Treatment of frontotemporal dementia. Curr Treat Options Neurol 2014;16:319.
6. Rohrer JD, Nicholas JM, Cash DM, et al. Presymptomatic cognitive and neuroanatomical changes in genetic frontotemporal dementia in the Genetic Frontotemporal dementia Initiative (GENFI) study: a cross-sectional analysis. Lancet Neurol 2015;14:253-262.
7. Agosta F, Scola E, Canu E, et al. White matter damage in frontotemporal lobar degeneration spectrum. Cereb Cortex 2012;22:2705-2714.
8. Borroni B, Alberici A, Premi E, et al. Brain magnetic resonance imaging structural changes in a pedigree of asymptomatic progranulin mutation carriers. Rejuvenation Res 2008;11:585-595.
9. Dopper EG, Rombouts SA, Jiskoot LC, et al. Structural and functional brain connectivity in presymptomatic familial frontotemporal dementia. Neurology 2014;83:e19-26.
10. Zhang Y, Tartaglia MC, Schuff N, et al. MRI signatures of brain macrostructural atrophy and microstructural degradation in frontotemporal lobar degeneration subtypes. J Alzheimers Dis 2013;33:431-444.
11. Lam BY, Halliday GM, Irish M, Hodges JR, Piguet O. Longitudinal white matter changes in frontotemporal dementia subtypes. Hum Brain Mapp 2014;35:3547-3557.
12. Mahoney CJ, Ridgway GR, Malone IB, et al. Profiles of white matter tract pathology in frontotemporal dementia. Hum Brain Mapp 2014;35:4163-4179.
13. Borroni B, Brambati SM, Agosti C, et al. Evidence of white matter changes on diffusion tensor imaging in frontotemporal dementia. Arch Neurol 2007;64:246-251.
14. Farb NA, Grady CL, Strother S, et al. Abnormal network connectivity in frontotemporal dementia: evidence for prefrontal isolation. Cortex 2013;49:1856-1873.
15. Lee SE, Khazenzon AM, Trujillo AJ, et al. Altered network connectivity in frontotemporal dementia with C9orf72 hexanucleotide repeat expansion. Brain 2014;137:3047-3060.
16. Whitwell JL, Josephs KA, Avula R, et al. Altered functional connectivity in asymptomatic MAPT subjects: a comparison to bvFTD. Neurology 2011;77:866-874.
17. Zhou J, Greicius MD, Gennatas ED, et al. Divergent network connectivity changes in behavioural variant frontotemporal dementia and Alzheimer's disease. Brain 2010;133:1352-1367.

18. Borroni B, Alberici A, Cercignani M, et al. Granulin mutation drives brain damage and reorganization from preclinical to symptomatic FTLD. Neurobiol Aging 2012;33:2506-2520.

19. Agosta F, Sala S, Valsasina P, et al. Brain network connectivity assessed using graph theory in frontotemporal dementia. Neurology 2013;81:134-143.

20. Filippi M, Agosta F, Scola E, et al. Functional network connectivity in the behavioral variant of frontotemporal dementia. Cortex 2013;49:2389-2401.

21. Hallam BJ, Jacova C, Hsiung GY, et al. Early neuropsychological characteristics of progranulin mutation carriers. J Int Neuropsychol Soc 2014;20:694-703.

22. Janssen JC, Schott JM, Cipolotti L, et al. Mapping the onset and progression of atrophy in familial frontotemporal lobar degeneration. J Neurol Neurosurg Psychiatry 2005;76:162-168.

23. Barandiaran M, Estanga A, Moreno F, et al. Neuropsychological features of asymptomatic c.709-1G>A progranulin mutation carriers. J Int Neuropsychol Soc 2012;18:1086-1090.

24. Geschwind DH, Robidoux J, Alarcon M, et al. Dementia and neurodevelopmental predisposition: cognitive dysfunction in presymptomatic subjects precedes dementia by decades in frontotemporal dementia. Ann Neurol 2001;50:741-746.

25. Rohrer JD, Warren JD, Barnes J, et al. Mapping the progression of progranulin-associated frontotemporal lobar degeneration. Nat Clin Pract Neurol 2008;4:455-460.

26. Kipps CM, Hodges JR. Theory of mind in frontotemporal dementia. Soc Neurosci 2006;1:235-244.

27. Torralva T, Roca M, Gleichgerrcht E, Bekinschtein T, Manes F. A neuropsychological battery to detect specific executive and social cognitive impairments in early frontotemporal dementia. Brain 2009;132:1299-1309.

28. Hodges JR. The Frontotemporal Dementia Syndromes. Chapter 1: overview of frontotemporal dementia. Cambridge, England: Cambridge University Press 2007.

29. Johnson SA, Stout JC, Solomon AC, et al. Beyond disgust: impaired recognition of negative emotions prior to diagnosis in Huntington's disease. Brain 2007;130:1732-1744.

30. Henley SM, Wild EJ, Hobbs NZ, et al. Defective emotion recognition in early HD is neuropsychologically and anatomically generic. Neuropsychologia 2008;46:2152-2160.

31. Milders M, Crawford JR, Lamb A, Simpson SA. Differential deficits in expression recognition in gene-carriers and patients with Huntington's disease. Neuropsychologia 2003;41:1484-1492.

32. Quiroz YT, Stern CE, Reiman EM, et al. Cortical atrophy in presymptomatic Alzheimer's disease presenilin 1 mutation carriers. J Neurol Neurosurg Psychiatry 2013;84:556-561.

33. Tabrizi SJ, Scahill RI, Owen G, et al. Predictors of phenotypic progression and disease onset in premanifest and early-stage Huntington's disease in the TRACK-HD study: analysis of 36-month observational data. Lancet Neurol 2013;12:637-649.

34. Whitwell JL, Jack CR, Jr., Boeve BF, et al. Voxel-based morphometry patterns of atrophy in FTLD with mutations in MAPT or PGRN. Neurology 2009;72:813-820.

35. Von Der Heide RJ, Skipper LM, Klobusicky E, Olson IR. Dissecting the uncinate fasciculus: disorders, controversies and a hypothesis. Brain 2013;136:1692-1707.

36. Novak MJ, Seunarine KK, Gibbard CR, et al. White matter integrity in premanifest and early Huntington's disease is related to caudate loss and disease progression. Cortex 2014;52:98-112.

37. Ringman JM, O'Neill J, Geschwind D, et al. Diffusion tensor imaging in preclinical and presymptomatic carriers of familial Alzheimer's disease mutations. Brain 2007;130:1767-1776.

38. Englund E, Brun A, Persson B. Correlations between histopathologic white matter changes and proton MR relaxation times in dementia. Alzheimer Dis Assoc Disord 1987;1:156-170.

39. Neumann M, Kwong LK, Truax AC, et al. TDP-43-positive white matter pathology in frontotemporal lobar degeneration with ubiquitin-positive inclusions. J Neuropathol Exp Neurol 2007;66:177-183.

APPENDIX

SUPPLEMENTARY METHODS

Subjects

At baseline 79 subjects were included, of whom one was unwilling to continue in the study at follow-up, one was unable to undergo follow-up neuropsychological assessment and two others refused the second MRI scan. Moreover, the follow-up MRI session was terminated prematurely by two subjects, resulting in a missing resting-state fMRI scan and two missing DTI scans. Furthermore, two subjects had major motion artefacts on their baseline MRI scans, and three subjects were excluded from resting-state fMRI analyses and eight from DTI analyses because of large artefacts on the follow-up scans. This resulted in neuropsychological data for 77 subjects, appropriate T1-weighted images for 74 participants, DTI for 64, and resting-state fMRI for 70 participants.

Neuropsychological assessment

All participants were screened at baseline and follow-up with the Mini-Mental State Examination (MMSE), the Beck Depression inventory (BDI), and an extensive neuropsychological assessment including the Dutch version of the Rey Auditory Verbal Learning Test (RAVLT), Visual Association Test (VAT), WAIS III subtests digit span, similarities, and block design, Trailmaking Test (TMT), Stroop colour-word test, categorical and letter fluency, modified Wisconsin Card Sorting Test (WCST), Letter Digit Substitution Test (LDST), Boston Naming Test (BNT), Semantic Association Test (SAT), ScreeLing phonological, clock drawing (Royall), Ekman faces, and Happé Cartoons.[e12]

SUPPLEMENTARY TABLE

Supplementary table 1. Neuropsychological performance

	Controls (n=37)		*Carriers (n=40)*	
Domain, test	*Baseline*	*Follow-up*	*Baseline*	*Follow-up*
Language				
BNT	53.5±4.2	55.5±3.7	54.6±4.0	55.6±4.4
SAT	27.8±1.4	28.3±1.3	27.5±1.9	28.1±1.6
Categorical fluency - animals	24.0±5.0	24.7±6.5	23.8±6.1	23.9±5.3
Categorical fluency -occupations	17.5±4.4	17.9±5.0	17.6±5.0	18.1±4.5
Letter fluency	32.5±9.8	39.8±14.1	36.1±12.2	40.8±14.9
Screeling phonological	23.5±0.8	23.7±.7	23.8±0.4	23.9±0.3
Memory				
RAVLT learning	42.4±9.9	50.1±8.7	46.1±10.3	51.6±10.0
RAVLT recall	8.4±3.2	10.4±2.6	9.3±3.5	10.9±3.2
RAVLT recognition	28.5±2.1	29.8±4.6	29.1±1.5	29.2±1.2
VAT	11.8±0.6	11.9±0.3	11.5±1.2	11.6±1.0
Digit Span	14.7±3.5	15.3±3.3	15.6±3.6	15.8±4.1
Attention, mental speed and executive function				
TMT A	32.0±15.3	30.9±13.0	31.2±11.8	31.1±11.1
TMT B†	68.5±29.7	70.6±35.0	70.9±39.6	71.2±38.0
Stroop card I	47.0±8.2	47.7±8.5	45.0±8.6	45.2±8.0
Stroop card II	58.8±10.8	59.1±11.1	59.7±12.0	59.1±11.8
Stroop card III	94.0±23.0	92.9±24.5	94.8±24.6	92.8±24.4
WCST concepts	5.5±0.9	5.3±1.6	5.7±0.8	5.4±1.3
- perseverative errors†	1.0±1.6	0.9±1.5	1.4±2.5	.8±1.5
- non-perseverative errors†	6.6±5.0	4.0±2.8	4.6±3.7	3.6±2.7
LDST	34.5±7.0	34.8±8.3	33.2±6.6	32.4±6.3
Visuospatial and -constructive function				
Block Design	36.7±14.3	39.4±13.7	34.2±15.1	36.9±14.6
Clock drawing	12.5±1.4	12.9±1.1	12.2±1.6	12.9±0.8
Social cognition				
Ekman faces (total)	45.6±6.6	46.6±6.4	47.0±5.7	47.5±5.8
- anger	7.8±2.1	8.1±2.1	8.6±1.7	8.3±1.8
- disgust	6.5±2.7	7.2±2.5	6.7±2.2	7.4±2.2

- fear	5.8±2.5	6.1±2.3	6.5±2.3	6.2±2.4
- happiness	9.9±0.3	9.9±0.3	9.9±0.3	9.9±0.3
- sadness	6.8±1.9	6.8±2.0	6.7±2.0	6.8±1.8
- surprise	8.8±1.6	8.4±1.3	8.6±1.3	8.9±1.2
Happé cartoons (total)	23.3±5.7	25.3±5.7	25.5±5.3	25.7±5.9
- TOM	11.7±3.4	12.6±3.2	12.7±3.4	13.1±3.5
- non-TOM	11.7±2.9	12.7±2.7	12.8±2.8	12.6±2.9
Similarities	24.7±4.8	25.1±5.2	25.9±4.7	26.1±4.8

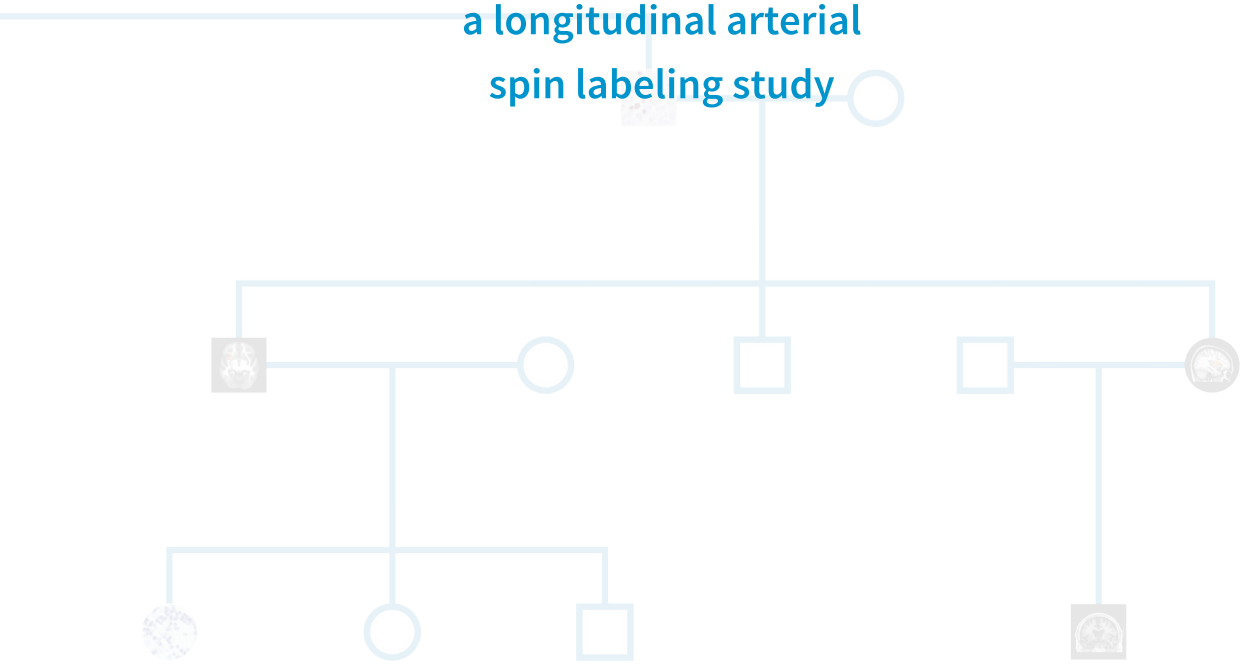
Values denote mean ± SD. †Scores were log-transformed due to non-normality.

SUPPLEMENTARY REFERENCES

1. Smith SM, Jenkinson M, Woolrich MW, et al. Advances in functional and structural MR image analysis and implementation as FSL. Neuroimage 2004;23 Suppl 1:S208-219.
2. Smith SM, Nichols TE. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. Neuroimage 2009;44:83-98.
3. Makris N, Goldstein JM, Kennedy D, et al. Decreased volume of left and total anterior insular lobule in schizophrenia. Schizophr Res 2006;83:155-171.
4. Frazier JA, Chiu S, Breeze JL, et al. Structural brain magnetic resonance imaging of limbic and thalamic volumes in pediatric bipolar disorder. Am J Psychiatry 2005;162:1256-1265.
5. Desikan RS, Segonne F, Fischl B, et al. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. Neuroimage 2006;31:968-980.
6. Goldstein JM, Seidman LJ, Makris N, et al. Hypothalamic abnormalities in schizophrenia: sex effects and genetic vulnerability. Biol Psychiatry 2007;61:935-945.
7. Mori S, Wakana S, Nagae-Poetscher L, Van Zijl P. MRI atlas of human white matter. Elsevier 2005.
8. Wakana S, Caprihan A, Panzenboeck MM, et al. Reproducibility of quantitative tractography methods applied to cerebral white matter. Neuroimage 2007;36:630-644.
9. Hua K, Zhang J, Wakana S, et al. Tract probability maps in stereotaxic spaces: analyses of white matter anatomy and tract-specific quantification. Neuroimage 2008;39:336-347.
10. Worsley K. Statistical analysis of activation images. CH 14, in Functional MRI: An Introduction to Methods, eds P Jezzard, PM Matthew, and SM Smith OUP. 2001.
11. Verhage F. [Intelligence and age: Research on Dutch people aged twelve to seventy-seven years old]. Assen: van Gorcum 1964.
12. Lezak M, Howieson D, Loring D. Neuropsychological Assessment (4th ed). New York: Oxford University Press 2004.

CHAPTER 3.3

Cerebral blood flow in presymptomatic familial frontotemporal dementia: a longitudinal arterial spin labeling study



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In preparation.

ABSTRACT

Introduction: Frontotemporal dementia (FTD) is characterized by behavioral disturbances and language problems. Familial forms can be caused by genetic defects in *microtubule-associated protein tau* (*MAPT*), *progranulin* (*GRN*), and *C9orf72.* In light of upcoming clinical trials with potential disease-modifying agents, the development of sensitive biomarkers to evaluate such agents is crucial. Presymptomatic carriers of the abovementioned genetic defects provide an ideal study population to investigate biomarkers in the earliest stage of the disease. In the current study we used longitudinal arterial spin labeling in presymptomatic carriers of *MAPT* and *GRN* mutations to investigate the earliest changes in cerebral blood flow.

Methods: Healthy first-degree relatives of patients with a *MAPT* or *GRN* mutation underwent arterial spin labeling MRI at baseline and follow-up after two years. We investigated cross-sectional and longitudinal differences in cerebral blood flow between mutation carriers ($n = 34$) and controls without a mutation ($n = 31$).

Results: We found no cross-sectional group differences in cerebral blood flow between mutation carriers and controls. However, mutation carriers showed a significantly stronger decrease in cerebral blood flow in frontal, temporal, parietal, and subcortical areas over time, which was most significant for mutation carriers in close proximity to their estimated symptom onset. Moreover, when restricting cross-sectional analyses to these areas, we found lower cerebral blood flow in carriers compared with controls at follow-up.

Conclusion: We demonstrated longitudinal alterations in cerebral blood flow in presymptomatic carriers of *MAPT* and *GRN* mutations, which was related to approaching the estimated symptom onset. Therefore, arterial spin labeling could have the potential to serve as a sensitive biomarker for disease progression in the presymptomatic stage of FTD in future clinical trials.

INTRODUCTION

Frontotemporal dementia (FTD) is the second most common form of presenile dementia, characterized by behavioral disturbances and language disorders, which is caused by neurodegeneration of the frontal and temporal lobes.[1] Mutations in the *microtubule-associated protein tau* (*MAPT*), *progranulin* (*GRN*) and *C9orf72,* and, less frequently, *CHMP2B* and *VCP* genes can cause an autosomal dominant form of FTD.[2-7] There is currently no therapy available to prevent or cure the disease, but knowledge on the pathophysiological disease process is rapidly growing. Therefore, clinical trials with disease-modifying agents are upcoming, urging the need for sensitive biomarkers to evaluate such therapies.[8] We have previously shown changes in neuropsychological performance, grey matter volume, white matter integrity and functional connectivity in the presymptomatic stage of FTD.

Positron emission tomography with 18F-fluorodeoxyglucose (FDG-PET) is often suggested as a useful biomarker for the earliest stages of FTD. Hypometabolism in the frontal and anterior temporal lobes, and subcortical regions, is consistently seen in FTD patients,[9-12] which progresses over time.[12] Moreover, presymptomatic *GRN* mutation carriers already showed regional hypometabolism on FDG-PET, supporting its potential to detect functional brain changes at a very early stage.[13] However, FDG-PET has several serious disadvantages to be used as a biomarker for FTD, including the high costs, limited availability of PET-scanners and the need for exposure to a radioactive tracer.[14]

Arterial spin labeling MRI (ASL) provides a non-invasive measure of brain perfusion by magnetically labeling water protons in arterial blood and thereby creating an endogenous tracer of cerebral blood flow (CBF), which is assumed to be tightly coupled to brain metabolism.[14] ASL studies in patients with FTD have provided highly similar results as FDG-PET studies, with hypoperfusion in bilateral frontal lobes, the anterior cingulate cortex (ACC), insula, and thalamus compared with controls.[15-19] Besides one small study in presymptomatic *CHMP2B* mutation carriers showing widespread hypoperfusion in hippocampus, temporal, parietal, and occipital lobes by means of spin echo,[20] no studies investigating CBF in the presymptomatic stage of FTD have been performed thus far.

In the current study we used longitudinal ASL to investigate the earliest changes in CBF in presymptomatic carriers of *MAPT* and *GRN* mutations and to explore whether ASL has the potential to serve as a sensitive biomarker to detect FTD and track disease progression in the earliest disease stage.

METHODS

This study is part of a larger project investigating biomarkers in individuals at risk for FTD. In- and exclusion criteria have previously been described.[21] In short, all participants had a 50% risk to carry either a *MAPT* or a *GRN* mutation, and DNA of all subjects was screened for these mutations,[22] resulting in a group of presymptomatic mutation carriers and a group of controls without a mutation. All participants were studied at baseline and two-years follow-up. In total, 73 participants underwent baseline and follow-up ASL-MRI scans, however, eight subjects had to be excluded from the analyses because of labeling errors or major artefacts at baseline (n = 3) or follow-up (n = 5). We investigated cross-sectional differences in CBF between mutation carriers and controls at baseline and follow-up. Moreover, we investigated between-group differences in longitudinal CBF changes. Subsequently, we examined group differences in association with expected onset age, and we evaluated single-subject data. Expected onset age was defined as the mean onset age of all affected persons per family. Participants were categorized into four groups: less than five, five to ten, ten to fifteen, or more than fifteen years younger than their expected onset age. The local ethics committee approved the study and all participants provided written informed consent.

Neuropsychological assessment

All participants were screened using the Mini-Mental State Examination (MMSE), the Beck Depression inventory (BDI) and an extensive neuropsychological assessment including the Dutch version of the Rey Auditory Verbal Learning Test (RAVLT), Visual Association Test (VAT), WAIS III subtests digit span, similarities, and block design, Trailmaking Test (TMT), Stroop color-word test, categorical and letter fluency, modified Wisconsin Card Sorting Test (WCST), Letter Digit Substitution Test (LDST), Boston Naming Test (BNT), Semantic Association Test (SAT), ScreeLing phonological, clock drawing (Royall), Ekman faces, and Happé Cartoons.[23] Conversion to symptomatic FTD was defined as the presence of

behavioral or language symptoms as reported by informants in combination with cognitive deficits in at least two cognitive domains at neuropsychological assessment.

Image acquisition

MRI scans were acquired using a Philips 3.0 Tesla Achieve MRI scanner (Philips Medical Systems, Best, The Netherlands) using an eight-channel SENSE head coil. We obtained pseudo-continuous ASL scans using single-shot echo-planar imaging (EPI) with a background suppression scheme, consisting of a saturation pulse directly before labeling and inversion pulses at 1680 and 2830 ms after the saturation pulse. The following acquisition parameters were applied: repetition time = 4020 ms, echo time = 14 ms, label duration = 1650 ms, postlabeling delay = 1525 ms, 17 slices, voxel size = 3 x 3 x 7 mm, 40 pairs of label and control images, total scan duration = 5.5 minutes. The labeling plane was oriented perpendicular to the carotid arteries. Furthermore, we acquired whole brain T1-weighted images, as described previously.[21]

Image analyses

We used Matrix laboratory (MATLAB, http://www.mathworks.nl/products/matlab/) to create perfusion-weighted images by pairwise subtraction of tag images from control images. MRI analyses were carried out in FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk).[24]

Perfusion-weighted images were motion corrected, brain-extracted, aligned to the anatomical scans and to MNI-152 (T1 standard brain averaged over 152 subjects; Montreal Neurological Institute, Montreal, QC, Canada) standard space, and smoothed with an isotropic Gaussian kernel of 3.4 mm. The derived CBF images were divided by the mean perfusion in the occipital pole, a region typically not affected in FTD,[9, 16] to account for global variations in CBF independent of the disease process. We subtracted follow-up CBF images from baseline maps per subject in order to obtain maps of change in perfusion over time. We investigated cross-sectional differences between mutation carriers and controls at baseline and follow-up, and longitudinal group differences by means of permutation-based testing using 5,000 permutations, applying a 2-sample *t*-test with age, gender and voxel-wise grey matter volume as confound regressors, to compare regional CBF between mutation carriers and controls. We thresholded the statistical images

at $p < 0.05$, corrected for multiple comparisons using threshold-free cluster enhancement.[25] The analyses were restricted to grey matter voxels that were covered by the ASL scan in all participants.

Statistical analyses

We analyzed demographic features using independent samples *t*-tests and Pearson's χ^2 tests in SPSS 21.0 for Windows (SPSS, Chicago, IL), applying a significance level of $p < 0.05$. Group differences for the different age categories based on estimated onset age were analyzed using independent samples *t*-tests.

RESULTS

Demographic features

The groups of mutation carriers *(n* = 34) and controls (*n* = 31) did not differ in age, time between MRI scans, years till expected symptom onset, gender, level of education, and scores on the MMSE and BDI (Table 1). Two mutation carriers, one with a *MAPT* mutation and one with a *GRN* mutation have converted to the clinical stage of FTD during the follow-up period, as described in more detail previously.

Table 1. Demographic features

	Controls (n=31)	*Carriers (n=34)*	*p-value*
Age t1, y	51.0 (10.3)	50.1 (9.7)	0.696
Interval t1-t2, y	2.3 (0.1)	2.2 (0.1)	0.460
Years till expected onset age t1, y	-7.4 (8.9)	-7.4 (8.4)	0.988
Females	52%	62%	0.409
GRN mutation†	-	68%	-
Level of education‡	5.3 (1.0)	5.7 (0.8)	0.064
MMSE score t2	29.2 (1.3)	28.7 (1.9)	0.231
MMSE Δt1-t2	0.0 (1.3)	0.6 (1.6)	0.109
BDI score t2	3.5 (3.7)	2.9 (4.0)	0.592
BDI Δt1-t2	0.1 (2.8)	0.1 (3.2)	0.943

Values denote mean (SD) or percentage of subjects.

†Remaining mutation carriers have a *MAPT* mutation.

‡Level of education was determined on a Dutch 7-point scale ranging from 1 (less than elementary school) to 7 (university or technical college).[33]

Cerebral blood flow

Cross-sectional analyses revealed no significant differences in CBF between mutation carriers and controls at baseline or follow-up. However, in longitudinal analyses mutation carriers

showed a significantly stronger decrease in CBF over time compared with controls in widespread frontal, temporal, parietal, and subcortical regions (Figure 1, Table 2). This finding was more significant for mutation carriers closer to their expected onset age (> 15 years younger $p = 0.234$; 10 - 15 years younger $p = 0.089$; 5 - 10 years younger $p = 0.041$; < 5 years younger $p = 0.013$). Moreover, the *GRN* carrier who converted to clinical FTD showed the strongest decline in CBF in all significant clusters, with the second strongest decline in the converted *MAPT* carrier in three out of five significant clusters including frontal and right medial temporal regions (clusters 2, 3, and 5) (Figure 2). However, the abovementioned findings were not solely driven by the converters, as excluding them from the analyses still resulted in, albeit smaller, significant differences between carriers and controls.

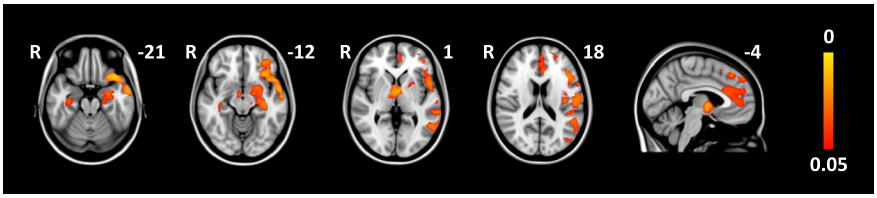

Figure 1. Significant longitudinal CBF differences between mutation carriers and controls. Maps illustrate clusters of significantly stronger decline in CBF over time in mutation carriers compared with controls. Color bar represents *p*-values.

Restricting the cross-sectional analyses to the significant clusters in the longitudinal analyses revealed lower CBF in mutation carriers compared with controls at follow-up in the superior frontal gyrus, left temporal pole, frontal orbital cortex, middle and superior temporal gyrus, right temporal fusiform cortex, parahippocampal gyrus, hippocampus, and thalamus (Figure 3, Table 3), whereas we still found no group differences at baseline. Again, without the two converters, these differences were smaller, but still significant.

Table 2. Clusters of significant longitudinal CBF differences between mutation carriers and controls.

Decrease over time	*Cluster*	*Region*	*Sixe (voxels)*	*x, y ,z*	*Minimum p-value*
Controls > carriers		None			
Carriers > controls	1	Temporal pole L	7417	-38, 20, -24	0.010
		Middle, superior temporal gyrus L			
		Insula L			
		Planum polare L			
		Frontal orbital cortex L			
		Frontal pole L			
		Inferior, middle, superior frontal gyrus L			
		Frontal, central, parietal operculum cortex L			
		Supramarginal gyrus L			
		Precentral gyrus L			
		Postcentral gyrus L			
		Angular gyrus L			
		Lateral occipital cortex L			
	2	Superior frontal gyrus L+R	1897	0, 32, 48	0.027
		Frontal pole			
		Anterior cingulate cortex			
		Paracingulate gyrus			
	3	Thalamus L+R	1802	0, -6, -2	0.015
		Parahippocampal gyrus L			
		Hippocampus L			
		Amygdala L			
		Putamen L			
		Pallidum L			
	4	Temporal fusiform cortex R	203	36, -20, -22	0.036
		Parahippocampal gyrus R			
		Hippocampus R			
	5	Frontal pole L	11	-16, 62, 6	0.048

L, left; R, right

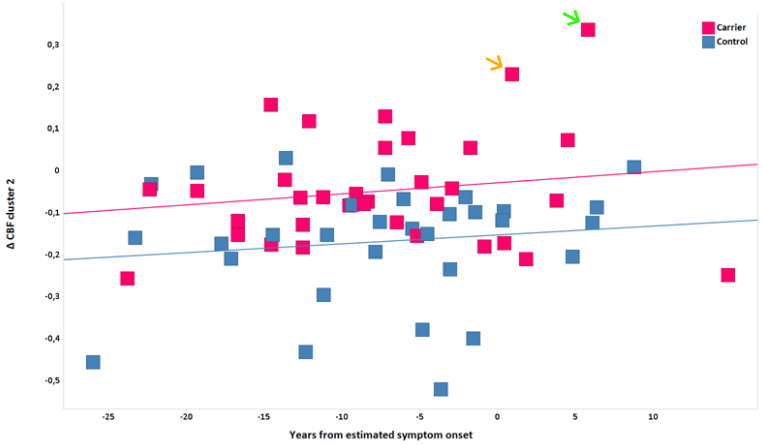

Figure 2. Individual values of decline in CBF over time in cluster 2 (superior frontal gyrus, frontal pole, anterior cingulate cortex, paracingulate gyrus). Pink squares represent mutation carriers, blue squares are controls. The yellow arrow points to the converted subject with a *MAPT* mutation and the green arrow to the converter with a *GRN* mutation.

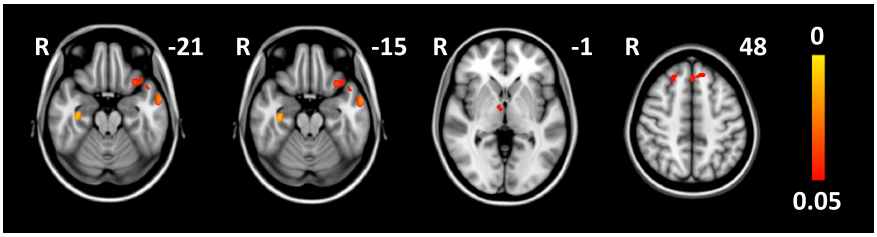

Figure 3. Differences in CBF between mutation carriers and controls at follow-up. Maps illustrate significant differences in CBF between mutation carriers and controls at follow-up when restricting the analysis to the significant clusters in the longitudinal analysis. Color bar represents *p*-values.

Table 3. Clusters of significant differences in CBF between mutation carriers and controls at follow-up when restricting the analysis to the significant clusters in the longitudinal analysis.

CBF at follow-up	*Cluster*	*Region*	*Sixe (voxels)*	*x, y ,z*	*Minimum p-value*
Controls > carriers	1	Temporal pole L	699	-38, 8, -36	0.008
		Frontal orbital cortex L			
		Middle, superior temporal gyrus L			
	2	Temporal fusiform cortex R	155	36, -20, -22	0.013
		Parahippocampal gyrus R			
		Hippocampus R			
	3	Superior frontal gyrus L	68	0, 32, 46	0.042
	4	Thalamus R	55	0, -6, -6	0.045
	5	Superior frontal gyrus R	41	22, 32, 48	0.046
	6	Superior frontal gyrus	6	6, 20, 52	0.048
Carriers > controls		None			

L, left; R, right

DISCUSSION

The present study is the first to show that a significant decrease in CBF in frontal, temporal, parietal and subcortical regions during two years of follow-up can be detected by ASL in presymptomatic carriers of *GRN* and *MAPT* mutations compared with controls. These differences were most significant in the carriers that were approaching their estimated symptom onset, and the two subjects that have converted to clinically manifest FTD showed the strongest decline in perfusion in these regions. While we found no cross-sectional group differences at baseline, mutation carriers demonstrated significantly lower CBF in these regions at follow-up. These findings underline the value of ASL in detecting early changes in brain perfusion in FTD, which could be used to evaluate future therapies in clinical trials.

Our findings of a longitudinal CBF decline in frontal and subcortical regions in mutation carriers compared with controls is in line with previous cross-sectional ASL studies in patients with FTD, and an FDG-PET study in presymptomatic *GRN* mutation carriers.[13, 15-19] Frontal hypoperfusion has been correlated with behavioral disturbances.[16] In contrast to most previous studies, we also found longitudinal

decrease in temporal and parietal CBF in the current presymptomatic mutation carriers. The discrepancy regarding temporal hypoperfusion between the present and previous studies, might be explained by incomplete brain coverage in previous ASL studies.[16, 18] The present finding of temporal hypoperfusion in presymptomatic carriers is supported by FDG-PET studies in patients with FTD, demonstrating hypometabolism in the anterior temporal lobes.[9, 10] The posterior temporal and parietal involvement is likely to be driven by the *GRN* mutation carriers, as *GRN* mutations have consistently been shown to affect more posteriorly located brain regions as well.[26-28] Not surprisingly, the *GRN* converter only, and not the converted subject with a *MAPT* mutation, showed the strongest decline in these areas. Conversely, no parietal hypometabolism was found in a previous FDG-PET study in presymptomatic *GRN* mutation carriers, and the authors suggested that parietal damage is a later phenomenon in the disease process than frontotemporal involvement.[13] The lack of significant parietal hypoperfusion in the cross-sectional analyses, in the presence of reduced frontotemporal perfusion, supports this notion.

In contrast to the present study, a more widespread hypoperfusion pattern including temporal, parietal, and occipital lobes was found using spin-echo MRI in presymptomatic *CHMP2B* mutation carriers. However, this likely reflects grey matter atrophy in this genetic group, as correction for grey matter volume was not applied in these subjects, who were previously shown to have generalized atrophy in the presymptomatic stage.

Our longitudinal analyses suggest that ASL is capable of measuring disease progression, possibly even at the individual level. However, based on current findings CBF does not provide an accurate predictor for disease conversion at baseline, since values of the two converted subjects were within the normal range at baseline. This contrasts findings in Alzheimer's disease (AD) showing an association between baseline perfusion and clinical conversion to dementia in patients with mild cognitive impairment.[29] The low number of converters in the current study might have hampered the identification of baseline predictors. However, it also possible that changes in CBF occur earlier in AD compared to FTD, which has previously been suggested in a study showing hypoperfusion in AD relative to FTD.[16]

Several major advantages of ASL over FDG-PET for the use in future clinical trials

were already mentioned, including the lower costs, its non-invasiveness, there is no need for radiation exposure, and MRI scanners are more widely available compared with PET-scanners. Moreover, ASL can be easily combined with other MRI techniques in a single session. Other MRI studies in the present cohort have demonstrated presymptomatic alterations in grey matter volume, white matter integrity and functional connectivity, using T1-weighted images, DTI, and resting-state fMRI, respectively. Perhaps a multimodal approach could further improve their sensitivity for measuring therapy effects in future clinical trials. A drawback of ASL is the huge diversity in acquisition methods. Recently, an international consortium has published recommendations for the implementation of ASL,[30] which will hopefully result in a more standardized use of ASL, which is crucial for its use in clinical trials.

Limitations to our study include the small number of converted subjects and the lack of participants with *C9orf72* repeat expansions. Another important issue in both ASL and FDG-PET is the choice of a reference region to correct for normal global variations in cerebral perfusion. Mean global perfusion is often used for normalization, however, this has been shown to result in regions of artefactual hyperperfusion.[17, 31, 32] Therefore, normalization to a non-affected reference region seems to be more appropriate. In the current study we have used the occipital pole as a reference region, since this region is typically spared in FTD,[9, 16] although it cannot be completely excluded that this choice of reference has influenced our results. The cerebellum is also often used as a reference region,[31] but the field-of-view in our ASL protocol did not cover the cerebellum.

To conclude, we demonstrated longitudinal alterations in CBF in presymptomatic carriers of *MAPT* and *GRN* mutations over two years of time, which appear to be related to approaching symptom onset. These findings suggest that ASL could provide a sensitive biomarker of disease progression in the presymptomatic stage of FTD, which can be useful for future clinical trials.

REFERENCES

1. Rascovsky K, Hodges JR, Knopman D, Mendez MF, Kramer JH, Neuhaus J, et al. Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. Brain. 2011 Sep;134(Pt 9):2456-77.
2. Renton AE, Majounie E, Waite A, Simon-Sanchez J, Rollinson S, Gibbs JR, et al. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron. 2011 Oct 20;72(2):257-68.
3. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron. 2011 Oct 20;72(2):245-56.
4. Baker M, Mackenzie IR, Pickering-Brown SM, Gass J, Rademakers R, Lindholm C, et al. Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. Nature. 2006 Aug 24;442(7105):916-9.
5. Hutton M, Lendon CL, Rizzu P, Baker M, Froelich S, Houlden H, et al. Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. Nature. 1998 Jun 18;393(6686):702-5.
6. Skibinski G, Parkinson NJ, Brown JM, Chakrabarti L, Lloyd SL, Hummerich H, et al. Mutations in the endosomal ESCRTIII-complex subunit CHMP2B in frontotemporal dementia. Nat Genet. 2005 Aug;37(8):806-8.
7. Watts GD, Wymer J, Kovach MJ, Mehta SG, Mumm S, Darvish D, et al. Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia is caused by mutant valosin-containing protein. Nat Genet. 2004 Apr;36(4):377-81.
8. Tsai RM, Boxer AL. Treatment of frontotemporal dementia. Curr Treat Options Neurol. 2014 Nov;16(11):319.
9. Ishii K, Sakamoto S, Sasaki M, Kitagaki H, Yamaji S, Hashimoto M, et al. Cerebral glucose metabolism in patients with frontotemporal dementia. J Nucl Med. 1998 Nov;39(11):1875-8.
10. Jeong Y, Cho SS, Park JM, Kang SJ, Lee JS, Kang E, et al. 18F-FDG PET findings in frontotemporal dementia: an SPM analysis of 29 patients. J Nucl Med. 2005 Feb;46(2):233-9.
11. Diehl J, Grimmer T, Drzezga A, Riemenschneider M, Forstl H, Kurz A. Cerebral metabolic patterns at early stages of frontotemporal dementia and semantic dementia. A PET study. Neurobiol Aging. 2004 Sep;25(8):1051-6.
12. Grimmer T, Diehl J, Drzezga A, Forstl H, Kurz A. Region-specific decline of cerebral glucose metabolism in patients with frontotemporal dementia: a prospective 18F-FDG-PET study. Dement Geriatr Cogn Disord. 2004;18(1):32-6.
13. Jacova C, Hsiung GY, Tawankanjanachot I, Dinelle K, McCormick S, Gonzalez M, et al. Anterior brain glucose hypometabolism predates dementia in progranulin mutation carriers. Neurology. 2013 Oct 8;81(15):1322-31.
14. Wolk DA, Detre JA. Arterial spin labeling MRI: an emerging biomarker for Alzheimer's disease and other neurodegenerative conditions. Curr Opin Neurol. 2012 Aug;25(4):421-8.

15. Binnewijzend MA, Kuijer JP, van der Flier WM, Benedictus MR, Moller CM, Pijnenburg YA, et al. Distinct perfusion patterns in Alzheimer's disease, frontotemporal dementia and dementia with Lewy bodies. Eur Radiol. 2014 Sep;24(9):2326-33.

16. Du AT, Jahng GH, Hayasaka S, Kramer JH, Rosen HJ, Gorno-Tempini ML, et al. Hypoperfusion in frontotemporal dementia and Alzheimer disease by arterial spin labeling MRI. Neurology. 2006 Oct 10;67(7):1215-20.

17. Hu WT, Wang Z, Lee VM, Trojanowski JQ, Detre JA, Grossman M. Distinct cerebral perfusion patterns in FTLD and AD. Neurology. 2010 Sep 7;75(10):881-8.

18. Shimizu S, Zhang Y, Laxamana J, Miller BL, Kramer JH, Weiner MW, et al. Concordance and discordance between brain perfusion and atrophy in frontotemporal dementia. Brain Imaging Behav. 2010 Mar;4(1):46-54.

19. Zhang Y, Schuff N, Ching C, Tosun D, Zhan W, Nezamzadeh M, et al. Joint assessment of structural, perfusion, and diffusion MRI in Alzheimer's disease and frontotemporal dementia. Int J Alzheimers Dis. 2011;2011:546871.

20. Lunau L, Mouridsen K, Rodell A, Ostergaard L, Nielsen JE, Isaacs A, et al. Presymptomatic cerebral blood flow changes in CHMP2B mutation carriers of familial frontotemporal dementia (FTD-3), measured with MRI. BMJ Open. 2012;2(2):e000368.

21. Dopper EG, Rombouts SA, Jiskoot LC, den Heijer T, de Graaf JR, de Koning I, et al. Structural and functional brain connectivity in presymptomatic familial frontotemporal dementia. Neurology. 2014 Jul 8;83(2):e19-26.

22. Seelaar H, Kamphorst W, Rosso SM, Azmani A, Masdjedi R, de Koning I, et al. Distinct genetic forms of frontotemporal dementia. Neurology. 2008 Oct 14;71(16):1220-6.

23. Lezak M, D. H, D. L. Neuropsychological Assessment (4th ed.). New York: Oxford Univeristy Press 2004. 2004.

24. Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TE, Johansen-Berg H, et al. Advances in functional and structural MR image analysis and implementation as FSL. Neuroimage. 2004;23 Suppl 1:S208-19.

25. Smith SM, Nichols TE. Threshold-free cluster enhancement: addressing problems of smoothing, threshold dependence and localisation in cluster inference. Neuroimage. 2009 Jan 1;44(1):83-98.

26. Le Ber I, Camuzat A, Hannequin D, Pasquier F, Guedj E, Rovelet-Lecrux A, et al. Phenotype variability in progranulin mutation carriers: a clinical, neuropsychological, imaging and genetic study. Brain. 2008 Mar;131(Pt 3):732-46.

27. Rohrer JD, Warren JD, Omar R, Mead S, Beck J, Revesz T, et al. Parietal lobe deficits in frontotemporal lobar degeneration caused by a mutation in the progranulin gene. Arch Neurol. 2008 Apr;65(4):506-13.

28. Seelaar H, Papma JM, Garraux G, de Koning I, Reijs AE, Valkema R, et al. Brain perfusion patterns in familial frontotemporal lobar degeneration. Neurology. 2011 Jul 26;77(4):384-92.

29. Chao LL, Buckley ST, Kornak J, Schuff N, Madison C, Yaffe K, et al. ASL perfusion MRI predicts cognitive decline and conversion from MCI to dementia. Alzheimer Dis Assoc Disord. 2010 Jan-

Mar;24(1):19-27.

30. Alsop DC, Detre JA, Golay X, Gunther M, Hendrikse J, Hernandez-Garcia L, et al. Recommended implementation of arterial spin-labeled perfusion MRI for clinical applications: A consensus of the ISMRM perfusion study group and the European consortium for ASL in dementia. Magn Reson Med. 2014 Apr 8.

31. Dukart J, Perneczky R, Forster S, Barthel H, Diehl-Schmid J, Draganski B, et al. Reference cluster normalization improves detection of frontotemporal lobar degeneration by means of FDG-PET. PLoS One. 2013;8(2):e55415.

32. Yakushev I, Landvogt C, Buchholz HG, Fellgiebel A, Hammers A, Scheurich A, et al. Choice of reference area in studies of Alzheimer's disease using positron emission tomography with fluorodeoxyglucose-F18. Psychiatry Res. 2008 Nov 30;164(2):143-53.

33. Verhage F. Intelligence and age: Research on Dutch people aged twelve to seventy-seven years old. Assen: van Gorcum. 1964.

CHAPTER 4

General discussion

Frontotemporal lobar degeneration (FTLD) is the second most common cause of presenile dementia, characterized by behavioral disturbances and language deficits, typically presenting before the age of 65 years. An autosomal dominant familial form of FTLD exists in 10 - 30%, with mutations in *microtubule-associated protein tau* (*MAPT*) and *progranulin* (*GRN*), and the *C9orf72* repeat expansion as the most common genetic causes. The genetic forms of FTLD have largely increased our knowledge about the disease process, which is crucial for the development of disease-modifying therapies. Moreover, in the light of upcoming clinical trials for such agents, the development of sensitive biomarkers for early FTLD is needed. Presymptomatic carriers of FTLD causing mutations provide an ideal population to investigate these biomarkers at the earliest stage of the disease. The aim of the current thesis was to expand current knowledge on phenotypical characteristics of genetic FTLD and to investigate presymptomatic changes in familial FTLD using neuropsychological assessment and MRI.

Phenotypical variability of FTLD

The clinical spectrum of FTLD ranges from frontotemporal dementia with motor neuron disease (FTD-MND), semantic dementia (SD), behavioral variant of frontotemporal dementia (bvFTD), progressive non-fluent aphasia (PNFA), corticobasal syndrome (CBS) to progressive supranuclear palsy (PSP). FTD-MND and SD are most commonly associated with FTLD with accumulation of transactive response DNA binding protein of 43 kDa (FTLD-TDP) pathology, whereas PNFA, CBS, and PSP are caused by FTLD with tau-positive inclusions (FTLD-tau) in general. In contrast, bvFTD can be caused by either FTLD-TDP, FTLD-tau or FTLD-FET.[1, 2] Moreover, the FTLD presentation can mimic Alzheimer's disease (AD),[3] especially in patients older than 65 years,[4] or psychiatric disorders.[5] Conversely, several other disorders sometimes manifest a frontal disease presentation, thereby mimicking FTLD. Such FTLD-like presentations have been reported in AD,[6-8] corticobasal degeneration (CBD),[9] PSP,[10-12] prion diseases,[13-16] multiple sclerosis,[17, 18] CADASIL,[19] voltage-gated K-channel encephalopathy,[20] and neurofilamentopathy.[21] Due to this large clinical and pathological variation it can be challenging to diagnose FTLD during life.

In **Chapter 2.1** we reported a novel c.314dup *GRN* mutation in a patient with an atypical symmetric CBS presentation with walking disabilities and postural

instability, symmetric rigidity of his arms, non-fluent aphasia, behavioral disturbances and forgetfulness. Neuroimaging revealed symmetrical frontoparietal atrophy and hypoperfusion. FTLD-TDP type A pathology was demonstrated at autopsy, which prompted us to perform genetic screening in this patient despite the lack of a positive family history for neurodegenerative diseases. Whereas *GRN* mutations have been suggested as the most common cause of autosomal dominant CBS, they are far less common in sporadic cases.[22] CBS was originally regarded as the clinical presentation of CBD pathology, characterized by neural and glial inclusions of 4-repeat tau.[23] However, it is increasingly recognized that other pathologies, including AD, PSP, Creutzfeldt-Jakob disease, FTLD-tau, and FTLD-TDP (sometimes with *GRN* mutations) can also present as CBS, and that CBD can also present with behavioral disturbances or language difficulties.[9, 24-28] Therefore, in 2014 novel criteria for CBD have been proposed incorporating four clinical phenotypes, including CBS, frontal behavioral-spatial syndrome, non-fluent/agrammatic variant of primary progressive aphasia, and PSP. Two sets of criteria have been proposed: clinical research criteria for probable CBD and less restrictive criteria for possible CBD, which should still emphasize on tau-based pathologies.[28] However, the first studies to validate these new criteria have shown that they still lack the ability to accurately differentiate tau-based CBS from non-tau pathologies including AD and FTLD-TDP.[27, 29] It turns out that it is still very difficult to define specific features to predict the underlying pathology for patients presenting with CBS. Therefore, biomarkers such as cerebrospinal fluid or tracers for positron emission tomography are needed for pathological predictions, which is crucial for upcoming protein-based therapeutic trials.

C9orf72 repeat expansion

In **Chapter 2.2** we have demonstrated the clinical heterogeneity of the *C9orf72* repeat expansion in a Dutch FTD cohort. The clinical presentation was bvFTD in 81% of patients and PPA in 19%, with concomitant signs of amyotrophic lateral sclerosis (ALS) or Parkinsonism in 17% and 14%, respectively.

The observed clinical diversity in our study has been confirmed and expanded by others studies. The most common motor neuron disease (MND) presentation of *C9orf72* repeat expansions is ALS, but a few cases of primary lateral sclerosis, progressive muscular atrophy, and monomelic amyotrophy have been reported.[30-32] *C9orf72* repeat expansions in MND have been associated with

younger age at onset, shorter disease duration, more frequent bulbar symptom onset and behavioral or cognitive deficits than MND cases without a *C9orf72* repeat expansion.[32-34] BvFTD is the most frequent FTLD presentation of the *C9orf72* repeat expansion. Other presentations include language variants PNFA and SD, an amnestic syndrome compatible with clinical AD, Parkinsonian syndromes, sometimes fulfilling criteria for Parkinson's disease, CBS, or PSP, and psychiatric disorders, including psychosis.[5, 30, 31, 34-39] Compared to other FTLD cases, psychiatric symptoms, and concomitant MND are more common in *C9orf72* repeat expansion carriers.[5, 34, 36, 39] Interestingly, the repeat expansion has also been reported in several patients with the FTD phenocopy syndrome, characterized by behavioral disturbances that are typical for bvFTD, but without imaging abnormalities and clinical progression over time, which was previously considered to be unlikely of neurodegenerative nature.[40] Thorough evaluation of family history in such cases is crucial to identify potential *C9orf72* repeat expansion carriers. The large variation in clinical presentation might reflect different expansion sizes across tissues or result from other genetic modifiers.[41] Additional correlation studies between expansion length and clinical phenotype are needed to resolve this.

Besides the large variation in clinical symptoms, we also reported that the age of onset widely varies among *C9orf72* repeat expansion carriers, ranging from 27 to 80 years. Intra-familial differences in onset age up to 20 years have been reported.[37] Moreover, survival time after onset ranges from 3 to 96 months in ALS and from 1 to 22 years in FTLD.[35] Several genetic modifiers for age of onset and survival time have been reported.[42, 43] Moreover, genetic anticipation has been suggested as additional explanation for the variation in onset age, similar to other repeat expansion disorders, such as Huntington's disease. However, this hypothesis could not be confirmed thus far, since accurate estimation of exact repeat sizes is difficult due to instability of the repeat.[30, 31, 37, 44]

Pathologically, *C9orf72* repeat expansions are associated TDP-43 pathology, most commonly consistent with either FTLD-TDP type B or classical ALS, but sometimes with (additional) FTLD-TDP type A or, rarely, type C.[5, 34, 45] TDP-43-positive inclusions are commonly detected in the frontal and temporal cortex, hippocampus, pyramidal motor system, and subcortical regions, in a highly symmetrical pattern.[34, 45] The distribution of TDP-43-pathology has been found

to correlate with the clinical presentation.[46] Additional to TDP-43-pathology, we reported ubiquitin- and p62-positive, but TDP-43-negative inclusions, in the granular layer of the cerebellum. These inclusions have been demonstrated to be a highly consistent and specific finding among *C9orf72* repeat expansion carriers, and are most prominent in the cerebellum and hippocampus.[31,47] These inclusions have been demonstrated to contain dipeptide repeats (DPR), resulting from unconventional non-ATG-initiated translation of the expanded GGGGCC repeat.[48, 49] Finally, intranuclear RNA foci in the frontal cortex, hippocampus, and cerebellum are a consistent finding in *C9orf72* brains.[45] The lack of *C9orf72*-specific RNA foci in our study, could be due to the different fluorescence *in situ* hybridization protocol.

The mechanism by which the *C9orf72* repeat expansion leads to neurodegeneration is currently unclear. Three different mechanisms have been suggested:

1) Haploinsufficiency leading to loss of normal function of C9orf72 protein, which is supported by findings of reduced RNA levels of at least one of the C9orf72 transcripts and a zebrafish model suggesting that loss of C9orf72 function may lead to motor neuron degeneration. However, the failure to detect other loss-of-function mutations in *C9orf72*, the report of a patient with a homozygous repeat expansion, not exhibiting more severe or other clinical features compared to heterozygous carriers, and a *C9orf72* knockout mouse model without any signs of motor neuron disease, demonstrate that a loss-of-function mechanism alone cannot fully explain the *C9orf72* phenotype.[5, 31, 35, 45, 50, 51]
2) Formation of toxic RNA foci that sequester RNA-binding proteins, such as TDP-43, thereby interfering with their normal function in regulation of RNA processing. As stated above, the presence of intranuclear RNA foci has convincingly been demonstrated using fluorescence *in situ* hybridization in brains with the *C9orf72* repeat expansion and binding screens have revealed that the repeat binds the RNA-binding proteins FUS, Pur α, and hnRNP A3.[5, 35] An RNA-only *C9orf72* mouse model showed ubiquitin-positive inclusions, favoring a gain-of-function mechanism. However, no obvious neuronal loss or behavioral deficits were observed in this model. Additional studies using this mouse model will hopefully further clarify the role of RNA toxicity in the disease mechanism of the repeat expansion. [52]
3) The third proposed mechanism is toxicity of the abovementioned DPR

aggregates in the brain. Repeat-associated non-ATG-initiated (RAN) translation of the expanded repeat results in formation of five different polypeptides, composed of repeating units of two amino acids that form insoluble aggregates, which are found in the brains of patients with the repeat expansion. Drosophila models with pure versus RNA-only repeats, leading to RNA foci in both models, but DPR solely in the pure repeat model, revealed neurodegeneration in the pure repeat model only. These findings confirm a neurotoxic role for the DPR.[53] However, an additional role of toxic RNA foci cannot be ruled out based on these data.

Very recently, a *C9orf72* mouse model has been developed, in which nuclear RNA foci, DPR, TDP-43 pathology and neuronal cell loss were demonstrated. Importantly, these mice also exhibited behavioral disturbances. This mouse model can therefore be highly useful in further deciphering the disease mechanism of the *C9orf72* repeat expansion and for testing potential therapies.[54]

In **Chapter 2.3** we investigated the worldwide frequency of the *C9orf72* repeat expansion. There is a large geographical variation in frequencies, which is the highest in Finland. On average, the *C9orf72* repeat expansion is found in 25% and 36% of familial FTLD and ALS, respectively. Moreover, the repeat expansion was demonstrated in 6% of both sporadic FTLD and ALS, making it worldwide the most frequent genetic cause of both sporadic diseases identified thus far. We found the *C9orf72* repeat expansion in 18% of familial FTLD patients and 2% of sporadic FTLD in the Netherlands, thereby explaining 54% of familial FTLD together with *MAPT* and *GRN* mutations. The repeat expansion was demonstrated in 37% and 6% of familial and sporadic ALS in the Netherlands, respectively.[32] Mutations in *C9orf72*, *TADBRP*, *FUS*/*TLS*, *ANG*, and *SOD1* together explain 48% of familial ALS.[55] We and others reported a common risk haplotype that was partly or fully shared by repeat expansion carriers, suggesting that the repeat expansion occurred about 1,500 years ago in a common ancestor of all current repeat expansion carriers.[56] However, later studies have provided evidence against this hypothesis and proposed that the risk haplotype merely provides a genetic background giving increased risk of developing a repeat expansion.[34, 57]

The discovery of the *C9orf72* repeat expansions demonstrates how much we can learn about possible disease mechanisms from genetic forms of FTLD, which is

crucial for finding treatment targets to prevent or cure the disease. Since there still exist familial forms of FTLD, without one of the known genetic defects, additional studies are needed to identify the causative genetic defect in those families. A recent study showed that whole exome sequencing combined with proteomics is a successful approach for this purpose.[21]

Treatment of FTLD

There is currently no therapy available to prevent or cure FTLD. Treatment is limited to symptom management with mainly antipsychotics or serotonin-reuptake inhibitors, generally without convincing evidence from randomized controlled trials.[58] However, increased insight in FTLD pathophysiology has recently led to the development of several potential protein-specific disease-modifying therapies.

Proposed therapeutic strategies for FTLD-tau include inhibition of tau aggregation, inhibition of phosphorylation, reduction of tau levels, and microtubule stabilization.[58] Methylthioninium has been identified as a tau aggregation inhibitor and was studied in a phase II trial in patients with AD. This study showed that methylthioninium can be safely administered and has a positive effect on cognition and brain perfusion.[59] Currently, phase III double-blind placebo-controlled clinical trials with an improved form of this drug, LMTX, are ongoing in both AD and bvFTD.[60, 61]

In carriers of *GRN* mutations the level of progranulin in serum and plasma is reduced due to haploinsufficiency.[62-65] Recently, a histone deacetylase (HDAC) inhibitor named suberoylanilide hydroxamic acid (SAHA) has been identified as an enhancer of *GRN* expression, thereby restoring progranulin levels in animal models and cultured cells from human *GRN* carriers.[66] FRM-0334 is another HDAC inhibitor, with better brain penetrance compared to SAHA, which has also proven to increase progranulin levels. At this moment, a phase II clinical trial has started to investigate the safety, tolerability, and pharmacodynamics effects of this drug in both symptomatic and presymptomatic carriers of a *GRN* mutation.[67, 68] Nimodipine is another drug that might have the potential to normalize progranulin levels, which is currently under investigation in a phase I trial.[69]

Antisense oligonucleotide therapies are the most promising treatment strategy

regarding the *C9orf72* repeat expansion at this moment. This therapy is based on the assumption that the toxic RNA foci play a crucial role in the disease mechanism. These antisense oligonucleotides have been demonstrated to reduce formation of RNA foci and thereby sequestration of RNA binding proteins.[70] However, it remains to be investigated whether such therapy is actually able to interfere with the disease process in patients with the *C9orf72* repeat expansion.

Biomarkers of FTLD

In the light of these therapeutic interventional trials, appropriate biomarkers to evaluate the effect of such therapies are urgently needed. In **Chapters 3.1-3.3** we demonstrated presymptomatic brain alterations using neuropsychological assessment, volumetric MRI, diffusion tensor imaging (DTI), resting-state functional MRI (fMRI), and arterial spin labeling (ASL) in carriers of *GRN* and *MAPT* mutations.

Neuropsychological assessment

We have demonstrated presymptomatic longitudinal alterations in performance on tasks for executive functioning and social cognition, which were most prominent in mutation carriers within five years before their estimated onset age. It has previously been suggested that impaired executive functions and social cognition are very sensitive measures in early bvFTD.[71] Presymptomatic cognitive dysfunctions were previously demonstrated in FTLD mutation carriers, mainly in tasks for attention, executive functioning, or language,[72-77] although other studies did not confirm this.[78-81] Importantly, none of these studies included tasks for social cognition.[72-81] Our results of presymptomatic deficits in social cognition are in line with a study in a large cohort of healthy individuals, showing an association between low scores on a theory of mind task and development of bvFTD during follow-up.[82] These results together confirm that social cognition tasks are sensitive in the earliest stage of FTD and emphasize that such tasks should be incorporated in the standard neuropsychological test battery.

It remains to be elucidated whether the three major genetic groups differ in their earliest neuropsychological deficits. Two studies in presymptomatic *GRN* carriers suggested early deficits on tasks reflecting parietal functioning, such as visuospatial dysfunction or dyscalculia,[73, 77] which is in line with a previous study showing parietal dysfunction in patients with *GRN* mutations versus other FTD

patients.[83] However, other studies in presymptomatic *GRN* mutations, including the present, revealed executive, language, or social cognition deficits, in the absence of parietal dysfunction.[76] Perhaps, early parietal dysfunction is merely associated with certain phenotypical presentations of *GRN* mutations, such as CBS.

Volumetric MRI

We found right insular atrophy starting around five years before estimated symptom onset in presymptomatic *MAPT* and *GRN* carriers. This is in line with findings from a large international consortium pointing at the insula as first affected brain region across the three major genetic groups.[72] Moreover, the frontoinsula has consistently been found to be among the first atrophied regions in symptomatic bvFTD,[84] and frontoinsula atrophy has been correlated with behavioral symptoms, including loss of empathy and eating abnormalities.[85, 86] The frontoinsula, together with the anterior cingulate cortex (ACC), has previously been shown to serve as a major hub in the salience network, which is involved in emotional processing, and is typically affected in early bvFTD.[87, 88] It has been suggested that the early involvement of frontoinsula and ACC reflects selective vulnerability of Von Economo neurons (VENs). VENs are large bipolar projection neurons that are restrictedly located in the frontoinsula and ACC, are more abundant in the right than left hemisphere, and are unique to mammals with complex social behavior, suggesting a role of these neurons in social functioning.[89] Several small studies have indeed shown selective sensitivity of VENs in the ACC and frontoinsula in FTD.[89-92] However, future studies are needed to further explore the exact role of VENs in the FTD disease process.

Although all genetic subtypes have shown early involvement of the insula, the distribution of grey matter atrophy has been shown to vary across the different genetic groups. *MAPT* mutations are typically associated with symmetric atrophy most prominent in the temporal lobes.[93-95] The hippocampus and amygdala were demonstrated to be the first affected brain regions in presymptomatic *MAPT* carriers, followed by the temporal lobes.[72, 96] *GRN* mutations are characterized by asymmetric atrophy with involvement of the posterior temporal and parietal lobes, posterior cingulate cortex and precuneus.[93-95] Several studies have shown presymptomatic atrophy in the frontal, temporal and/or parietal lobes in *GRN*

mutation carriers.[73, 74, 80, 97, 98] The largest study thus far revealed the insula as first affected region, with subsequent atrophy of the temporal and parietal lobes.[72] *C9orf72* repeat expansions have been associated with a symmetrical widespread pattern of atrophy, involving frontal, temporal, parietal, and occipital lobes, thalamus, and cerebellum.[38, 93, 99, 100] Presymptomatic carriers of *C9orf72* repeat expansions showed atrophy in the thalamus, insula, and posterior cortical areas, which was detectable 25 years before estimated symptom onset already, possibly suggesting a developmental origin of atrophy in this genetic subtype.[34, 72]

Diffusion tensor imaging

We demonstrated early damage of the right uncinate fasciculus in the presymptomatic stage at baseline and loss of white matter integrity of the left uncinate fasciculus over two years follow-up in mutation carriers who were within five years before their estimated symptom onset. The uncinate fasciculus, connecting the orbitofrontal cortex and anterior temporal lobes,[101] has previously been identified as the tract that most accurately differentiates FTLD patients from controls,[102] and has been shown to play a role in social behavior.[101, 103] Moreover, we demonstrated that the mutation carriers that developed symptoms during the follow-up period had the worst DTI values in the bilateral uncinate fasciculi and the forceps minor at baseline already, suggesting that these DTI measures could be used as baseline predictors of conversion to the symptomatic disease stage. Moreover, the moment when the first white matter changes are detectable using DTI, might be the ideal time point to start a therapeutic intervention in order to prevent clinical manifestation of the disease.

At baseline we reported widespread white matter changes in *MAPT* carriers, but no white matter changes in the separate *GRN* analyses, and suggested that this could reflect a mutation-specific distribution of white matter damage. However, when incorporating our follow-up findings, it seems more likely that the baseline results for the relatively small *MAPT* subgroup where largely driven by the *MAPT* carrier that converted to clinical FTD, and had the strongest white matter impairment at baseline already. Studies in FTLD patients comparing the three major genetic subtypes using DTI are lacking to date. One study compared a small groups of patients with a *MAPT* mutation or the *C9orf72* repeat expansion and showed more extensive white matter damage in the *MAPT* group, including the left and right

uncinate fasciculus, corpus callosum, fornix, and bilateral inferior and superior longitudinal fasciculi, whereas in the *C9orf72* patients white matter impairment had a more dorsal distribution and was restricted to the corpus callosum and cingulum.[104] No previous DTI studies have been performed in the presymptomatic stage of carriers of *MAPT* mutations and *C9orf72* repeat expansions. White matter loss in patients with *GRN* and *MAPT* mutations was compared in one study using voxel-based morphometry and revealed white matter loss in the inferior longitudinal fasciculus, superior longitudinal fasciculus, inferior fronto-occipital fasciculus, cingulum, corpus callosum and brainstem in the *GRN* group and involvement of the bilateral fornices and uncinate fasciculus in patients with *MAPT* mutations.[94] However, no study has investigated patients with *GRN* mutations using DTI thus far. Two groups did perform DTI in small groups of presymptomatic *GRN* mutation carriers and revealed reduced FA in the left uncinate and inferior fronto-occipital fasciculus and increased DA in the right cingulum, superior longitudinal fasciculus and corticospinal tract, respectively.[79, 80] As DTI seems to provide an early marker for clinical disease manifestation based on our results, it would be interesting to further explore the distribution of white matter impairment for each genetic group in more detail in future studies.

Regarding the different DTI measures more studies are needed to define an accurate interpretation of each parameter. Previous studies suggested that radial diffusivity is the most sensitive parameter for FTLD,[102, 104] whereas fractional anisotropy is more accurate in differentiating FTLD from AD.[104] In our study, fractional anisotropy values most accurately discriminated the mutation carriers that were about to develop clinical symptoms from the other mutation carriers, but differences were identified with all parameters. Since the number of converters was limited, more studies are needed to investigate which DTI parameter is the most sensitive predictor of disease conversion.

For the analysis of DTI we have used a popular approach named tract-based spatial statistics (TBSS),[105] that has overcome certain alignment issues of previously applied methods by projecting maximum fractional anisotropy values from main white matter tracts to a central skeleton, common to all subjects, to which the analysis is restricted.[106, 107] This strongly reduces the risk of including non-white matter tissue in the analyses. This method is highly popular due to its ease of use

and straightforward interpretability. However, a major limitation is that only the central line of a tract is examined, thereby limiting the anatomical information about white matter changes. Tractography techniques can be used to investigate the full extent of certain white matter tracts, and thus allowing better anatomical characterization of results.[106, 107] Future studies are needed to dissolve which analytical technique is most useful in presymptomatic FTLD. Perhaps it would be most accurate to use a combination of both techniques.

Resting-state fMRI

We have demonstrated that alterations in frontoinsula connectivity within the salience network (SN) and in posterior cingulate cortex connectivity within the default mode network (DMN) can be detected many years before symptom onset using resting-state fMRI. Reduced SN connectivity is a consistent finding in patients with FTD,[78, 88, 108-112] whereas both increases and decreases in DMN connectivity in FTD have been found across studies.[78, 88, 108, 110-113]

Several studies have investigated specific functional connectivity alterations in the symptomatic or presymptomatic stage for the different genetic forms of FTLD. Presymptomatic *MAPT* carriers were shown to have reduced DMN connectivity between a precuneus seed and the lateral temporal and medial prefrontal cortices and increased connectivity between the precuneus seed and the medial parietal lobe, whereas no SN alterations were found by investigating a seed in the frontoinsula.[112] No studies have investigated functional connectivity in the symptomatic stage of *MAPT* mutation carriers specifically.

Reduced SN connectivity in the bilateral frontal lobe and enhanced DMN connectivity in the left angular gyrus, temporal lobe and posterior cingulate cortex were demonstrated in FTD patients with a *GRN* mutation using independent component analysis (ICA).[78] Results regarding presymptomatic *GRN* mutation carriers differ across studies, all using ICA: one study investigated the SN and DMN and revealed increased SN connectivity in the medial frontal cortex and no DMN alterations;[78] another study investigated the DMN, a ventral and dorsal SN, executive network, attentive network and bilateral frontoparietal networks and showed decreased connectivity of the superior parietal cortex in the frontoparietal network and increased right precentral gyrus connectivity in the executive network, without changes in the other networks;[81] a third study found

no functional connectivity changes in the DMN, SN, bilateral executive networks and the sensorimotor network.[80]

Patients with the *C9orf72* repeat expansion were studied using seed-based analyses, with seeds in the frontoinsula, right angular gyrus, and right precentral gyrus to investigate the SN, DMN and sensorimotor network (typically affected in ALS), respectively. This revealed reduced connectivity between the frontoinsula and ACC, medial superior frontal gyri, anterior insulae, and thalami and between the right precentral gyrus and bilateral precentral and postcentral gyri, insulae, striatum and thalami, without changes in right angular gyrus connectivity.[100] No resting-state fMRI studies have been conducted in the presymptomatic stage in *C9orf72* repeat expansion carriers thus far.

Since all of the abovementioned studies had relatively small study groups and applied different analytical strategies, it is difficult to draw firm conclusions about mutation-specific connectivity alterations, as demonstrated by the large variation of results across the three studies in presymptomatic *GRN* mutation carriers.

Currently three different analytical approaches are commonly applied: seed-based analyses, ICA, and graph theory.[114]

In seed-based analysis a specific region of interest is chosen as seed region for the analysis. Spontaneous fluctuations in BOLD signal within this region are entered as regressor in a general linear model analysis, in order to investigate functional connectivity between the seed and each other voxel in the brain. BOLD signals in the white matter and cerebrospinal fluid are thought to be influenced by noise related to cardiac and respiratory signals, and can therefore be included in the model as confound regressors. Since we had a clear hypothesis about involvement of the anterior midcingulate cortex and frontoinsula from studies in patients with FTLD, we chose this method in our study. However, an obvious disadvantage of this approach is the requisite of a priori defining a region of interest, thereby ignoring potential effects between other regions.[114, 115]

Using ICA, there is no need to define a region of interest. This method decomposes an fMRI data set into spatial components that consist of brain regions with similar BOLD signal fluctuations over time, thus regions that are functionally connected. These components include resting-state networks, but also components of noise signals. The user has to manually distinguish potential components of interest from noise components.[114, 115] Recently, a method named FMRIB's ICA-based

X-noiseifier (FIX), has been developed that allows for automatic classification and removal of structured noise-components, such as scanner artefacts, which could improve the quality of the data.[116]
Graph theory analysis is a relatively new approach in which the brain is regarded as one large functional network. It requires defining certain functionally homogeneous brain regions, named nodes. Subsequently, functional connectivity between all possible pairs of nodes is investigated, resulting in a so-called connectivity matrix. Measures of interest include the number of connections of a node and average length of node-to-node connections for example. This technique demonstrated a small world topology in the brain, meaning that there are certain critical nodes, named hubs, with a large number of connections, thereby providing a connection of their neighboring nodes with many other nodes using only few connections.[114, 115]

It remains to be investigated which technique is most appropriate to be used for clinical applications. Harmonization of analytical strategies across resting-state fMRI studies would enable a more accurate comparison of results.

A relatively new technique under investigation is pharmacological resting-state fMRI, which studies the influence of pharmacological challenges on functional connectivity.[117] It has been hypothesized that cholinergic and serotonergic enhancement using pharmacological agents will affect functional connectivity and that this might aid to the differential diagnosis between different types of dementia at an early disease stage. However, studies investigating this hypothesis are lacking to date.

Arterial spin labeling

Using ASL, we found a longitudinal decline in cerebral blood flow (CBF) in frontal, temporal, parietal and subcortical regions in presymptomatic *MAPT* and *GRN* mutations carriers, with the strongest decline for the subjects that converted to clinical FTD during the follow-up period. Frontal and subcortical hypoperfusion is a consistent finding in patients with FTD.[118-121] Temporal hypoperfusion is not commonly reported in ASL studies of FTD, but this might reflect incomplete brain coverage in previous studies, since temporal hypometabolism has been demonstrated in FTD using position emission tomography with 18F-fluorodeoxyglucose (FDG-PET).[122, 123] We assumed that our finding of parietal

hypoperfusion is related to the high proportion of *GRN* mutation carriers in our cohort, since *GRN* mutation are typically associated with a more posterior distribution of pathology.[94, 95, 124] However, no studies have investigated CBF differences using ASL across the three major genetic subtypes of FTLD thus far. A previous study by our group compared brain perfusion in patients with familial FTLD-TDP (in some due to a *GRN* mutation) and patients with a *MAPT* mutation using SPECT and demonstrated hypoperfusion in the precuneus, inferior parietal lobe and right frontal lobe in familial FTLD-TDP, and hypoperfusion in the left temporal and inferior frontal gyri in the *MAPT* group.[125] Accordingly, *GRN* mutations were previously shown to be associated with hypoperfusion in right dorsolateral frontal, posterior temporal and inferior parietal cortices, hippocampus and posterior cingulate cortex compared to other FTD patients using SPECT.[83] One FDG-PET study investigated presymptomatic and early symptomatic *GRN* mutation carriers and showed asymmetric hypometabolism in frontal and anterior temporal lobes.[126] Other studies investigating brain perfusion or metabolism in the genetic groups are lacking. Therefore, additional studies comparing larger groups of genetic FTLD subtypes are needed to elucidate mutation-specific changes.

FDG-PET is the most commonly applied method to investigate brain function in clinical practice. Despite the fact that ASL has several major advantages over FDG-PET, including lower costs, non-invasiveness, absence of radiation exposure, wide availability of MRI scanners, and the possibility to combine ASL with other MRI techniques in a single session,[127] several methodological issues in the acquisition and processing of ASL scans have hampered its clinical application.[128] For instance, the choice of labeling approach has been an important issue. The two most common methods are continuous labeling and pulsed labeling. In continuous ASL, arterial blood is continuously labeled using a long radiofrequency pulse as it passes through a particular labeling plane, whereas in pulsed ASL a short radiofrequency pulse is applied to label a thick slab of arterial blood. Continuous ASL has proven to provide a higher signal to noise ratio, whereas pulsed ASL has a better labeling efficiency. Therefore, pseudo-continuous ASL has been developed, which combines the advantages of both techniques by applying many short pulses at a high rate.[128-130] Moreover, the wider availability of 3 Tesla MRI scanners has improved the quality of ASL acquisition, since higher field strength is associated with higher signal to noise ratio.[127] Recently, Alsop *et al*[128] have published a

consensus statement on recommended implementations of ASL of the brain in the clinical setting, which will hopefully result in a more harmonized use of ASL across studies, thereby enhancing interpretability of findings and making ASL more suitable for clinical application.

Regarding the development of PET, in analogy with ^{11}C-PIB-PET allowing in vivo detection of β-amyloid plaques for AD,[131] there is a search for PET ligands for FTLD pathology. Several compounds binding tau have been developed, and the first exploratory studies in FTD with *MAPT* mutations with [F-18]-T807 were promising.[132] Perhaps this could provide a very early marker for tau pathology, however additional research is needed to investigate its applicability.

CONCLUSION

Knowledge about genetics in FTLD has learned us a lot about the pathophysiology of the disease, which is crucial for the development of disease-modifying agents. In the current study, we have expanded the phenotypical characterization of genetic FTLD in both the symptomatic and presymptomatic disease stage.

We reported an atypical presentation of symmetrical CBS due to a *GRN* mutation in a patient without a positive family history, thereby further expanding the great diversity in clinical manifestations of *GRN* mutations and demonstrating that genetic screening should also be considered in sporadic cases. Secondly, we provided a clinicopathological characterization of the *C9orf72* repeat expansion, which is associated with a variety of clinical presentations, including bvFTD, PPA, ALS, with FTLD-TDP type B as the most common pathological diagnosis. Furthermore, we have demonstrated that this repeat expansion is a frequent cause of both familial and sporadic FTLD and ALS. Additional studies are needed to elucidate the pathomechanism of the *C9orf72* repeat expansion, hopefully providing a target for a therapeutic intervention. Moreover, there still remain families with autosomal dominant FTLD, for which the causing genetic defect remains to be determined.

Finally, we have demonstrated that using neuropsychological assessment and MRI presymptomatic changes can be detected in carriers of *GRN* and *MAPT*

mutations. Our study suggests that the first brain changes in presymptomatic FTLD can be detected using resting-state fMRI, even more than fifteen years before expected symptom onset. However, these functional connectivity alterations were not predictive for clinical disease manifestation. Conversely, changes on volumetric MRI, DTI, and ASL seem to strongly accelerate in close proximity to the onset of symptoms. Therefore, DTI might provide an accurate baseline predictor of conversion to the clinical disease stage. Moreover, the moment when white matter impairment is detectable using DTI, might be the ideal starting point of a therapy to prevent further disease progression, and thereby clinical disease manifestation. Overall, these various MRI methods seem to provide sensitive biomarkers for presymptomatic FTLD, and could be used to measure effects of therapy in future clinical trials. Perhaps a multimodal approach would give the highest accuracy. Additional and larger studies are needed to explore the specific distribution of imaging abnormalities in the presymptomatic stage of FTLD for each genetic subtype, especially since current therapy approaches are directed at specific pathophysiological targets for each mutation. Finally, harmonization of MRI acquisition and analysis techniques would improve interpretability results across different studies.

As the ultimate goal is to find a treatment for patients with FTLD, the ongoing trials with LMTX in patients with tau pathology and SAHA in patients with *GRN* mutations are of extraordinary importance. A positive therapeutic effect of one of these agents would give an enormous boost to the research field of FTLD and AD, as these conditions are generally considered untreatable thus far.

REFERENCES

1. Josephs KA, Hodges JR, Snowden JS, Mackenzie IR, Neumann M, Mann DM, Dickson DW. Neuropathological background of phenotypical variability in frontotemporal dementia. Acta Neuropathol. 2011 Aug;122(2):137-53.
2. Rohrer JD, Lashley T, Schott JM, Warren JE, Mead S, Isaacs AM, Beck J, Hardy J, de Silva R, Warrington E, Troakes C, Al-Sarraj S, King A, Borroni B, Clarkson MJ, Ourselin S, Holton JL, Fox NC, Revesz T, Rossor MN, Warren JD. Clinical and neuroanatomical signatures of tissue pathology in frontotemporal lobar degeneration. Brain. 2011 Sep;134(Pt 9):2565-81.
3. Hodges JR, Davies RR, Xuereb JH, Casey B, Broe M, Bak TH, Kril JJ, Halliday GM. Clinicopathological correlates in frontotemporal dementia. Ann Neurol. 2004 Sep;56(3):399-406.
4. Baborie A, Griffiths TD, Jaros E, Momeni P, McKeith IG, Burn DJ, Keir G, Larner AJ, Mann DM, Perry R. Frontotemporal dementia in elderly individuals. Arch Neurol. 2012 Aug;69(8):1052-60.
5. Yokoyama JS, Sirkis DW, Miller BL. C9ORF72 hexanucleotide repeats in behavioral and motor neuron disease: clinical heterogeneity and pathological diversity. Am J Neurodegener Dis. 2014;3(1):1-18.
6. Mendez MF, Joshi A, Tassniyom K, Teng E, Shapira JS. Clinicopathologic differences among patients with behavioral variant frontotemporal dementia. Neurology. 2013 Feb 5;80(6):561-8.
7. Balasa M, Gelpi E, Antonell A, Rey MJ, Sanchez-Valle R, Molinuevo JL, Llado A, Neurological Tissue Bank/University of Barcelona/Hospital Clinic NTBUBHCCG. Clinical features and APOE genotype of pathologically proven early-onset Alzheimer disease. Neurology. 2011 May 17;76(20):1720-5.
8. Forman MS, Farmer J, Johnson JK, Clark CM, Arnold SE, Coslett HB, Chatterjee A, Hurtig HI, Karlawish JH, Rosen HJ, Van Deerlin V, Lee VM, Miller BL, Trojanowski JQ, Grossman M. Frontotemporal dementia: clinicopathological correlations. Ann Neurol. 2006 Jun;59(6):952-62.
9. Lee SE, Rabinovici GD, Mayo MC, Wilson SM, Seeley WW, DeArmond SJ, Huang EJ, Trojanowski JQ, Growdon ME, Jang JY, Sidhu M, See TM, Karydas AM, Gorno-Tempini ML, Boxer AL, Weiner MW, Geschwind MD, Rankin KP, Miller BL. Clinicopathological correlations in corticobasal degeneration. Ann Neurol. 2011 Aug;70(2):327-40.
10. Donker Kaat L, Boon AJ, Kamphorst W, Ravid R, Duivenvoorden HJ, van Swieten JC. Frontal presentation in progressive supranuclear palsy. Neurology. 2007 Aug 21;69(8):723-9.
11. Hassan A, Parisi JE, Josephs KA. Autopsy-proven progressive supranuclear palsy presenting as behavioral variant frontotemporal dementia. Neurocase. 2012;18(6):478-88.
12. Respondek G, Stamelou M, Kurz C, Ferguson LW, Rajput A, Chiu WZ, van Swieten JC, Troakes C, Al Sarraj S, Gelpi E, Gaig C, Tolosa E, Oertel WH, Giese A, Roeber S, Arzberger T, Wagenpfeil S, Hoglinger GU, Movement Disorder Society-endorsed PSPSG. The phenotypic spectrum of progressive supranuclear palsy: a retrospective multicenter study of 100 definite cases. Mov Disord. 2014 Dec;29(14):1758-66.
13. Giovagnoli AR, Di Fede G, Aresi A, Reati F, Rossi G, Tagliavini F. Atypical frontotemporal dementia as a new clinical phenotype of Gerstmann-Straussler-Scheinker disease with the PrP-P102L mutation. Description of a previously unreported Italian family. Neurol Sci. 2008 Dec;29(6):405-

10.

14. Jansen C, Parchi P, Capellari S, Strammiello R, Dopper EG, van Swieten JC, Kamphorst W, Rozemuller AJ. A second case of Gerstmann-Straussler-Scheinker disease linked to the G131V mutation in the prion protein gene in a Dutch patient. J Neuropathol Exp Neurol. 2011 Aug;70(8):698-702.
15. Kumar N, Boeve BF, Boot BP, Orr CF, Duffy J, Woodruff BK, Nair AK, Ellison J, Kuntz K, Kantarci K, Jack CR, Jr., Westmoreland BF, Fields JA, Baker M, Rademakers R, Parisi JE, Dickson DW. Clinical characterization of a kindred with a novel 12-octapeptide repeat insertion in the prion protein gene. Arch Neurol. 2011 Sep;68(9):1165-70.
16. Woulfe J, Kertesz A, Frohn I, Bauer S, George-Hyslop PS, Bergeron C. Gerstmann-Straussler-Scheinker disease with the Q217R mutation mimicking frontotemporal dementia. Acta Neuropathol. 2005 Sep;110(3):317-9.
17. Curral R, Correia R, Lopes R, Maia D, Rio E, Bastos-Leite AJ. Dementia in multiple sclerosis: report of a case with cortical gray matter involvement and frontotemporal-like clinical features. Psychiatry Res. 2012 May 31;202(2):172-4.
18. Zarei M, Chandran S, Compston A, Hodges J. Cognitive presentation of multiple sclerosis: evidence for a cortical variant. J Neurol Neurosurg Psychiatry. 2003 Jul;74(7):872-7.
19. Alexander SK, Brown JM, Graham A, Nestor PJ. CADASIL presenting with a behavioural variant frontotemporal dementia phenotype. J Clin Neurosci. 2014 Jan;21(1):165-7.
20. Molloy A, Cassidy E, Ryan A, O OT. VGKC positive autoimmune encephalopathy mimicking dementia. BMJ Case Rep. 2011;2011.
21. Wong TH, Chiu WZ, Breedveld GJ, Li KW, Verkerk AJ, Hondius D, Hukema RK, Seelaar H, Frick P, Severijnen LA, Lammers GJ, Lebbink JH, van Duinen SG, Kamphorst W, Rozemuller AJ, Netherlands Brain B, Bakker EB, International Parkinsonism Genetics N, Neumann M, Willemsen R, Bonifati V, Smit AB, van Swieten J. PRKAR1B mutation associated with a new neurodegenerative disorder with unique pathology. Brain. 2014 May;137(Pt 5):1361-73.
22. Siuda J, Fujioka S, Wszolek ZK. Parkinsonian syndrome in familial frontotemporal dementia. Parkinsonism Relat Disord. 2014 Sep;20(9):957-64.
23. Dickson DW, Bergeron C, Chin SS, Duyckaerts C, Horoupian D, Ikeda K, Jellinger K, Lantos PL, Lippa CF, Mirra SS, Tabaton M, Vonsattel JP, Wakabayashi K, Litvan I, Office of Rare Diseases of the National Institutes of H. Office of Rare Diseases neuropathologic criteria for corticobasal degeneration. J Neuropathol Exp Neurol. 2002 Nov;61(11):935-46.
24. Boeve BF, Maraganore DM, Parisi JE, Ahlskog JE, Graff-Radford N, Caselli RJ, Dickson DW, Kokmen E, Petersen RC. Pathologic heterogeneity in clinically diagnosed corticobasal degeneration. Neurology. 1999 Sep 11;53(4):795-800.
25. Wadia PM, Lang AE. The many faces of corticobasal degeneration. Parkinsonism Relat Disord. 2007;13 Suppl 3:S336-40.
26. Tartaglia MC, Sidhu M, Laluz V, Racine C, Rabinovici GD, Creighton K, Karydas A, Rademakers R, Huang EJ, Miller BL, DeArmond SJ, Seeley WW. Sporadic corticobasal syndrome due to FTLD-TDP. Acta Neuropathol. 2010 Mar;119(3):365-74.

27. Ouchi H, Toyoshima Y, Tada M, Oyake M, Aida I, Tomita I, Satoh A, Tsujihata M, Takahashi H, Nishizawa M, Shimohata T. Pathology and sensitivity of current clinical criteria in corticobasal syndrome. Mov Disord. 2014 Feb;29(2):238-44.

28. Armstrong MJ, Litvan I, Lang AE, Bak TH, Bhatia KP, Borroni B, Boxer AL, Dickson DW, Grossman M, Hallett M, Josephs KA, Kertesz A, Lee SE, Miller BL, Reich SG, Riley DE, Tolosa E, Troster AI, Vidailhet M, Weiner WJ. Criteria for the diagnosis of corticobasal degeneration. Neurology. 2013 Jan 29;80(5):496-503.

29. Alexander SK, Rittman T, Xuereb JH, Bak TH, Hodges JR, Rowe JB. Validation of the new consensus criteria for the diagnosis of corticobasal degeneration. J Neurol Neurosurg Psychiatry. 2014 Aug;85(8):925-9.

30. Boeve BF, Boylan KB, Graff-Radford NR, DeJesus-Hernandez M, Knopman DS, Pedraza O, Vemuri P, Jones D, Lowe V, Murray ME, Dickson DW, Josephs KA, Rush BK, Machulda MM, Fields JA, Ferman TJ, Baker M, Rutherford NJ, Adamson J, Wszolek ZK, Adeli A, Savica R, Boot B, Kuntz KM, Gavrilova R, Reeves A, Whitwell J, Kantarci K, Jack CR, Jr., Parisi JE, Lucas JA, Petersen RC, Rademakers R. Characterization of frontotemporal dementia and/or amyotrophic lateral sclerosis associated with the GGGGCC repeat expansion in C9ORF72. Brain. 2012 Mar;135(Pt 3):765-83.

31. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, Nicholson AM, Finch NA, Flynn H, Adamson J, Kouri N, Wojtas A, Sengdy P, Hsiung GY, Karydas A, Seeley WW, Josephs KA, Coppola G, Geschwind DH, Wszolek ZK, Feldman H, Knopman DS, Petersen RC, Miller BL, Dickson DW, Boylan KB, Graff-Radford NR, Rademakers R. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron. 2011 Oct 20;72(2):245-56.

32. van Rheenen W, van Blitterswijk M, Huisman MH, Vlam L, van Doormaal PT, Seelen M, Medic J, Dooijes D, de Visser M, van der Kooi AJ, Raaphorst J, Schelhaas HJ, van der Pol WL, Veldink JH, van den Berg LH. Hexanucleotide repeat expansions in C9ORF72 in the spectrum of motor neuron diseases. Neurology. 2012 Aug 28;79(9):878-82.

33. Byrne S, Elamin M, Bede P, Shatunov A, Walsh C, Corr B, Heverin M, Jordan N, Kenna K, Lynch C, McLaughlin RL, Iyer PM, O'Brien C, Phukan J, Wynne B, Bokde AL, Bradley DG, Pender N, Al-Chalabi A, Hardiman O. Cognitive and clinical characteristics of patients with amyotrophic lateral sclerosis carrying a C9orf72 repeat expansion: a population-based cohort study. Lancet Neurol. 2012 Mar;11(3):232-40.

34. Rohrer JD, Isaacs AM, Mizielinska S, Mead S, Lashley T, Wray S, Sidle K, Fratta P, Orrell RW, Hardy J, Holton J, Revesz T, Rossor MN, Warren JD. C9orf72 expansions in frontotemporal dementia and amyotrophic lateral sclerosis. Lancet Neurol. 2015 Mar;14(3):291-301.

35. Cruts M, Gijselinck I, Van Langenhove T, van der Zee J, Van Broeckhoven C. Current insights into the C9orf72 repeat expansion diseases of the FTLD/ALS spectrum. Trends Neurosci. 2013 Aug;36(8):450-9.

36. Dobson-Stone C, Hallupp M, Bartley L, Shepherd CE, Halliday GM, Schofield PR, Hodges JR, Kwok JB. C9ORF72 repeat expansion in clinical and neuropathologic frontotemporal dementia cohorts. Neurology. 2012 Sep 4;79(10):995-1001.

37. Hsiung GY, DeJesus-Hernandez M, Feldman HH, Sengdy P, Bouchard-Kerr P, Dwosh E, Butler R, Leung B, Fok A, Rutherford NJ, Baker M, Rademakers R, Mackenzie IR. Clinical and pathological features of familial frontotemporal dementia caused by C9ORF72 mutation on chromosome 9p. Brain. 2012 Mar;135(Pt 3):709-22.

38. Mahoney CJ, Beck J, Rohrer JD, Lashley T, Mok K, Shakespeare T, Yeatman T, Warrington EK, Schott JM, Fox NC, Rossor MN, Hardy J, Collinge J, Revesz T, Mead S, Warren JD. Frontotemporal dementia with the C9ORF72 hexanucleotide repeat expansion: clinical, neuroanatomical and neuropathological features. Brain. 2012 Mar;135(Pt 3):736-50.

39. Snowden JS, Rollinson S, Thompson JC, Harris JM, Stopford CL, Richardson AM, Jones M, Gerhard A, Davidson YS, Robinson A, Gibbons L, Hu Q, DuPlessis D, Neary D, Mann DM, Pickering-Brown SM. Distinct clinical and pathological characteristics of frontotemporal dementia associated with C9ORF72 mutations. Brain. 2012 Mar;135(Pt 3):693-708.

40. Khan BK, Yokoyama JS, Takada LT, Sha SJ, Rutherford NJ, Fong JC, Karydas AM, Wu T, Ketelle RS, Baker MC, Hernandez MD, Coppola G, Geschwind DH, Rademakers R, Lee SE, Rosen HJ, Rabinovici GD, Seeley WW, Rankin KP, Boxer AL, Miller BL. Atypical, slowly progressive behavioural variant frontotemporal dementia associated with C9ORF72 hexanucleotide expansion. J Neurol Neurosurg Psychiatry. 2012 Apr;83(4):358-64.

41. van Blitterswijk M, DeJesus-Hernandez M, Rademakers R. How do C9ORF72 repeat expansions cause amyotrophic lateral sclerosis and frontotemporal dementia: can we learn from other noncoding repeat expansion disorders? Curr Opin Neurol. 2012 Dec;25(6):689-700.

42. van Blitterswijk M, Mullen B, Wojtas A, Heckman MG, Diehl NN, Baker MC, DeJesus-Hernandez M, Brown PH, Murray ME, Hsiung GY, Stewart H, Karydas AM, Finger E, Kertesz A, Bigio EH, Weintraub S, Mesulam M, Hatanpaa KJ, White CL, 3rd, Neumann M, Strong MJ, Beach TG, Wszolek ZK, Lippa C, Caselli R, Petrucelli L, Josephs KA, Parisi JE, Knopman DS, Petersen RC, Mackenzie IR, Seeley WW, Grinberg LT, Miller BL, Boylan KB, Graff-Radford NR, Boeve BF, Dickson DW, Rademakers R. Genetic modifiers in carriers of repeat expansions in the C9ORF72 gene. Mol Neurodegener. 2014;9:38.

43. Gallagher MD, Suh E, Grossman M, Elman L, McCluskey L, Van Swieten JC, Al-Sarraj S, Neumann M, Gelpi E, Ghetti B, Rohrer JD, Halliday G, Van Broeckhoven C, Seilhean D, Shaw PJ, Frosch MP, Alafuzoff I, Antonell A, Bogdanovic N, Brooks W, Cairns NJ, Cooper-Knock J, Cotman C, Cras P, Cruts M, De Deyn PP, DeCarli C, Dobson-Stone C, Engelborghs S, Fox N, Galasko D, Gearing M, Gijselinck I, Grafman J, Hartikainen P, Hatanpaa KJ, Highley JR, Hodges J, Hulette C, Ince PG, Jin LW, Kirby J, Kofler J, Kril J, Kwok JB, Levey A, Lieberman A, Llado A, Martin JJ, Masliah E, McDermott CJ, McKee A, McLean C, Mead S, Miller CA, Miller J, Munoz DG, Murrell J, Paulson H, Piguet O, Rossor M, Sanchez-Valle R, Sano M, Schneider J, Silbert LC, Spina S, van der Zee J, Van Langenhove T, Warren J, Wharton SB, White CL, 3rd, Woltjer RL, Trojanowski JQ, Lee VM, Van Deerlin V, Chen-Plotkin AS. TMEM106B is a genetic modifier of frontotemporal lobar degeneration with C9orf72 hexanucleotide repeat expansions. Acta Neuropathol. 2014 Mar;127(3):407-18.

44. Renton AE, Majounie E, Waite A, Simon-Sanchez J, Rollinson S, Gibbs JR, Schymick JC, Laaksovirta H, van Swieten JC, Myllykangas L, Kalimo H, Paetau A, Abramzon Y, Remes AM, Kaganovich A, Scholz SW, Duckworth J, Ding J, Harmer DW, Hernandez DG, Johnson JO, Mok K,

Ryten M, Trabzuni D, Guerreiro RJ, Orrell RW, Neal J, Murray A, Pearson J, Jansen IE, Sondervan D, Seelaar H, Blake D, Young K, Halliwell N, Callister JB, Toulson G, Richardson A, Gerhard A, Snowden J, Mann D, Neary D, Nalls MA, Peuralinna T, Jansson L, Isoviita VM, Kaivorinne AL, Holtta-Vuori M, Ikonen E, Sulkava R, Benatar M, Wuu J, Chio A, Restagno G, Borghero G, Sabatelli M, Consortium I, Heckerman D, Rogaeva E, Zinman L, Rothstein JD, Sendtner M, Drepper C, Eichler EE, Alkan C, Abdullaev Z, Pack SD, Dutra A, Pak E, Hardy J, Singleton A, Williams NM, Heutink P, Pickering-Brown S, Morris HR, Tienari PJ, Traynor BJ. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron. 2011 Oct 20;72(2):257-68.

45. Mackenzie IR, Frick P, Neumann M. The neuropathology associated with repeat expansions in the C9ORF72 gene. Acta Neuropathol. 2014 Mar;127(3):347-57.

46. Mackenzie IR, Arzberger T, Kremmer E, Troost D, Lorenzl S, Mori K, Weng SM, Haass C, Kretzschmar HA, Edbauer D, Neumann M. Dipeptide repeat protein pathology in C9ORF72 mutation cases: clinico-pathological correlations. Acta Neuropathol. 2013 Dec;126(6):859-79.

47. Al-Sarraj S, King A, Troakes C, Smith B, Maekawa S, Bodi I, Rogelj B, Al-Chalabi A, Hortobagyi T, Shaw CE. p62 positive, TDP-43 negative, neuronal cytoplasmic and intranuclear inclusions in the cerebellum and hippocampus define the pathology of C9orf72-linked FTLD and MND/ALS. Acta Neuropathol. 2011 Dec;122(6):691-702.

48. Ash PE, Bieniek KF, Gendron TF, Caulfield T, Lin WL, Dejesus-Hernandez M, van Blitterswijk MM, Jansen-West K, Paul JW, 3rd, Rademakers R, Boylan KB, Dickson DW, Petrucelli L. Unconventional translation of C9ORF72 GGGGCC expansion generates insoluble polypeptides specific to c9FTD/ALS. Neuron. 2013 Feb 20;77(4):639-46.

49. Mori K, Weng SM, Arzberger T, May S, Rentzsch K, Kremmer E, Schmid B, Kretzschmar HA, Cruts M, Van Broeckhoven C, Haass C, Edbauer D. The C9orf72 GGGGCC repeat is translated into aggregating dipeptide-repeat proteins in FTLD/ALS. Science. 2013 Mar 15;339(6125):1335-8.

50. van der Zee J, Gijselinck I, Dillen L, Van Langenhove T, Theuns J, Engelborghs S, Philtjens S, Vandenbulcke M, Sleegers K, Sieben A, Baumer V, Maes G, Corsmit E, Borroni B, Padovani A, Archetti S, Perneczky R, Diehl-Schmid J, de Mendonca A, Miltenberger-Miltenyi G, Pereira S, Pimentel J, Nacmias B, Bagnoli S, Sorbi S, Graff C, Chiang HH, Westerlund M, Sanchez-Valle R, Llado A, Gelpi E, Santana I, Almeida MR, Santiago B, Frisoni G, Zanetti O, Bonvicini C, Synofzik M, Maetzler W, Vom Hagen JM, Schols L, Heneka MT, Jessen F, Matej R, Parobkova E, Kovacs GG, Strobel T, Sarafov S, Tournev I, Jordanova A, Danek A, Arzberger T, Fabrizi GM, Testi S, Salmon E, Santens P, Martin JJ, Cras P, Vandenberghe R, De Deyn PP, Cruts M, Van Broeckhoven C, van der Zee J, Gijselinck I, Dillen L, Van Langenhove T, Theuns J, Philtjens S, Sleegers K, Baumer V, Maes G, Corsmit E, Cruts M, Van Broeckhoven C, van der Zee J, Gijselinck I, Dillen L, Van Langenhove T, Philtjens S, Theuns J, Sleegers K, Baumer V, Maes G, Cruts M, Van Broeckhoven C, Engelborghs S, De Deyn PP, Cras P, Engelborghs S, De Deyn PP, Vandenbulcke M, Vandenbulcke M, Borroni B, Padovani A, Archetti S, Perneczky R, Diehl-Schmid J, Synofzik M, Maetzler W, Muller Vom Hagen J, Schols L, Synofzik M, Maetzler W, Muller Vom Hagen J, Schols L, Heneka MT, Jessen F, Ramirez A, Kurzwelly D, Sachtleben C, Mairer W, de Mendonca A, Miltenberger-Miltenyi G, Pereira S, Firmo C, Pimentel J, Sanchez-Valle R, Llado A, Antonell A, Molinuevo J, Gelpi E, Graff C, Chiang HH, Westerlund M, Graff C, Kinhult Stahlbom A, Thonberg H, Nennesmo I, Borjesson-Hanson A,

Nacmias B, Bagnoli S, Sorbi S, Bessi V, Piaceri I, Santana I, Santiago B, Santana I, Helena Ribeiro M, Rosario Almeida M, Oliveira C, Massano J, Garret C, Pires P, Frisoni G, Zanetti O, Bonvicini C, Sarafov S, Tournev I, Jordanova A, Tournev I, Kovacs GG, Strobel T, Heneka MT, Jessen F, Ramirez A, Kurzwelly D, Sachtleben C, Mairer W, Jessen F, Matej R, Parobkova E, Danel A, Arzberger T, Maria Fabrizi G, Testi S, Ferrari S, Cavallaro T, Salmon E, Santens P, Cras P, European Early-Onset Dementia C. A pan-European study of the C9orf72 repeat associated with FTLD: geographic prevalence, genomic instability, and intermediate repeats. Hum Mutat. 2013 Feb;34(2):363-73.

51. Koppers M, Blokhuis AM, Westeneng HJ, Terpstra ML, Zundel CA, Vieira de Sa R, Schellevis RD, Waite AJ, Blake DJ, Veldink JH, van den Berg LH, Jeroen Pasterkamp R. C9orf72 ablation in mice does not cause motor neuron degeneration or motor deficits. Ann Neurol. 2015 Jun 5.

52. Hukema RK, Riemslagh FW, Melhem S, van der Linde HC, Severijnen L, Edbauer D, Maas A, Charlet-Berguerand N, Willemsen R, van Swieten JC. A new inducible transgenic mouse model for C9orf72-associated GGGGCC repeat expansion supports a gain-of-function mechanism in C9orf72 associated ALS and FTD. Acta Neuropathol Commun. 2014 Dec 13;2(1):166.

53. Mizielinska S, Gronke S, Niccoli T, Ridler CE, Clayton EL, Devoy A, Moens T, Norona FE, Woollacott IO, Pietrzyk J, Cleverley K, Nicoll AJ, Pickering-Brown S, Dols J, Cabecinha M, Hendrich O, Fratta P, Fisher EM, Partridge L, Isaacs AM. C9orf72 repeat expansions cause neurodegeneration in Drosophila through arginine-rich proteins. Science. 2014 Sep 5;345(6201):1192-4.

54. Chew J, Gendron TF, Prudencio M, Sasaguri H, Zhang YJ, Castanedes-Casey M, Lee CW, Jansen-West K, Kurti A, Murray ME, Bieniek KF, Bauer PO, Whitelaw EC, Rousseau L, Stankowski JN, Stetler C, Daughrity LM, Perkerson EA, Desaro P, Johnston A, Overstreet K, Edbauer D, Rademakers R, Boylan KB, Dickson DW, Fryer JD, Petrucelli L. Neurodegeneration. C9ORF72 repeat expansions in mice cause TDP-43 pathology, neuronal loss, and behavioral deficits. Science. 2015 Jun 5;348(6239):1151-4.

55. van Blitterswijk M, van Es MA, Hennekam EA, Dooijes D, van Rheenen W, Medic J, Bourque PR, Schelhaas HJ, van der Kooi AJ, de Visser M, de Bakker PI, Veldink JH, van den Berg LH. Evidence for an oligogenic basis of amyotrophic lateral sclerosis. Hum Mol Genet. 2012 Sep 1;21(17):3776-84.

56. Mok K, Traynor BJ, Schymick J, Tienari PJ, Laaksovirta H, Peuralinna T, Myllykangas L, Chio A, Shatunov A, Boeve BF, Boxer AL, DeJesus-Hernandez M, Mackenzie IR, Waite A, Williams N, Morris HR, Simon-Sanchez J, van Swieten JC, Heutink P, Restagno G, Mora G, Morrison KE, Shaw PJ, Rollinson PS, Al-Chalabi A, Rademakers R, Pickering-Brown S, Orrell RW, Nalls MA, Hardy J. Chromosome 9 ALS and FTD locus is probably derived from a single founder. Neurobiol Aging. 2012 Jan;33(1):209 e3-8.

57. Beck J, Poulter M, Hensman D, Rohrer JD, Mahoney CJ, Adamson G, Campbell T, Uphill J, Borg A, Fratta P, Orrell RW, Malaspina A, Rowe J, Brown J, Hodges J, Sidle K, Polke JM, Houlden H, Schott JM, Fox NC, Rossor MN, Tabrizi SJ, Isaacs AM, Hardy J, Warren JD, Collinge J, Mead S. Large C9orf72 hexanucleotide repeat expansions are seen in multiple neurodegenerative syndromes and are more frequent than expected in the UK population. Am J Hum Genet. 2013 Mar 7;92(3):345-53.

58. Tsai RM, Boxer AL. Treatment of frontotemporal dementia. Curr Treat Options Neurol. 2014 Nov;16(11):319.

59. Wischik CM, Staff RT, Wischik DJ, Bentham P, Murray AD, Storey JM, Kook KA, Harrington CR. Tau aggregation inhibitor therapy: an exploratory phase 2 study in mild or moderate Alzheimer's disease. J Alzheimers Dis. 2015 Jan 1;44(2):705-20.

60. https://clinicaltrials.gov/ct2/show/NCT01626378.

61. http://taurx.com/007-clinical-study.html.

62. Coppola G, Karydas A, Rademakers R, Wang Q, Baker M, Hutton M, Miller BL, Geschwind DH. Gene expression study on peripheral blood identifies progranulin mutations. Ann Neurol. 2008 Jul;64(1):92-6.

63. Finch N, Baker M, Crook R, Swanson K, Kuntz K, Surtees R, Bisceglio G, Rovelet-Lecrux A, Boeve B, Petersen RC, Dickson DW, Younkin SG, Deramecourt V, Crook J, Graff-Radford NR, Rademakers R. Plasma progranulin levels predict progranulin mutation status in frontotemporal dementia patients and asymptomatic family members. Brain. 2009 Mar;132(Pt 3):583-91.

64. Ghidoni R, Benussi L, Glionna M, Franzoni M, Binetti G. Low plasma progranulin levels predict progranulin mutations in frontotemporal lobar degeneration. Neurology. 2008 Oct 14;71(16):1235-9.

65. Sleegers K, Brouwers N, Van Damme P, Engelborghs S, Gijselinck I, van der Zee J, Peeters K, Mattheijssens M, Cruts M, Vandenberghe R, De Deyn PP, Robberecht W, Van Broeckhoven C. Serum biomarker for progranulin-associated frontotemporal lobar degeneration. Ann Neurol. 2009 May;65(5):603-9.

66. Cenik B, Sephton CF, Dewey CM, Xian X, Wei S, Yu K, Niu W, Coppola G, Coughlin SE, Lee SE, Dries DR, Almeida S, Geschwind DH, Gao FB, Miller BL, Farese RV, Jr., Posner BA, Yu G, Herz J. Suberoylanilide hydroxamic acid (vorinostat) up-regulates progranulin transcription: rational therapeutic approach to frontotemporal dementia. J Biol Chem. 2011 May 6;286(18):16101-8.

67. http://www.forumpharma.com/content/innovation-pipeline/hdac-program.

68. https://clinicaltrials.gov/ct2/show/NCT02149160.

69. https://clinicaltrials.gov/ct2/show/NCT01835665.

70. Riboldi G, Zanetta C, Ranieri M, Nizzardo M, Simone C, Magri F, Bresolin N, Comi GP, Corti S. Antisense oligonucleotide therapy for the treatment of C9ORF72 ALS/FTD diseases. Mol Neurobiol. 2014 Dec;50(3):721-32.

71. Torralva T, Roca M, Gleichgerrcht E, Bekinschtein T, Manes F. A neuropsychological battery to detect specific executive and social cognitive impairments in early frontotemporal dementia. Brain. 2009 May;132(Pt 5):1299-309.

72. Rohrer JD, Nicholas JM, Cash DM, van Swieten J, Dopper E, Jiskoot L, van Minkelen R, Rombouts SA, Cardoso MJ, Clegg S, Espak M, Mead S, Thomas DL, De Vita E, Masellis M, Black SE, Freedman M, Keren R, MacIntosh BJ, Rogaeva E, Tang-Wai D, Tartaglia MC, Laforce R, Jr., Tagliavini F, Tiraboschi P, Redaelli V, Prioni S, Grisoli M, Borroni B, Padovani A, Galimberti D, Scarpini E, Arighi A, Fumagalli G, Rowe JB, Coyle-Gilchrist I, Graff C, Fallstrom M, Jelic V, Stahlbom AK, Andersson C, Thonberg H, Lilius L, Frisoni GB, Pievani M, Bocchetta M, Benussi L, Ghidoni R, Finger E, Sorbi S, Nacmias B, Lombardi G, Polito C, Warren JD, Ourselin S, Fox NC, Rossor MN. Presymptomatic cognitive and neuroanatomical changes in genetic frontotemporal dementia in the Genetic Frontotemporal dementia Initiative (GENFI) study: a cross-sectional analysis. Lancet Neurol.

2015 Feb 3.

73. Rohrer JD, Warren JD, Barnes J, Mead S, Beck J, Pepple T, Boyes R, Omar R, Collinge J, Stevens JM, Warrington EK, Rossor MN, Fox NC. Mapping the progression of progranulin-associated frontotemporal lobar degeneration. Nat Clin Pract Neurol. 2008 Aug;4(8):455-60.

74. Janssen JC, Schott JM, Cipolotti L, Fox NC, Scahill RI, Josephs KA, Stevens JM, Rossor MN. Mapping the onset and progression of atrophy in familial frontotemporal lobar degeneration. J Neurol Neurosurg Psychiatry. 2005 Feb;76(2):162-8.

75. Geschwind DH, Robidoux J, Alarcon M, Miller BL, Wilhelmsen KC, Cummings JL, Nasreddine ZS. Dementia and neurodevelopmental predisposition: cognitive dysfunction in presymptomatic subjects precedes dementia by decades in frontotemporal dementia. Ann Neurol. 2001 Dec;50(6):741-6.

76. Barandiaran M, Estanga A, Moreno F, Indakoetxea B, Alzualde A, Balluerka N, Marti Masso JF, Lopez de Munain A. Neuropsychological features of asymptomatic c.709-1G>A progranulin mutation carriers. J Int Neuropsychol Soc. 2012 Nov;18(6):1086-90.

77. Hallam BJ, Jacova C, Hsiung GY, Wittenberg D, Sengdy P, Bouchard-Kerr P, Slack P, Rademakers R, Baker M, Chow TW, Levine B, Feldman HH, Mackenzie IR. Early neuropsychological characteristics of progranulin mutation carriers. J Int Neuropsychol Soc. 2014 Aug;20(7):694-703.

78. Borroni B, Alberici A, Cercignani M, Premi E, Serra L, Cerini C, Cosseddu M, Pettenati C, Turla M, Archetti S, Gasparotti R, Caltagirone C, Padovani A, Bozzali M. Granulin mutation drives brain damage and reorganization from preclinical to symptomatic FTLD. Neurobiol Aging. 2012 Oct;33(10):2506-20.

79. Borroni B, Alberici A, Premi E, Archetti S, Garibotto V, Agosti C, Gasparotti R, Di Luca M, Perani D, Padovani A. Brain magnetic resonance imaging structural changes in a pedigree of asymptomatic progranulin mutation carriers. Rejuvenation Res. 2008 Jun;11(3):585-95.

80. Pievani M, Paternico D, Benussi L, Binetti G, Orlandini A, Cobelli M, Magnaldi S, Ghidoni R, Frisoni GB. Pattern of structural and functional brain abnormalities in asymptomatic granulin mutation carriers. Alzheimers Dement. 2014 Oct;10(5 Suppl):S354-S63 e1.

81. Premi E, Formenti A, Gazzina S, Archetti S, Gasparotti R, Padovani A, Borroni B. Effect of TMEM106B polymorphism on functional network connectivity in asymptomatic GRN mutation carriers. JAMA Neurol. 2014 Feb;71(2):216-21.

82. Pardini M, Emberti Gialloreti L, Mascolo M, Benassi F, Abate L, Guida S, Viani E, Dal Monte O, Schintu S, Krueger F, Cocito L. Isolated theory of mind deficits and risk for frontotemporal dementia: a longitudinal pilot study. J Neurol Neurosurg Psychiatry. 2013 Jul;84(7):818-21.

83. Le Ber I, Camuzat A, Hannequin D, Pasquier F, Guedj E, Rovelet-Lecrux A, Hahn-Barma V, van der Zee J, Clot F, Bakchine S, Puel M, Ghanim M, Lacomblez L, Mikol J, Deramecourt V, Lejeune P, de la Sayette V, Belliard S, Vercelletto M, Meyrignac C, Van Broeckhoven C, Lambert JC, Verpillat P, Campion D, Habert MO, Dubois B, Brice A, French research network on FF-M. Phenotype variability in progranulin mutation carriers: a clinical, neuropsychological, imaging and genetic study. Brain. 2008 Mar;131(Pt 3):732-46.

84. Pan PL, Song W, Yang J, Huang R, Chen K, Gong QY, Zhong JG, Shi HC, Shang HF. Gray matter

atrophy in behavioral variant frontotemporal dementia: a meta-analysis of voxel-based morphometry studies. Dement Geriatr Cogn Disord. 2012;33(2-3):141-8.

85. Rankin KP, Gorno-Tempini ML, Allison SC, Stanley CM, Glenn S, Weiner MW, Miller BL. Structural anatomy of empathy in neurodegenerative disease. Brain. 2006 Nov;129(Pt 11):2945-56.

86. Woolley JD, Gorno-Tempini ML, Seeley WW, Rankin K, Lee SS, Matthews BR, Miller BL. Binge eating is associated with right orbitofrontal-insular-striatal atrophy in frontotemporal dementia. Neurology. 2007 Oct 2;69(14):1424-33.

87. Seeley WW, Menon V, Schatzberg AF, Keller J, Glover GH, Kenna H, Reiss AL, Greicius MD. Dissociable intrinsic connectivity networks for salience processing and executive control. J Neurosci. 2007 Feb 28;27(9):2349-56.

88. Zhou J, Greicius MD, Gennatas ED, Growdon ME, Jang JY, Rabinovici GD, Kramer JH, Weiner M, Miller BL, Seeley WW. Divergent network connectivity changes in behavioural variant frontotemporal dementia and Alzheimer's disease. Brain. 2010 May;133(Pt 5):1352-67.

89. Seeley WW, Carlin DA, Allman JM, Macedo MN, Bush C, Miller BL, Dearmond SJ. Early frontotemporal dementia targets neurons unique to apes and humans. Ann Neurol. 2006 Dec;60(6):660-7.

90. Kim EJ, Sidhu M, Gaus SE, Huang EJ, Hof PR, Miller BL, DeArmond SJ, Seeley WW. Selective frontoinsular von Economo neuron and fork cell loss in early behavioral variant frontotemporal dementia. Cereb Cortex. 2012 Feb;22(2):251-9.

91. Santillo AF, Nilsson C, Englund E. von Economo neurones are selectively targeted in frontotemporal dementia. Neuropathol Appl Neurobiol. 2013 Aug;39(5):572-9.

92. Santillo AF, Englund E. Greater loss of von Economo neurons than loss of layer II and III neurons in behavioral variant frontotemporal dementia. Am J Neurodegener Dis. 2014;3(2):64-71.

93. Whitwell JL, Weigand SD, Boeve BF, Senjem ML, Gunter JL, DeJesus-Hernandez M, Rutherford NJ, Baker M, Knopman DS, Wszolek ZK, Parisi JE, Dickson DW, Petersen RC, Rademakers R, Jack CR, Jr., Josephs KA. Neuroimaging signatures of frontotemporal dementia genetics: C9ORF72, tau, progranulin and sporadics. Brain. 2012 Mar;135(Pt 3):794-806.

94. Rohrer JD, Ridgway GR, Modat M, Ourselin S, Mead S, Fox NC, Rossor MN, Warren JD. Distinct profiles of brain atrophy in frontotemporal lobar degeneration caused by progranulin and tau mutations. Neuroimage. 2010 Nov 15;53(3):1070-6.

95. Whitwell JL, Avula R, Senjem ML, Kantarci K, Weigand SD, Samikoglu A, Edmonson HA, Vemuri P, Knopman DS, Boeve BF, Petersen RC, Josephs KA, Jack CR, Jr. Gray and white matter water diffusion in the syndromic variants of frontotemporal dementia. Neurology. 2010 Apr 20;74(16):1279-87.

96. Miyoshi M, Shinotoh H, Wszolek ZK, Strongosky AJ, Shimada H, Arakawa R, Higuchi M, Ikoma Y, Yasuno F, Fukushi K, Irie T, Ito H, Suhara T. In vivo detection of neuropathologic changes in presymptomatic MAPT mutation carriers: a PET and MRI study. Parkinsonism Relat Disord. 2010 Jul;16(6):404-8.

97. Cruchaga C, Fernandez-Seara MA, Seijo-Martinez M, Samaranch L, Lorenzo E, Hinrichs A, Irigoyen J, Maestro C, Prieto E, Marti-Climent JM, Arbizu J, Pastor MA, Pastor P. Cortical atrophy and language network reorganization associated with a novel progranulin mutation. Cereb

Cortex. 2009 Aug;19(8):1751-60.

98. Moreno F, Sala-Llonch R, Barandiaran M, Sanchez-Valle R, Estanga A, Bartres-Faz D, Sistiaga A, Alzualde A, Fernandez E, Marti Masso JF, Lopez de Munain A, Indakoetxea B. Distinctive age-related temporal cortical thinning in asymptomatic granulin gene mutation carriers. Neurobiol Aging. 2013 May;34(5):1462-8.

99. Sha SJ, Takada LT, Rankin KP, Yokoyama JS, Rutherford NJ, Fong JC, Khan B, Karydas A, Baker MC, DeJesus-Hernandez M, Pribadi M, Coppola G, Geschwind DH, Rademakers R, Lee SE, Seeley W, Miller BL, Boxer AL. Frontotemporal dementia due to C9ORF72 mutations: clinical and imaging features. Neurology. 2012 Sep 4;79(10):1002-11.

100. Lee SE, Khazenzon AM, Trujillo AJ, Guo CC, Yokoyama JS, Sha SJ, Takada LT, Karydas AM, Block NR, Coppola G, Pribadi M, Geschwind DH, Rademakers R, Fong JC, Weiner MW, Boxer AL, Kramer JH, Rosen HJ, Miller BL, Seeley WW. Altered network connectivity in frontotemporal dementia with C9orf72 hexanucleotide repeat expansion. Brain. 2014 Nov;137(Pt 11):3047-60.

101. Von Der Heide RJ, Skipper LM, Klobusicky E, Olson IR. Dissecting the uncinate fasciculus: disorders, controversies and a hypothesis. Brain. 2013 Jun;136(Pt 6):1692-707.

102. Agosta F, Scola E, Canu E, Marcone A, Magnani G, Sarro L, Copetti M, Caso F, Cerami C, Comi G, Cappa SF, Falini A, Filippi M. White matter damage in frontotemporal lobar degeneration spectrum. Cereb Cortex. 2012 Dec;22(12):2705-14.

103. Hornberger M, Geng J, Hodges JR. Convergent grey and white matter evidence of orbitofrontal cortex changes related to disinhibition in behavioural variant frontotemporal dementia. Brain. 2011 Sep;134(Pt 9):2502-12.

104. Mahoney CJ, Ridgway GR, Malone IB, Downey LE, Beck J, Kinnunen KM, Schmitz N, Golden HL, Rohrer JD, Schott JM, Rossor MN, Ourselin S, Mead S, Fox NC, Warren JD. Profiles of white matter tract pathology in frontotemporal dementia. Hum Brain Mapp. 2014 Aug;35(8):4163-79.

105. Smith SM, Jenkinson M, Johansen-Berg H, Rueckert D, Nichols TE, Mackay CE, Watkins KE, Ciccarelli O, Cader MZ, Matthews PM, Behrens TE. Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. Neuroimage. 2006 Jul 15;31(4):1487-505.

106. Preti MG, Baglio F, Lagana MM, Griffanti L, Nemni R, Clerici M, Bozzali M, Baselli G. Assessing corpus callosum changes in Alzheimer's disease: comparison between tract-based spatial statistics and atlas-based tractography. PLoS One. 2012;7(4):e35856.

107. Jones DK, Cercignani M. Twenty-five pitfalls in the analysis of diffusion MRI data. NMR Biomed. 2010 Aug;23(7):803-20.

108. Agosta F, Sala S, Valsasina P, Meani A, Canu E, Magnani G, Cappa SF, Scola E, Quatto P, Horsfield MA, Falini A, Comi G, Filippi M. Brain network connectivity assessed using graph theory in frontotemporal dementia. Neurology. 2013 Jul 9;81(2):134-43.

109. Day GS, Farb NA, Tang-Wai DF, Masellis M, Black SE, Freedman M, Pollock BG, Chow TW. Salience network resting-state activity: prediction of frontotemporal dementia progression. JAMA Neurol. 2013 Oct;70(10):1249-53.

110. Farb NA, Grady CL, Strother S, Tang-Wai DF, Masellis M, Black S, Freedman M, Pollock BG, Campbell KL, Hasher L, Chow TW. Abnormal network connectivity in frontotemporal dementia: evidence for prefrontal isolation. Cortex. 2013 Jul-Aug;49(7):1856-73.

111. Filippi M, Agosta F, Scola E, Canu E, Magnani G, Marcone A, Valsasina P, Caso F, Copetti M, Comi G, Cappa SF, Falini A. Functional network connectivity in the behavioral variant of frontotemporal dementia. Cortex. 2013 Oct;49(9):2389-401.

112. Whitwell JL, Josephs KA, Avula R, Tosakulwong N, Weigand SD, Senjem ML, Vemuri P, Jones DT, Gunter JL, Baker M, Wszolek ZK, Knopman DS, Rademakers R, Petersen RC, Boeve BF, Jack CR, Jr. Altered functional connectivity in asymptomatic MAPT subjects: a comparison to bvFTD. Neurology. 2011 Aug 30;77(9):866-74.

113. Rytty R, Nikkinen J, Paavola L, Abou Elseoud A, Moilanen V, Visuri A, Tervonen O, Renton AE, Traynor BJ, Kiviniemi V, Remes AM. GroupICA dual regression analysis of resting state networks in a behavioral variant of frontotemporal dementia. Front Hum Neurosci. 2013;7:461.

114. Barkhof F, Haller S, Rombouts SA. Resting-state functional MR imaging: a new window to the brain. Radiology. 2014 Jul;272(1):29-49.

115. Lee MH, Smyser CD, Shimony JS. Resting-state fMRI: a review of methods and clinical applications. AJNR Am J Neuroradiol. 2013 Oct;34(10):1866-72.

116. Salimi-Khorshidi G, Douaud G, Beckmann CF, Glasser MF, Griffanti L, Smith SM. Automatic denoising of functional MRI data: combining independent component analysis and hierarchical fusion of classifiers. Neuroimage. 2014 Apr 15;90:449-68.

117. Khalili-Mahani N, Zoethout RM, Beckmann CF, Baerends E, de Kam ML, Soeter RP, Dahan A, van Buchem MA, van Gerven JM, Rombouts SA. Effects of morphine and alcohol on functional brain connectivity during "resting state": a placebo-controlled crossover study in healthy young men. Hum Brain Mapp. 2012 May;33(5):1003-18.

118. Binnewijzend MA, Kuijer JP, van der Flier WM, Benedictus MR, Moller CM, Pijnenburg YA, Lemstra AW, Prins ND, Wattjes MP, van Berckel BN, Scheltens P, Barkhof F. Distinct perfusion patterns in Alzheimer's disease, frontotemporal dementia and dementia with Lewy bodies. Eur Radiol. 2014 Sep;24(9):2326-33.

119. Du AT, Jahng GH, Hayasaka S, Kramer JH, Rosen HJ, Gorno-Tempini ML, Rankin KP, Miller BL, Weiner MW, Schuff N. Hypoperfusion in frontotemporal dementia and Alzheimer disease by arterial spin labeling MRI. Neurology. 2006 Oct 10;67(7):1215-20.

120. Hu WT, Wang Z, Lee VM, Trojanowski JQ, Detre JA, Grossman M. Distinct cerebral perfusion patterns in FTLD and AD. Neurology. 2010 Sep 7;75(10):881-8.

121. Shimizu S, Zhang Y, Laxamana J, Miller BL, Kramer JH, Weiner MW, Schuff N. Concordance and discordance between brain perfusion and atrophy in frontotemporal dementia. Brain Imaging Behav. 2010 Mar;4(1):46-54.

122. Ishii K, Sakamoto S, Sasaki M, Kitagaki H, Yamaji S, Hashimoto M, Imamura T, Shimomura T, Hirono N, Mori E. Cerebral glucose metabolism in patients with frontotemporal dementia. J Nucl Med. 1998 Nov;39(11):1875-8.

123. Jeong Y, Cho SS, Park JM, Kang SJ, Lee JS, Kang E, Na DL, Kim SE. 18F-FDG PET findings in frontotemporal dementia: an SPM analysis of 29 patients. J Nucl Med. 2005 Feb;46(2):233-9.

124. Rohrer JD, Warren JD, Omar R, Mead S, Beck J, Revesz T, Holton J, Stevens JM, Al-Sarraj S, Pickering-Brown SM, Hardy J, Fox NC, Collinge J, Warrington EK, Rossor MN. Parietal lobe deficits in frontotemporal lobar degeneration caused by a mutation in the progranulin gene.

Arch Neurol. 2008 Apr;65(4):506-13.

125. Seelaar H, Papma JM, Garraux G, de Koning I, Reijs AE, Valkema R, Rozemuller AJ, Salmon E, van Swieten JC. Brain perfusion patterns in familial frontotemporal lobar degeneration. Neurology. 2011 Jul 26;77(4):384-92.

126. Jacova C, Hsiung GY, Tawankanjanachot I, Dinelle K, McCormick S, Gonzalez M, Lee H, Sengdy P, Bouchard-Kerr P, Baker M, Rademakers R, Sossi V, Stoessl AJ, Feldman HH, Mackenzie IR. Anterior brain glucose hypometabolism predates dementia in progranulin mutation carriers. Neurology. 2013 Oct 8;81(15):1322-31.

127. Wolk DA, Detre JA. Arterial spin labeling MRI: an emerging biomarker for Alzheimer's disease and other neurodegenerative conditions. Curr Opin Neurol. 2012 Aug;25(4):421-8.

128. Alsop DC, Detre JA, Golay X, Gunther M, Hendrikse J, Hernandez-Garcia L, Lu H, Macintosh BJ, Parkes LM, Smits M, van Osch MJ, Wang DJ, Wong EC, Zaharchuk G. Recommended implementation of arterial spin-labeled perfusion MRI for clinical applications: A consensus of the ISMRM perfusion study group and the European consortium for ASL in dementia. Magn Reson Med. 2014 Apr 8.

129. Nezamzadeh M, Matson GB, Young K, Weiner MW, Schuff N. Improved pseudo-continuous arterial spin labeling for mapping brain perfusion. J Magn Reson Imaging. 2010 Jun;31(6):1419-27.

130. Detre JA, Rao H, Wang DJ, Chen YF, Wang Z. Applications of arterial spin labeled MRI in the brain. J Magn Reson Imaging. 2012 May;35(5):1026-37.

131. Klunk WE, Engler H, Nordberg A, Wang Y, Blomqvist G, Holt DP, Bergstrom M, Savitcheva I, Huang GF, Estrada S, Ausen B, Debnath ML, Barletta J, Price JC, Sandell J, Lopresti BJ, Wall A, Koivisto P, Antoni G, Mathis CA, Langstrom B. Imaging brain amyloid in Alzheimer's disease with Pittsburgh Compound-B. Ann Neurol. 2004 Mar;55(3):306-19.

132. Ghetti B, Oblak AL, Boeve BF, Johnson KA, Dickerson BC, Goedert M. Invited review: Frontotemporal dementia caused by microtubule-associated protein tau gene (MAPT) mutations: a chameleon for neuropathology and neuroimaging. Neuropathol Appl Neurobiol. 2015 Feb;41(1):24-46.

CHAPTER 5

Summary & samenvatting

SUMMARY

Frontotemporal lobar degeneration (FTLD) is the second most common cause of presenile dementia, and is characterized by neurodegeneration of the frontal and temporal lobes. The disease typically presents with behavioral disturbances and language difficulties that occur before the age of 65 years. An autosomal dominant familial form of FTLD can be caused by mutations in *microtubule-associated protein tau* (*MAPT*) or *progranulin* (*GRN*), and the *chromosome 9 open reading frame 72* (*C9orf72*) repeat expansion. There currently is no therapy available to prevent or cure FTLD. In order to develop a disease-modifying therapy, knowledge concerning the pathophysiological process is crucial to identify proper treatment targets. The familial forms of FTLD can learn us a lot about the disease process by enabling us to explore clinical and pathological characteristics in relation to genetic defects. With the development of potential disease-modifying therapies, sensitive biomarkers to evaluate the effects of treatment in early FTLD are essential. Presymptomatic carriers of the abovementioned genetic defects provide a unique study population to investigate biomarkers at the earliest stage of the disease, at a time of minimal brain damage.

In **Chapter 1** current knowledge about genetic forms of FTLD and MRI biomarkers are presented and the aims of the current study are outlined:

1) To expand current knowledge on phenotypical characteristics in the clinical stage of genetic FTLD (**Chapter 2**).
2) To investigate presymptomatic changes in familial FTLD using neuropsychological assessment and MRI (**Chapter 3**).

Chapter 2.1 reports a novel c.314dup *GRN* mutation in a patient that presented with an atypical symmetric corticobasal syndrome with symmetric frontoparietal atrophy and hypoperfusion at neuroimaging. FTLD-type A pathology at autopsy prompted us to perform genetic screening in this patient without a positive family history for neurodegenerative disorders, revealing this novel mutation. We concluded that *GRN* mutations are an important cause of corticobasal syndrome and suggested that screening for *GRN* mutations should be considered in patients with corticobasal syndrome without a positive family history.

Chapter 2.2 describes the clinical and pathological characteristics of the *C9orf72* repeat expansion in a Dutch frontotemporal dementia (FTD) cohort. We found the *C9orf72* repeat expansion in 18% of patients with familial FTD and 2% of sporadic FTD in the Netherlands, thereby explaining 54% of familial FTD together with *MAPT* and *GRN* mutations. The most common clinical presentation was the behavioral variant of FTD, but presentations with primary progressive aphasia, and concomitant amyotrophic lateral sclerosis (ALS) or signs of Parkinsonism were also demonstrated. Pathological examination revealed FTLD with transactive response DNA binding protein of 43 kDa (TDP-43) positive inclusions, consistent with FTLD-TDP type B, but with additional cerebellar p62-positive, TDP-43-negative inclusions.

In **Chapter 2.3** we estimated the global frequency of the *C9orf72* repeat expansion, and found a large geographical variation in frequencies, which was highest in Finland. Worldwide, the *C9orf72* repeat expansion was found in 25% of familial FTLD and 36% of familial ALS. Moreover, we demonstrated the repeat expansion in 6% of both sporadic FTLD and ALS, suggesting that this is the most frequent genetic cause of these diseases identified thus far.

In **Chapter 3.1** we investigated presymptomatic carriers of *GRN* and *MAPT* mutations using neuropsychological investigation, volumetric MRI, diffusion tensor imaging (DTI) and resting-state fMRI and demonstrated that reduced white matter integrity of the uncinate fasciculus, and reduced functional connectivity within the salience network can be detected in the presymptomatic stage of FTLD.

Chapter 3.2 describes longitudinal findings within the same group of presymptomatic mutation carriers after two years of follow-up. We showed that resting-state fMRI has the potential to detect changes in functional connectivity within the salience and default mode network more than fifteen years before expected symptom onset. Carriers within five years prior to their estimated symptom onset also showed longitudinal alterations in executive functions and social cognition, insular grey matter volume and white matter integrity within the uncinate fasciculus. Interestingly, the two mutation carriers that converted to clinical bvFTD during follow-up showed the strongest white matter integrity loss in the uncinate fasciculi and forceps minor at baseline already, suggesting that

this MRI technique might have the potential to serve as a baseline predictor for conversion to clinically manifest disease.

In **Chapter 3.3** we investigated cerebral blood flow in the same individuals by means of arterial spin labeling and showed a longitudinal decline in cerebral blood flow in frontal, temporal, parietal, and subcortical brain regions in presymptomatic mutation carriers compared to controls, which was most apparent for mutation carriers approaching their estimated age of symptom onset.

Our findings suggest that MRI is a powerful tool to detect FTLD and track disease progression in the presymptomatic stage. This is important for future clinical trials, in which these techniques can be used to evaluate the effect of therapies.

To conclude, in the current study we expanded the phenotypical characterization of genetic FTLD in both the symptomatic and presymptomatic disease stage. In **Chapter 4** the main findings of this study and their implications are discussed in light of literature and suggestions for future research are provided. An important development is that there are currently two ongoing clinical trials with potential therapies for FTLD: LMTX for patients with tau pathology and SAHA for patients with *GRN* mutations. These trials provide hope that we will be able to prevent or cure this devastating disorder in the future.

SAMENVATTING

Frontotemporale lobaire degeneratie (FTLD) is de tweede meest voorkomende vorm van preseniele dementie en wordt gekenmerkt door neurodegeneratie van de frontale en temporale hersenkwabben. De ziekte presenteert zich typisch met gedragsveranderingen en taalproblemen die voor het 65e levensjaar optreden. Een autosomaal dominante familiaire vorm van FTLD kan veroorzaakt worden door mutaties in *microtubule-associated protein tau* (*MAPT*) of *progranulin* (*GRN*) en de *chromosome 9 open reading frame 72* (*C9orf72*) repeat-expansie. Er is momenteel geen therapie beschikbaar die FTLD kan voorkomen of genezen. Voor de ontwikkeling van een therapie voor de ziekte is kennis van het pathofysiologisch proces cruciaal om een geschikt aangrijpingspunt voor medicatie te vinden. De familiaire vormen van FTLD kunnen ons veel leren over het ziekteproces door klinische en pathologische kenmerken in relatie tot de genetische defecten te onderzoeken. Bij de ontwikkeling van potentiële behandelingen voor de ziekte, zijn sensitieve biomarkers om het effect van dergelijke medicatie te beoordelen essentieel. Presymptomatische dragers van bovengenoemde erfelijke afwijkingen vormen een unieke studiepopulatie om biomarkers in een zo vroeg mogelijk ziektestadium, op het moment van minimale hersenschade, te onderzoeken.

In **hoofdstuk 1** wordt de huidige kennis omtrent genetische vormen van FTLD en MRI biomarkers beschreven en worden de doelen van de huidige studie gepresenteerd:

1) Uitbreiding van de huidige kennis van fenotypische karakteristieken in het klinische ziektestadium van erfelijke FTLD (**Hoofdstuk 2**).
2) Onderzoeken van presymptomatische hersenveranderingen in familiaire FTLD met behulp van neuropsychologisch onderzoek en MRI (**Hoofdstuk 3**).

In **hoofdstuk 2.1** rapporteren wij een nieuwe c.314dup *GRN* mutatie in een patiënt die zich presenteerde met een atypisch, symmetrisch corticobasaal syndroom met symmetrische atrofie en hypoperfusie op beeldvorming. FTLD-type A pathologie bij obductie bracht ons ertoe om bij deze patiënt, zonder positieve familieanamnese voor neurodegeneratieve aandoeningen, genetische screening uit te voeren, waarbij deze nieuwe mutatie werd aangetoond. Wij concludeerden dat *GRN* mutaties een belangrijke oorzaak zijn van corticobasaal syndroom en dat

screening op *GRN* mutaties ook overwogen zou moeten worden bij patiënten met een corticobasaal syndroom zonder positieve familieanamnese.

Hoofdstuk 2.2 beschrijft de klinische en pathologische kenmerken van de *C9orf72* repeat-expansie in een Nederlands frontotemporale dementie (FTD) cohort. We hebben de *C9orf72* repeat-expansie aangetoond bij 18% van de patiënten met familiaire FTD en 2% van de patiënten met sporadische FTD in Nederland. Hiermee wordt, samen met mutaties in *MAPT* en *GRN,* nu 54% van de familiaire FTD in Nederland verklaard. De meest voorkomende klinische presentatie was de gedragsvariant van FTD, maar presentaties met primaire progressieve afasie en bijkomende amyotrofische laterale sclerose (ALS) of tekenen van Parkinsonisme kwamen ook voor. Pathologisch onderzoek toonde FTLD met inclusies van het transactive response DNA binding protein van 43 kDa (TDP-43), maar daarnaast ook p62-positieve, TDP-43-negatieve inclusies in het cerebellum.

In **hoofdstuk 2.3** hebben wij getracht de wereldwijde frequentie van de *C9orf72* repeat-expansie te schatten. We vonden een grote geografische variatie in frequentie, die het hoogste was in Finland. Gemiddeld werd de repeat-expansie gevonden bij 25% van de familiaire FTLD en 36% van de familiaire ALS. Daarnaast vonden wij de repeat-expansie bij 6% van zowel sporadische FTLD en ALS, hetgeen suggereert dat dit de meest voorkomende geïdentificeerde genetische oorzaak van beide sporadische ziektes is.

In **hoofdstuk 3.1** hebben wij presymptomatische dragers van *GRN* en *MAPT* mutaties onderzocht met behulp van neuropsychologisch onderzoek, volumetrische MRI, diffusion tensor imaging (DTI) en resting-state fMRI. Wij hebben laten zien dat verminderde integriteit van de fasciculus uncinatus en verminderde functionele connectiviteit in het salience netwerk aan te tonen zijn in de presymptomatische fase van FTLD.

Hoofdstuk 3.2 beschrijft de longitudinale bevindingen in dezelfde groep presymptomatische mutatiedragers na twee jaar follow-up. We hebben aangetoond dat met behulp van resting-state fMRI, al meer dan 15 jaar voor de te verwachten klinische ziektemanifestatie, veranderingen in functionele connectiviteit in het salience netwerk gevonden kunnen worden. Mutatiedragers

binnen 5 jaar voor hun verwachte klinische ziektemanifestatie lieten daarnaast longitudinale veranderingen zien in executieve functies en sociale cognitie, in grijze stof volume van de insula en in witte stof integriteit van de fasciculus uncinatus. Opmerkelijk is dat de twee mutatiedragers die gedurende de follow-up periode klachten van de ziekte hebben ontwikkeld, al bij het baseline onderzoek de meest afwijkende DTI waarden in de fasciculus uncinatus en forceps minor lieten zien. Dit suggereert dat deze MRI techniek wellicht een geschikte baseline voorspeller is van klinische ziektemanifestatie.

In **hoofdstuk 3.3** hebben wij de hersendoorbloeding van dezelfde individuen onderzocht met behulp van arterial spin labeling. Wij hebben een longitudinale achteruitgang in hersendoorbloeding in frontale, temporale, pariëtale en subcorticale hersengebieden aangetoond in mutatiedragers ten opzichte van controles, hetgeen het meest uitgesproken was voor mutatiedragers die hun verwachte leeftijd van klinische ziektemanifestatie naderen.

Onze bevindingen suggereren dat MRI een waardevol instrument is om FTLD aan te tonen en ziekteprogressie te meten in de presymptomatische fase. Dit is van groot belang voor toekomstige therapeutische trials, waarin deze MRI technieken gebruikt kunnen worden om het effect van een therapie te beoordelen.

Concluderend hebben wij in de huidige studie de fenotypische karakterisering van erfelijke FTLD uitgebreid in zowel de symptomatische als presymptomatisch ziektestadia. In **hoofdstuk 4** worden de belangrijkste bevindingen van deze studie en de implicaties hiervan besproken aan de hand van de literatuur en worden suggesties voor toekomstig onderzoek gegeven. Een belangrijke ontwikkeling is dat er momenteel twee klinische trials met potentiële behandelingen voor FTLD lopen: LMTX voor patiënten met tau-pathologie en SAHA voor patiënten met een *GRN* mutatie. Deze trials bieden hoop dat er in de toekomst een behandeling zal zijn, om deze ingrijpende ziekte te voorkomen of genezen.

Dankwoord

Curriculum vitae

List of publications

PhD Portfolio

List of abbreviations

DANKWOORD

Na altijd gedacht te hebben nooit onderzoek te willen doen, is gedurende mijn keuze-onderzoek toch mijn interesse voor de wetenschap gewekt en ben ik uiteindelijk in dit promotietraject beland. Hoewel er in de loop van mijn onderzoeksperiode zeker ook momenten van teleurstelling, frustratie of paniek zijn geweest, heb ik er al met al ontzettend veel plezier in gehad en had ik het voor geen goud willen missen. Uiteraard had ik dit proefschrift niet kunnen voltooien zonder hulp van anderen. In dit hoofdstuk wil ik dan ook graag stilstaan bij iedereen die een bijdrage aan mijn proefschrift heeft geleverd.

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CURRICULUM VITAE

Elise Geertruida Petronella Dopper was born on September 15th, 1987 in Rotterdam, the Netherlands. After finishing secondary school at the Emmauscollege in Rotterdam in 2005, she started Medical school at the Erasmus University Rotterdam. During her study she performed a research project on primary progressive aphasia, supervised by Prof.dr. J.C. van Swieten at the department of Neurology in the Erasmus Medical Center. She passed the "doctoraal" examination in 2009. In 2010, she started working on the research presented in this thesis at the departments of Neurology in the Erasmus Medical Center, Radiology in the Leiden University Medical Center, and Neurology of the VU Medical Center under supervision of Prof.dr. J.C. van Swieten and Prof.dr. S.A.R.B. Rombouts. In 2014 she started her internships in order to obtain her medical degree, after which she wishes to start a specialty training in Neurology at the Erasmus Medical Center.

LIST OF PUBLICATIONS

Dopper EG, Chalos V, Ghariq E, Den Heijer T, Hafkemeijer A, Jiskoot LC, De Koning I, Seelaar H, Van Minkelen R, Van Osch MJ, Rombouts SA, Van Swieten JC. Cerebral blood flow in presymptomatic familial frontotemporal dementia: a longitudinal arterial spin labeling study. *In preparation.*

Dopper EG*, Jiskoot LC*, Den Heijer T, Hafkemeijer A, De Koning I, Seelaar H, Veer IM, Van Buchem MA, Van Minkelen R, Rombouts SA, van Swieten JC. Longitudinal brain changes in presymptomatic familial frontotemporal dementia. *Submitted*.

Rohrer JD, Nicholas JM, Cash DM, van Swieten J, **Dopper E**, Jiskoot L, van Minkelen R, Rombouts SA, Cardoso MJ, Clegg S, Espak M, Mead S, Thomas DL, De Vita E, Masellis M, Black SE, Freedman M, Keren R, MacIntosh BJ, Rogaeva E, Tang-Wai D, Tartaglia MC, Laforce R, Jr., Tagliavini F, Tiraboschi P, Redaelli V, Prioni S, Grisoli M, Borroni B, Padovani A, Galimberti D, Scarpini E, Arighi A, Fumagalli G, Rowe JB, Coyle-Gilchrist I, Graff C, Fallstrom M, Jelic V, Stahlbom AK, Andersson C, Thonberg H, Lilius L, Frisoni GB, Pievani M, Bocchetta M, Benussi L, Ghidoni R, Finger E, Sorbi S, Nacmias B, Lombardi G, Polito C, Warren JD, Ourselin S, Fox NC, Rossor MN. Presymptomatic cognitive and neuroanatomical changes in genetic frontotemporal dementia in the Genetic Frontotemporal dementia Initiative (GENFI) study: a cross-sectional analysis. Lancet Neurol. 2015 Feb 3.

Dopper EG, Rombouts SA, Jiskoot LC, den Heijer T, de Graaf JR, de Koning I, Hammerschlag AR, Seelaar H, Seeley WW, Veer IM, van Buchem MA, Rizzu P, van Swieten JC. Structural and functional brain connectivity in presymptomatic familial frontotemporal dementia. Neurology. 2014 Jul 8;83(2):e19-26.

Dopper EG, Seelen M, De Jong FJ, Veldink JH, Van den Berg LH, Van Swieten JC. Repeat-expansie in het *C9orf72*-gen: link tussen frontotemporale dementie en amyotrofische laterale sclerose. Nederlands tijdschrift voor geneeskunde. 2013;157(A6271).

Dopper EG, Van Swieten JC. Primair progressieve afasie: frontotemporale lobaire degeneratie of de ziekte van Alzheimer? Tijdschrift voor neurologie en

neurochirurgie. 2012;113:101-7.

Ferrari R, Hernandez DG, Nalls MA, Rohrer JD, Ramasamy A, Kwok JB, Dobson-Stone C, Brooks WS, Schofield PR, Halliday GM, Hodges JR, Piguet O, Bartley L, Thompson E, Haan E, Hernandez I, Ruiz A, Boada M, Borroni B, Padovani A, Cruchaga C, Cairns NJ, Benussi L, Binetti G, Ghidoni R, Forloni G, Galimberti D, Fenoglio C, Serpente M, Scarpini E, Clarimon J, Lleo A, Blesa R, Waldo ML, Nilsson K, Nilsson C, Mackenzie IR, Hsiung GY, Mann DM, Grafman J, Morris CM, Attems J, Griffiths TD, McKeith IG, Thomas AJ, Pietrini P, Huey ED, Wassermann EM, Baborie A, Jaros E, Tierney MC, Pastor P, Razquin C, Ortega-Cubero S, Alonso E, Perneczky R, Diehl-Schmid J, Alexopoulos P, Kurz A, Rainero I, Rubino E, Pinessi L, Rogaeva E, St George-Hyslop P, Rossi G, Tagliavini F, Giaccone G, Rowe JB, Schlachetzki JC, Uphill J, Collinge J, Mead S, Danek A, Van Deerlin VM, Grossman M, Trojanowski JQ, van der Zee J, Deschamps W, Van Langenhove T, Cruts M, Van Broeckhoven C, Cappa SF, Le Ber I, Hannequin D, Golfier V, Vercelletto M, Brice A, Nacmias B, Sorbi S, Bagnoli S, Piaceri I, Nielsen JE, Hjermind LE, Riemenschneider M, Mayhaus M, Ibach B, Gasparoni G, Pichler S, Gu W, Rossor MN, Fox NC, Warren JD, Spillantini MG, Morris HR, Rizzu P, Heutink P, Snowden JS, Rollinson S, Richardson A, Gerhard A, Bruni AC, Maletta R, Frangipane F, Cupidi C, Bernardi L, Anfossi M, Gallo M, Conidi ME, Smirne N, Rademakers R, Baker M, Dickson DW, Graff-Radford NR, Petersen RC, Knopman D, Josephs KA, Boeve BF, Parisi JE, Seeley WW, Miller BL, Karydas AM, Rosen H, van Swieten JC, **Dopper EG**, Seelaar H, Pijnenburg YA, Scheltens P, Logroscino G, Capozzo R, Novelli V, Puca AA, Franceschi M, Postiglione A, Milan G, Sorrentino P, Kristiansen M, Chiang HH, Graff C, Pasquier F, Rollin A, Deramecourt V, Lebert F, Kapogiannis D, Ferrucci L, Pickering-Brown S, Singleton AB, Hardy J, Momeni P. Frontotemporal dementia and its subtypes: a genome-wide association study. Lancet Neurol. 2014 Jul;13(7):686-99.

Simon-Sanchez J*, **Dopper EG***, Cohn-Hokke PE, Hukema RK, Nicolaou N, Seelaar H, de Graaf JR, de Koning I, van Schoor NM, Deeg DJ, Smits M, Raaphorst J, van den Berg LH, Schelhaas HJ, De Die-Smulders CE, Majoor-Krakauer D, Rozemuller AJ, Willemsen R, Pijnenburg YA, Heutink P, van Swieten JC. The clinical and pathological phenotype of C9ORF72 hexanucleotide repeat expansions. Brain. 2012 Mar;135(Pt 3):723-35.

Majounie E*, Renton AE*, Mok K*, **Dopper EG***, Waite A*, Rollinson S*, Chio A*, Restagno G*, Nicolaou N*, Simon-Sanchez J*, van Swieten JC*, Abramzon Y, Johnson JO, Sendtner M, Pamphlett R, Orrell RW, Mead S, Sidle KC, Houlden H, Rohrer JD, Morrison KE, Pall H, Talbot K, Ansorge O, Chromosome ALSFTDC, French research network on FFA, Consortium I, Hernandez DG, Arepalli S, Sabatelli M, Mora G, Corbo M, Giannini F, Calvo A, Englund E, Borghero G, Floris GL, Remes AM, Laaksovirta H, McCluskey L, Trojanowski JQ, Van Deerlin VM, Schellenberg GD, Nalls MA, Drory VE, Lu CS, Yeh TH, Ishiura H, Takahashi Y, Tsuji S, Le Ber I, Brice A, Drepper C, Williams N, Kirby J, Shaw P, Hardy J, Tienari PJ*, Heutink P*, Morris HR*, Pickering-Brown S*, Traynor BJ*. Frequency of the C9orf72 hexanucleotide repeat expansion in patients with amyotrophic lateral sclerosis and frontotemporal dementia: a cross-sectional study. Lancet Neurol. 2012 Apr;11(4):323-30.

Dopper EG, Seelaar H, Chiu WZ, de Koning I, van Minkelen R, Baker MC, Rozemuller AJ, Rademakers R, van Swieten JC. Symmetrical corticobasal syndrome caused by a novel C.314dup progranulin mutation. J Mol Neurosci. 2011 Nov;45(3):354-8.

Rascovsky K, Hodges JR, Knopman D, Mendez MF, Kramer JH, Neuhaus J, van Swieten JC, Seelaar H, **Dopper EG**, Onyike CU, Hillis AE, Josephs KA, Boeve BF, Kertesz A, Seeley WW, Rankin KP, Johnson JK, Gorno-Tempini ML, Rosen H, Prioleau-Latham CE, Lee A, Kipps CM, Lillo P, Piguet O, Rohrer JD, Rossor MN, Warren JD, Fox NC, Galasko D, Salmon DP, Black SE, Mesulam M, Weintraub S, Dickerson BC, Diehl-Schmid J, Pasquier F, Deramecourt V, Lebert F, Pijnenburg Y, Chow TW, Manes F, Grafman J, Cappa SF, Freedman M, Grossman M, Miller BL. Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. Brain. 2011 Sep;134(Pt 9):2456-77.

Jansen C, Parchi P, Capellari S, Strammiello R, **Dopper EG**, van Swieten JC, Kamphorst W, Rozemuller AJ. A second case of Gerstmann-Straussler-Scheinker disease linked to the G131V mutation in the prion protein gene in a Dutch patient. J Neuropathol Exp Neurol. 2011 Aug;70(8):698-702.

PHD PORTFOLIO

Summary of PhD training and teaching

1. PhD training	Year	ECTS
General courses		
English-Language pitfalls for Dutch Academics, Leiden	2011	0.3
BROK, Rotterdam	2013	1.5
Specific courses		
FSL & FreeSurfer Course, Heidelberg, Germany	2010	2
SNP Course VII, The Erasmus Postgraduate School Molecular Medicine, Rotterdam	2010	2
Autumn School on Cognitive, Affective, and Nociceptive Functioning of the Anterior Cingulate Cortex, Oppurg, Germany, poster presentation	2010	1.5
Euro-CNS course in Neuropathology, Turin, Italy	2011	1.5
(Inter)national conferences/seminars		
16th Annual Meeting of the Organization for Human Brain Mapping, Barcelona, Spain	2010	1
Default Mode Network Conference, Barcelona, Spain	2010	1
7th International Conference on Frontotemporal Dementia, Indianapolis, USA, poster presentation	2010	1
Congress NWO Brain and cognition	2011	0.3
Mix & Match meeting Alzheimer Nederland, Utrecht	2011-2013	0.9
8th International Conference on Frontotemporal Dementia Manchester, United Kingdom, oral presentation	2012	2
Second European Conference on Clinical Neuroimaging, Lille, France, oral presentation	2013	2
Alzheimer's Association International Conference, Boston, USA, oral presentation	2013	2
9th International Conference on Frontotemporal Dementia, Vancouver, Canada	2014	1
Other		
Member of the Dutch 'FTD Expertgroep'	2010-2014	1
Department research meeting	2013	1

2. Teaching activities		
Lecturing		
Alzheimercafé, Nieuwegein	2012	0.5
Medilex congres Niet oud, toch dement, Amersfoort	2012	1
Alzheimercafé, Oud-Beijerland	2012	0.5
Symposium frontotemporale dementie, Amsterdam	2013	1
Groot Haags Geriatrie referaat, Den Haag	2013	1
Medilex congres Niet oud, toch dement, Driebergen	2013	1
Supervising Master's theses		
Four students	2010-2013	6
Total		33

LIST OF ABBREVIATIONS

AAO	Age at onset
ACC	Anterior cingulate cortex
AD	Alzheimer's disease
aFTLD-U	Atypical FTLD with ubiquinated inclusions
AGD	Argyrophilic grain disease
ALS	Amyotrophic lateral sclerosis
aMCC	Anterior midcingulate cortex
ASL	Arterial spin labeling
BDI	Beck Depression Inventory
BIBD	Basophilic inclusion body disease
BNT	Boston Naming Test
bvFTD	Behavioral variant of frontotemporal dementia
C9orf72	*Chromosome 9 open reading frame 72*
CBD	Corticobasal degeneration
CBF	Cerebral blood flow
CBS	Corticobasal syndrome
CHMP2B	*Charged multivesicular body protein 2B*
CJD	Creutzfeldt-Jakob disease
DA	Axial diffusivity
DMN	Default mode network
DN	Dystrophic neurites
DPR	Dipeptide repeats
DR	Radial diffusivity
DTI	Diffusion tensor imaging
EPI	Echo-planar imaging
EWS	Ewing's sarcoma protein
FA	Fractional anisotropy
FDG-PET	Positron emission tomography with 18F-fluorodeoxyglucose
FEAT	FMRI expert analysis tool
FIX	FMRIB's ICA-based X-noiseifier
fMRI	Functional MRI
FSL	FMRIB's Software Library

FTD	Frontotemporal dementia
FTLD	Frontotemporal lobar degeneration
FUS	Fused in sarcoma
GM	Grey matter
GRN	*Progranulin*
HD	Huntington's disease
HDAC	Histone deacetylase
ICA	Independent component analysis
LDST	Letter Digit Substitution Test
LPA	Logopenic progressive aphasia
MAPT	*Microtubule-associated protein tau*
MD	Mean diffusivity
MMSE	Mini-Mental State Examination
MND	Motor neuron disease
MSTD	Multiple system tauopathy with dementia
NCI	Neuronal cytoplasmic inclusions
NFTD	Neurofibrillary-tangle predominant dementia
NIFID	Neuronal intermediate filament inclusion disease
NII	Neuronal intranuclear inclusions
PCC	Posterior cingulate cortex
PCR	Polymerase chain reaction
PNFA	Progressive non-fluent aphasia
PPA	Primary progressive aphasia
PSP	Progressive supranuclear palsy
RAN	Repeat-associated non-ATG-initiated
RAVLT	Rey Auditory Verbal Learning Test
RBMT	Rivermead Behavioral Memory Test
SAHA	Suberoylanilide hydroxamic acid
SAT	Semantic Association Test
SD	Semantic dementia / standard deviation
SN	Salience network
SNP	Single-nucleotide polymorphism
STAI	State-Trait Anxiety Inventory
TAF15	TATA-binding protein associated factor 15

TARDBP	*Transactive response DNA binding protein*
TBSS	Tract-based spatial statistics
TDP-43	Transactive response DNA binding protein of 43 kDa
TE	Echo time
TFCE	Threshold-free cluster enhancement
TMT	Trail Making Test
TR	Repetition time
VAT	Visual Association Test
VBM	Voxel-based morphometry
VCP	*Valosin-containing protein*
VENs	Von Economo Neurons
WCST	Wisconsin Card Sorting Test
WM	White matter
WMT-GGI	White matter tauopathy with globular glial inclusions