

**Deep Phenotyping Through Biomarker
Profiling of Frontotemporal Dementia
(FTD) With and Without Co-morbid
Motor Neuron Degeneration: A
Multidimensional Approach**

PhD Thesis

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Declaration

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Signed

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Thesis Summary

The overall objective of this thesis, which is based on the central theme of biomarkers and progression in Neurodegeneration, was to perform deep phenotyping of Frontotemporal Dementia (FTD) and related disorders of motor neuron degeneration through multimodal biomarker profiling.

Incorporated into the deep phenotyping are clinical characterization, neurophysiological assessment, family aggregation analysis, genetics, imaging and outcome.

This project exploits a clinic-based, case control design integrating interconnected complementary cross-sectional and longitudinal approaches.

Results from the neurophysiology arm of this thesis included utilizing MUNIX to establish lower motor neuron repository, to study disease progression and to quantify lower motor neuron dysfunction in FTD. The aggregation of neuropsychiatric and neurodegenerative diseases including schizophrenia, suicide, learning disability and Motor Neuron Disease were also elevated in relatives of FTD patients compared to relatives of controls. A separate chapter in this thesis described the experience of our cognitive clinic over the study period. The multiparametric comparative Magnetic Resonance Imaging study described grey and white matter patterns across the FTD-ALS spectrum including the finding that FTD-ALS patients who tested negative for hexanucleotide repeats in C9orf72 had considerable extra-motor frontotemporal pathology, which is more prominent than that observed in C9orf72 positive patients.

Overall the biomarkers generated from this research are likely to represent valuable markers in future discovering of distinct disease subtypes within the FTD-ALS spectrum.

In the final section, results generated from this research are used to propose future research projects and key findings of this thesis are expected to have significant implications on both clinical care and future research in the field of FTD.

Data acquisition for this thesis

- Cognitive clinic study was designed by Taha Omer. Data was collected and analysed by Taha Omer.
- Family aggregation study and family aggregation databases were designed by Taha Omer. Data for the family aggregation study was collected and analysed by Taha Omer. The questionnaire used was designed by Susan Byrne.
- Genetics and Imaging studies were initiated by Taha Omer who also performed data collection. Genetic testing for known genes was carried out by Russell McLaughlin and Mark Doherty. Imaging data was analysed by Peter Bede.
- MUNIX studies: All studies were designed and initiated by Taha Omer, using SOPHIA protocol. All data were collected by Taha Omer. Databases were designed by Taha Omer. Data analysis was performed by Taha Omer and Bahman Nasserolelami.

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Abbreviations

AD:	Alzheimer Disease
ADM:	Abductor Digiti Minimi
AH:	Abductor Hallucis
ALS:	Amyotrophic Lateral Sclerosis
APB:	Abductor Pollicis Brevis
BB:	Biceps Brachii
BvFTD:	Behavioural variant Primary Progressive Aphasia
CBD:	Corticobasal Degeneration
CBS:	Corticobasal Syndrome
CMAP:	Compound Muscle Action Potential
CNS:	Central Nervous System
SvPPA:	Semantic variant Primary Progressive Aphasia
EDB:	Extensor Digitorum Brevis
EMG:	Electromyography
FTD:	Frontotemporal Dementia
MND:	Motor Neurone Disease
MUNE:	Motor Unit Number Estimate
MUNIX:	Motor Unit Number Index Estimation
MUs:	Motor Units

MUSIX: Motor Unit Size Index

NfvPPA: Nonfluent variant Primary Progressive Aphasia

PD: Parkinson Disease

PSP: Progressive Supranuclear Palsy

SIP: Surface Interference Pattern

TA: Tibialis Anterior

1 Chapter 1 - Introduction to Frontotemporal Dementia (FTD)

- Introduction to Motor Unit Number Index

(MUNIX) Estimation

- Introduction to Amyotrophic Lateral Sclerosis

(ALS)

- Introduction to Poliomyelitis

1.1 History, nomenclature and terminology of FTD

Frontotemporal dementia (FTD) is a clinically and pathologically heterogeneous syndrome, associated with progressive decline in behaviour and/or language caused by focal degeneration of the frontal and/or anterior temporal lobes. The FTD clinical syndromes are a common cause of early-onset dementia (below age of 65), with an incidence and prevalence comparable to classical Alzheimer disease (AD) in this age group (1), and are likely to be an underestimated causes of dementia due to case ascertainment difficulties. There are a number of reasons for case ascertainment difficulties in FTD; firstly, patients often present with subtle nonspecific behavioural symptoms making relatives less likely to seek medical opinion. Secondly, there are no dedicated specialist cognitive clinics or close relationship between health specialists caring for people with FTD (General practitioners, memory clinics, psychiatric services) in many countries resulting in decreased case detection rates. Arnold Pick described

the FTD syndrome in a series of clinical and anatomical papers more than 100 years ago. First, he described the presentation of focal language deterioration as a presentation of dementia in 1892 and helped to introduce the concept of a focal neurodegenerative disease:

“Simple progressive brain atrophy can lead to symptoms of local disturbance through local accentuation of the diffuse process” (2).

Subsequently, a histopathological substance forming characteristic argyrophilic neuronal inclusions was discovered; these inclusions were called “Pick bodies” (3).

Thereafter, these conditions did not attract much research interest until recently. The rekindling of interest was indicated by a series of seminal papers by Brun and Gustafson, and Mesulam’s description of slowly progressive aphasia in 1982 (4). He described what he called the syndrome with progressive aphasias without dementia. Also in the 1980’s, two groups in Lund, Sweden and Manchester, United Kingdom published separately large series of patients with frontotemporal atrophy and dementia associated with prominent behaviour and language difficulties and they coined the first consensus criteria for frontotemporal dementia. Recent progresses in neurobiology, histopathology, and molecular genetics have played a key role in dissecting the complex FTD syndrome.

The selective degeneration of the frontal and temporal regions, which correlate with behavioral and language symptoms, gives rise to the term FTD. First divided into three subtypes by Neary et al. in 1998 (5). The 1998 consensus group refining of diagnostic criteria, incorporating imaging and diagnostic discoveries that had been made since the first diagnostic criteria were made, separated the language from the behavioral syndrome. This paved the way for researchers to focus on different clinical variants resulting in significant advances in our current understanding of FTD phenotypes, molecular mechanisms, pathology and genetics.

FTD is now used to classify three syndromes that can be distinguished based on the early and predominant symptoms throughout the course of the disease: behavioral-variant FTD (bvFTD), semantic-variant primary progressive aphasia (svPPA) and nonfluent-variant primary progressive aphasia (nfvPPA).

The classification of these disorders is often considered to be confusing from a clinical perspective. This confusion is caused by the fact that different terminologies have been applied by different investigators in addition to significant clinical, pathological, and genetic overlap between FTD syndromes. There is also an increasing recognition of overlap between FTD and other neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS), progressive supranuclear palsy (PSP), and corticobasal syndrome (CBS).

In this thesis the term FTD is used for clinical syndromes including bvFTD, svPPA, and nfvPPA; and frontotemporal lobar degeneration (FTLD) as a pathologically comprehensive term for various pathological conditions.

1.2 Epidemiology of FTD

FTD is considered to be the third cause of neurodegenerative dementia across all ages, following AD and dementia with Lewy bodies (DLB) accounting for about 5-10% of all pathologically confirmed dementia (8). While FTD remains a disorder of the presenium with average age at onset of 50-60 years, it would appear that late-onset FTD is more common than previously approximated by Seelaar et al that 10% have an age of onset of 70 to 89 years (12). This fact is supported by a recent study based on the Swedish Registry for Dementia which reported that 70 % of FTD cases were 65 years or older at the time of diagnosis (152). Of note here is that the authors of this Swedish Registry study (152) justified use of age at diagnosis rather than age of onset (contrary most previous epidemiological studies) by their intention of avoiding

difficulties in estimating the time from onset to diagnosis. A recent study from the USA estimated that 60% of all FTD cases present in patients aged between 45 and 64 years (7).

Attempts to accurately estimate the incidence and prevalence of FTD in the general population is challenged by the difficulty in accurate diagnosis in population-based setting, and the variation in diagnostic criteria. Nonetheless, there are several population-based studies regarding the incidence and prevalence of FTD. The incidence of FTD is estimated to be between 2.7 and 4.0 cases per 100,000 person-years, based on epidemiological data from the United States, United Kingdom and the Netherlands (7,9,96). The prevalence of FTD has varied between several research studies. A population-based study from the Netherlands estimated 3-4 per 100,000 in the 45–64 age group, 9.4 per 100,000 for the 60–69 age group, and 3.8 per 100,000 at 70–79 age group (10); on the other hand, prevalence estimates of 15–22 cases per 100,000 in the United States (7), and 15 per 100,000 in the United Kingdom (1) have been made. These population studies show nearly equal distribution by gender, which contrasts with some clinical and neuropathology reports that suggest a male predominance for bvFTD.

FTD is frequently familial, with up to 40% of FTD syndrome patients having a suggestive family history, with about 10% of patients showing an autosomal dominant inheritance (11,12). Non-genetic risk factors have not yet been identified.

Mean survival in FTD has been estimated by some studies to range from 6–11 years from symptom onset with a total duration of illness from onset to death ranging from 2 to 20 years (7, 13-15). In one US centre, bvFTD is associated with the shortest survival (median 9 years from onset), svPPA with the longest survival (12 years), and nvPPA with intermediate survival (9.5 years) (13). Patients with nvPPA are typically older than those with bvFTD or svPPA. Motor involvement shortens survival as patients with FTD-

MND have the shortest survival with a mean of 3 years (115). The overall survival is shorter and cognitive and functional decline are more rapid in when compared to AD.

Prognosis can be highly variable and both clinical presentation and underlying pathology are significant determining factors. All FTD subtypes share an insidious onset and inexorably progressive, albeit variable decline.

Case under-ascertainment is a particular problem in FTD studies as FTD cases can easily be missed because of the pure behavioural nature of presentation in many patients. Such behavioural symptoms are often subtle and insidious. The ultimate prevalence study is a population based prospective one that involves door-to-door search for FTD cases (as it has been successfully done for Parkinsonism and Parkinson disease), but the rarity of this condition makes this undertaking not practical.

1.3 Differential diagnoses of FTD

The differential diagnosis in FTD is wide and includes other neurodegenerative diseases (Alzheimer's disease, Amyotrophic lateral sclerosis, Corticobasal degeneration, Progressive supranuclear palsy, prion disease; or less commonly, Parkinson's disease (PD), Huntington's disease (HD), Wilson's disease, and Multiple system atrophy (MSA)), other dementias (Vascular dementia and dementia with Lewy bodies), psychiatric disorders (Schizophrenia, Personality disorders, Mania, Depression, Asperger's syndrome, Huntington disease) and structural lesions affecting frontal and temporal lobes (tumour, infection, vascular lesions) (8,9,15,43).

1.4 Phenotypic characteristics and clinical syndromes of FTD

Despite its pathological heterogeneity, pure FTD can be classified into three clinical syndromes based on the early and predominant symptoms: bvFTD, svPPA and

nvPPA. Each clinical variant is associated with a distinct regional pattern of focal brain atrophy and, to some degree, a characteristic histopathology. Clinical overlap between the syndromes can occur, particularly later in the course as the disease spreads to involve the frontal and temporal lobes more diffusely.

There is a distinct typical topographic cerebral involvement for each clinical syndrome: bvFTD is associated with symmetric (or right-sided) frontal and anterior temporal dysfunction; nvPPA left frontotemporal dysfunction and svPPA anterior temporal (typically left more than right) deficits.

Pathological changes of FTD affect left cerebral hemisphere but less commonly, cases of isolated right frontal or temporal degeneration have been reported. Right-sided FTD patients present with prosopagnosia and failure to remember topographic relationships.

Clinical examination of FTD patients should focus on qualifying the cognitive deficits specific to each syndrome and identifying spared functions. Memory, orientation, visuospatial, and calculation domains are relatively well preserved early in FTD. Clinical examination should also detect the presence of any motor signs that may suggest an overlap syndrome (for instance; MND, CBS or PSP).

A battery of diagnostic tests (biochemical and metabolic laboratory tests, in depth neuropsychological assessment, electroencephalography, cerebrospinal fluid analysis and brain imaging) are often utilized before finalizing FTD diagnosis. The function of such tests is to exclude mimics and/or alternative diagnoses.

1.4.1 Behavioral-Variant Frontotemporal Dementia (bvFTD)

bvFTD is the most common of clinical syndromes among all FTD subtypes. It accounts for 55% of all FTD cases. Patients with bvFTD present with marked changes in personality and behaviour. The key features include an insidious onset and gradually progressive course, early behavioral disinhibition, early apathy or emotional blunting,

early stereotypic behaviour, alterations in eating behaviour, loss of sympathy or empathy, and dysexecutive neuropsychological profiles. Patients with bvFTD show a mixture of disinhibition and apathy (16). Apathy is characterized by loss of interest in personal affairs and responsibilities, social withdrawal, and loss of awareness of personal hygiene. Disinhibition is manifested by a large amount of socially inappropriate behaviours, such as shoplifting or making hurtful or insensitive remarks about others. Insight is impaired, with either obvious denial of illness or very shallow recognition of their problem. Stereotypic motor behaviours (rubbing, picking, throat clearing, pacing and wandering), idiosyncratic hoarding and collecting, change in eating behaviour (overeating and sweet food preference resulting in weight gain, loss of table manners) and hyperorality including oral exploration of inedible objects are also common and disease-specific symptoms. Psychotic symptoms such as delusions and hallucinations occur in a small minority of FTD-spectrum disorders. The most common cognitive symptoms are dysexecutive functions, such as poor judgment, less attention and distractibility, loss of the ability to plan, and disorganization (17). Patients show deficits on executive tasks, such as set shifting, mental flexibility, and response inhibition and abstract reasoning on cognitive testing. Visuospatial function is relatively preserved. Structural and functional neuroimaging studies have highlighted frontal atrophy, hypometabolism, and hypoperfusion in patients with bvFTD (18) (more details in imaging section). While the dorsolateral prefrontal cortex is often involved, the earliest changes occur in a medial paralimbic network that includes anterior cingulate, orbital frontal and frontoinsula cortices, and atrophy in these regions usually differentiates bvFTD from AD (18,19). The region of atrophy correlates with the clinical phenotype, with dorsomedial frontal atrophy associated with apathy and aberrant motor behaviour, and orbitofrontal atrophy associated with disinhibition (20).

Although episodic memory and visuospatial function is relatively preserved in contrast to AD patients, several groups reported anterograde amnesia in up to 10% of

pathology-confirmed bvFTD cases (21-23). The preponderance of primarily amnesic (versus behavioral) presentations in elderly subjects with bvFTD may be related to hippocampal sclerosis, which was reported in 43% of late onset FTLD cases (24). This might have resulted in diagnostic difficulties because health systems tolerate amnesic disturbances in the elderly, as MCI does not always progress to AD. Furthermore, although behavioural changes have higher predictive value for FTD, they are less likely to be recognized in the elderly population as those with behavioural symptoms may seem normal at the clinic and family members may make allowances for abnormal behaviour or may be unwilling to disclose actions/behaviours that are socially inappropriate.

An interesting group of patients is the "slow", "benign" or "non-progressive" bvFTD who progress very slowly over time. Patients in this group may not show atrophy on structural imaging or hypometabolism/hypoperfusion on functional imaging many years from symptom onset (100, 101). Moreover, behavioural decline in this group may be reported by carers but is often not measurable on cognitive testing (33), as cognitive testing is insensitive to behavioural changes and scales that focus primarily on behaviour needs a proxy. Previously undescribed neuropsychiatric syndrome with functional disruption of the same orbitofrontal-amygdala-polar network has been suggested as a possible diagnosis in this group of patients (102).

1.4.2 Semantic-Variant Primary Progressive Aphasia (svPPA)

svPPA is the most well defined form of FTD and is therefore relatively easier to diagnose. svPPA, also called semantic dementia (SD), is characterized by a fluent, anomia aphasia with or without behavioral changes with remarkable, often asymmetric degeneration of the anterior temporal lobes (25). svPPA accounts for 20% of all FTD cases. Patients with primarily left-sided predominant atrophy present initially with

progressive loss of word knowledge and meaning of words, objects and concepts; the so-called “semantic” knowledge. This manifests as a fluent aphasia with poor speech content and semantic paraphasic errors. Characteristically syntax, prosody and motor speech remain intact. When the disease disproportionately involves the right temporal lobe, deficits in knowledge of facial emotion, diminished recognition of familiar faces, and deficits in empathy for others predominate the clinical syndrome.

Anomia is the most common symptom. The inability to name an object is matched by the patient’s inability to give a detailed description of the object. In addition, patients with svPPA have a multimodal agnosia and are unable to recognize word meanings via written, auditory, olfactory and visual modalities (26). Patients with svPPA present surface dyslexia while reading, a condition in which the patient has difficulty reading words with irregular pronunciations, for example, yacht is pronounced “ya-ch.”

Patients with predominant right anterior temporal atrophy present with prosopagnosia and behavioral changes similar to bvFTD (28). Prosopagnosia and associative agnosia are the most prominent symptoms, but changes in personality and behaviour often precede these symptoms by years. There is a clear loss of empathy and interest in other people, and mental rigidity manifested with strict schedules and routines. Left-sided patients are reported more commonly than those with right-sided disease, approximately 3:1 in most centres (28-30), although this may be due to referral bias with the right-sided cases less likely to reach specialized centres.

svPPA is a very consistent syndrome in its presentation, its imaging and the underlying pathology. Imaging is very characteristic in SD; coronal MRI shows asymmetric atrophy of the temporal lobes, particularly of the anterior and inferior temporal area. The underlying pathology is that of TDP43 deposition, and it is rarely genetic.

1.4.3 Nonfluent-Variant Primary Progressive Aphasia (nfvPPA)

nfvPPA, affecting 25% of all FTD cases, is a progressive disorder of language expression and motor speech (31). Anatomically, it is associated with atrophy, hypometabolism and hypoperfusion of the left perisylvian area: frontal operculum, premotor and supplementary motor areas, and anterior insula (32). Patients present with slow, effortful speech, impaired production and comprehension of grammar, and motor speech deficits. Apraxia of speech, defined as difficulty initiating speech, a slow rate of speech or incorrect sequencing or omission of phonemes, is highly characteristic of nfvPPA, while dysarthria is more variably present. Comprehension is spared for single words and for all except the complex syntactic structures. Reading is nonfluent and effortful, while writing is agrammatic and features phonemic paraphasias. In addition to the aphasia, neuropsychological tests may show mild deficits in executive function, with relatively spared episodic memory and visuospatial function. Behavioral disturbances are less frequent when compared to bvFTD and svPPA, reflecting less damage in the orbitofrontal areas and the right hemisphere in general (33).

While Tau is the main protein causing pathology in this group, few patients have TDP43 pathology in their brain.

1.4.4 Logopenic variant PPA (lvPPA)

There are some patients with progressive language impairment who do not fit into the svPPA or nfvPPA criteria (Table): a third, more recently defined, subtype of PPA is the logopenic-variant PPA (lvPPA) (31,34). Patients with lvPPA are characterized by a slow rate of speech output, word-finding difficulties, deficits in sentence repetition, and occasional phonemic errors in spontaneous speech and naming, whereas motor speech, expressive grammar, and single-word comprehension are relatively preserved. The underlying pathology of most of lvPPA cases is AD.

A summary of the clinical features, distribution of atrophy, and underlying pathology of the three variants of Primary Progressive Aphasia is presented in the table below:

PPA variant	Core clinical features	Region of cortical atrophy	Common underlying pathology
Semantic variant	-Impaired single-word comprehension -Poor confrontation naming	Anterior and ventral temporal lobe	TDP-43
Non-fluent variant	Effortful speech with grammatical and phonemic errors	Left inferior frontal and insula	Tau
Logopenic variant	-Impaired single-word retrieval -Impaired repetition of phrases and sentences	Left posterior temporal and inferior parietal	AD

Table: Summary of Clinical features, Region of atrophy and Underlying pathology of Primary Progressive Aphasia variants

* Abbreviations: AD: Alzheimer disease, PPA: primary progressive aphasia, Tau: microtubule associated protein tau, TDP-43: transactive response (TAR) DNA-binding protein with molecular weight (Mw) of 43 kDa.

1.4.5 FTD Overlap Syndromes

Amyotrophic lateral sclerosis (ALS) and bvFTD are overlapping syndromes at the clinical, pathological, and genetic levels (35,36). While a proportion of patients presenting with ALS manifest cognitive and behavioral changes that may be severe enough to reach criteria for bvFTD (10–15%), a subgroup of patients (15%) with bvFTD develops features of ALS (37). The molecular pathology and genetics of the bvFTD-ALS spectrum are described in section 1.4.6. Because of this significant overlap between FTD and ALS, some authors suggest that FTD patients should undergo a neuromuscular evaluation with consideration for electromyography and, conversely, all ALS patients should be screened for evidence of cognitive and behavioral changes.

FTD clinical syndromes also show significant overlap with patients in whom the underlying pathology is corticobasal degeneration (CBD) or PSP (38). CBD is a syndrome of limb apraxia, axial and limb rigidity, dystonia, postural instability, ocular apraxia, the “alien limb phenomenon” and cortical sensory loss. PSP is described as a syndrome of supranuclear gaze palsies, axial-predominant parkinsonism and profound retropulsion. Pseudobulbar signs such as dysarthria, dysphagia, and pseudobulbar affect were also observed.

More recently, it has been increasingly recognized that many patients with the classical Corticobasal syndrome (CBS) exhibit AD at neuropathology (39). Additionally, the prominent behavioral, language and executive deficits associated with both conditions have been increasingly recognized (40,41). More than one-quarter of all clinical diagnoses of CBS cases show either nvPPA syndrome or bvFTD, while another third

exhibit an executive-motor syndrome with prominent deficits in executive control and Parkinsonism. Additionally, PSP often begins as a neuropsychiatric disorder or nfvPPA. Both are associated with prominent tau pathology and these syndromes should be considered within the pathological FTLD spectrum (42).

There is growing evidence for a possible relationship between FTD, ALS and traumatic brain injury or chronic traumatic encephalopathy (CTE): It has been shown that there is an increased prevalence of late-life cognitive impairment in retired NFL players (137). Head trauma has been reported as a risk factor for multiple degenerative conditions including FTD, AD, and PD (138), although definitive evidence is lacking.

1.4.6 FTD-MND overlap syndrome

FTD might co-occur with Motor Neurone Disease (MND), a syndrome known as FTD-MND. The recently rekindled awareness of the links or association between dementia and motor neurone disease was described in early literature of ALS (Marie and Reynolds in 1880s) (103). At present, medical treatment is extremely limited and etiological mechanisms underlying the condition are not fully understood.

Amyotrophic lateral sclerosis (ALS) is caused by degeneration of lower motor and pyramidal neurons, resulting in loss of voluntary muscle movement. FTD and ALS are strongly linked through common molecular, pathological and anatomical overlaps. In some patients FTD precedes ALS; in others ALS occurs first, while in a third group the two disorders begin simultaneously.

The FTD-MND overlap syndrome tends to transcend the traditional borders between the two conditions. An early psychotic phase characterised by vivid hallucinations and delusions and profound behavioural features characterizes this syndrome. The first author to posit a direct connection between MND and frontal lobe dysfunction was Braumuhl in 1932. Bak and Hodges wrote "In most cases the cognitive and behavioural

changes predate the appearance of the classical features of MND by months to years. Depending on the predominant symptoms the initial diagnosis can be that of behavioural variant FTD or progressive non-fluent aphasia ” (104). Abraham et al suggested that bulbar presentation is more often associated with dementia than other types of MND (105). FTD-MND is a more rapid disease but the electromyography (EMG) features are not different from that seen in classical MND (106). Death occurs in majority of cases within two to three years after onset and cases of substantially longer duration are rare.

A cross-sectional study aimed at identifying the frequency of ALS in FTD was conducted by Catherine Lomen-Hoerth et al (107). In this study, 36 consecutive FTD patients with no known diagnosis of ALS or family history of ALS were assessed clinically and electrophysiologically for the presence of ALS. Five patients (14%) in this group met the criteria for a definite diagnosis of ALS, two had EMG findings suggestive of denervation in one limb but did not have other changes to meet the criteria for diagnosis on ALS, and five patients had prominent fasciculations but no abnormalities by EMG studies. One of the patients with fasciculations and a normal EMG study progressed to definite ALS after one year of initial assessment. Thus, 36% of patients in this study met the diagnostic criteria for possible ALS (107).

In FTD-MND, the FTD syndrome is typically characterized by behavioral changes, but cases with prominent aphasia have been described (108). Although The MND domain is commonly characterized by bulbar problems such as swallowing or speech difficulties (109), limb weakness associated with fasciculations in the weak limb is also common. Signs of upper motor neuron disease or pyramidal tract signs, such as spasticity, hyper-reflexia, Babinski and clonus, are less common, but have been described (110). A clinical diagnosis of FTD-MND has almost perfect clinico-pathological association as described in the pathology section of this chapter.

1.5 Diagnostic criteria of FTD subtypes

The 1998 consensus diagnostic criteria by Neary et al were widely used in research and clinical practice until recently (5). In 2011, the International Behavioral Variant FTD Criteria Consortium (FTDC) proposed a revision of diagnostic and research criteria for bvFTD, which has higher sensitivity (43). The details of the new consensus criteria for bvFTD are shown in the table below (Table 1.1). According to the new criteria, the main feature of bvFTD is progressive deterioration of behaviour and/or cognition by observation or history provided by a knowledgeable informant. If this criterion is satisfied, there are three further levels of certainty for bvFTD: possible, probable, or definite. “Possible” bvFTD requires three out of six clinically discriminating features. “Probable” bvFTD meets the criteria of “possible” bvFTD and 1) a significant functional decline by caregiver report or evidenced at neuropsychological testing, and 2) imaging results consistent with bvFTD. “Definite” bvFTD requires the histopathological evidence of FTLD or the presence of a known pathogenic mutation. In the diagnostic evaluation of a patient with suspected FTD, as with any other neurodegenerative dementia, the clinician should first exclude treatable conditions that can mimic FTD and present in a similar way, such as metabolic disturbances, nutritional deficiencies, central nervous system (CNS) infections, vascular disease, normal or low intracranial pressure syndromes, and primary neoplastic conditions. These can be excluded by a careful medical history, physical examination, laboratory testing, and neuroimaging. Patients with FTD are sometimes misdiagnosed with a psychiatric disorder such as schizophrenia, major depression or bipolar affective disorder (44).

The following table summarizes the 2011 international consensus criteria for behavioural variant FTD (43):

Table 1.1: Diagnostic Criteria for bvFTD by the International Behavioral Variant FTD Criteria Consortium (FTDC)*

I. Neurodegenerative disease

The following symptom must be present to meet criteria for bvFTD: A. Shows progressive deterioration of behavior and/or cognition by observation or history (as provided by a knowledgeable informant)

II. Possible bvFTD

Three of the following behavioral/cognitive symptoms (A–F) must be present to meet criteria. Ascertainment requires that symptoms be persistent or recurrent, rather than single or rare events:

A. Early behavioral disinhibition (one of the following symptoms [A.1–A.3] must be present):

- A.1. Socially inappropriate behavior
- A.2. Loss of manners or decorum
- A.3. Impulsive, rash or careless actions

B. Early apathy or inertia (one of the following symptoms [B.1–B.2] must be present):

- B.1. Apathy
- B.2. Inertia

C. Early loss of sympathy or empathy (one of the following symptoms [C.1–C.2] must be present):

- C.1. Diminished response to other people's needs and feelings
- C.2. Diminished social interest, interrelatedness or personal warmth

D. Early perseverative, stereotyped or compulsive/ritualistic behavior (one of the following symptoms [D.1–D.3] must be present):

- D.1. Simple repetitive movements
- D.2. Complex, compulsive or ritualistic behaviors
- D.3. Stereotypy of speech

E. Hyperorality and dietary changes (one of the following symptoms [E.1–E.3] must be present):

E.1. Altered food preferences

E.2. Binge eating, increased consumption of alcohol or cigarettes

E.3. Oral exploration or consumption of inedible objects

F. Neuropsychological profile: executive/generation deficits with relative sparing of memory and visuospatial functions (all of the following symptoms [F.1–F.3] must be present):

F.1. Deficits in executive tasks

F.2. Relative sparing of episodic memory

F.3. Relative sparing of visuospatial skills

III. Probable bvFTD

All of the following symptoms (A–C) must be present to meet criteria:

A. Meets criteria for possible bvFTD

B. Exhibits significant functional decline (by caregiver report or as evidenced by Clinical Dementia Rating Scale or Functional Activities Questionnaire scores)

C. Imaging results consistent with bvFTD (one of the following [C.1–C.2] must be present):

C.1. Frontal and/or anterior temporal atrophy on MRI or CT

C.2. Frontal and/or anterior temporal hypometabolism or hypoperfusion on PET or SPECT

IV. Behavioral-variant FTD with definite FTLD pathology

Criterion A and either criterion B or C must be present to meet criteria:

A. Meets criteria for possible or probable bvFTD

B. Histopathological evidence of FTLD on biopsy or at postmortem

C. Presence of a known pathogenic mutation

V. Exclusionary criteria for bvFTD

Criteria A and B must be answered negatively for any bvFTD diagnosis. Criterion C can be positive for possible bvFTD but must be negative for probable bvFTD:

A. Pattern of deficits is better accounted for by other nondegenerative nervous system or medical disorders

B. Behavioral disturbance is better accounted for by a psychiatric diagnosis

C. Biomarkers strongly indicative of Alzheimer disease or other neurodegenerative process

***Source: Rascovsky, et al. 2011 (43).**

1.6 Neuropsychology of FTD

The criteria for diagnosing bvFTD have evolved over the last few years. A number of studies have been conducted looking for features differentiating FTD from Alzheimer's dementia and other FTD mimics. Loss of basic and social emotions, disinhibition, personal neglect, generalised loss of interest in activities, overeating, altered preference for sweet foods, wandering, pacing, and motor and verbal stereotypies constitute the most common behavioural changes (112). When symptoms are considered, Snowden et al reported that changes in emotion and eating, together with behavioural stereotypies differentiated FTD from Alzheimer's disease and vascular dementia with 97% accuracy (112). But at the level of objective neuropsychology testing, a meta-analytic review of the neuropsychological deficits in FTD and AD conducted by Hutchinson and Mathias concluded that none of the currently available neuropsychology tests showed acceptable low overlap between the scores of FTD and AD to confidently make a differential diagnosis (113).

The main sensitive symptoms of bvFTD include: Disinhibition, Social dysfunction and inappropriate behaviour (especially sexual behaviour) and isolation, Emotional blunting, Impulsivity, Rashness, Overspending of money, Loss of sympathy and empathy,

Change in appetite: in the form of overeating and sweet food preference, Executive problems (difficulty in planning and judging), Apathy and inertia.

Disinhibition, lack of empathy, perseveration and eating disorders are considered to be the four most discriminative features. Apathy and executive dysfunction on the other hand are less discriminative. This is because although apathy is very common in FTD but it is also common in Alzheimer's disease and almost all cortical dementias. Nonetheless, executive dysfunction including planning, organisation, judgement, problem solving and mental flexibility is characteristic of FTD. Both letter and categorical verbal fluency are usually impaired in bvFTD.

In svPPA patients semantic fluency is more impaired than letter fluency (35). Other cognitive domains that are usually well preserved in FTD include memory, visual perception and spatial skills (114).

Research exploring Theory of Mind (ToM), social cognition and emotional processing has enriched neuropsychological phenotype of FTD. One major deficit in FTD patients, which underlies lack of empathy and sympathy, is loss of the so-called theory of mind. ToM tests require the interpretation of social situations and imputing mental state to oneself and others.

Impairment in theory of mind (ToM) is also a key deficit in Autism and Asperger's syndrome. There are a number of neuropsychological tests incorporated in batteries and used for assessing theory of mind; such are the Faux Pa test and Reading the Eyes in the Mind test.

Testing recognition of emotion is relatively easier to administer and to interpret in clinical practice. The Florida Affect Battery is an example of a test used for this purpose. Recent research suggests that the neural basis for ToM, social cognition and empathy lies within the medio- and/or orbitofrontal cortex, cortical areas that are affected early in bvFTD (45).

A variety of rapid screening tests have been designed specifically for the purpose of discriminating FTD from non-FTD dementias such as the third version Addenbrooke's Cognitive Examination (*ACE-III*, Hsieh 2013) which is the most updated version of the ACE (Mathuranath 2000), the Executive Interview (Royall, Mahurin RK, and Gray, 1992), and the Frontal Assessment Battery (FAB, Dubois, Slachevsky, Litvan and Pillon B, 2000), and The Montreal Cognitive Assessment (MOCA, Nasreddine et al. 2005). However, these are screening tools and ideally the patient should be referred to a clinical psychologist for more detailed neuropsychological evaluation before making the diagnosis of FTD.

Precise characterization of neuropsychological profile of FTD patients is not easy, as such an attempt will be faced by two major problems: the weak clinicopathological correlation and contamination by cases with underlying Alzheimer's disease and other FTD mimics. Given the fact that FTD can frequently present with alterations in behaviour and personality, there should not be an over-reliance on cognitive testing in isolation but instead results of cognitive testing should be interpreted with caution taking in account patient's clinical presentation and imaging findings.

1.7 Neuroimaging of FTD

Neuroimaging is a standard tool that serves the aims of improving diagnostic certainty and excluding other differential diagnoses and FTD mimics. Quantitative MRI remains generally a research tool and embodies three main techniques: volumetric analysis of specific brain regions, Voxel Based Morphometry (VBM), and serial co-registration. The two methods of region of interest (ROI) analysis (volumetric analysis of specific brain regions) and voxel-based morphometry (VBM) are commonly used in research to analyse Magnetic resonance imaging (MRI) data. Both techniques are labour intensive and each has its own caveats. Both ROI and VBM methods are applicable to MRI,

positron emission tomography (PET) and single-photon emission computed tomography (SPECT).

Serial co-registration is a third MRI method that enables calculating the amount of change in whole brain volume by co-registering scans obtained at different time points. This makes it a possible method that can be used in longitudinal studies. Fluid-body registration was subsequently developed to allow regional assessment of changes using serial co-registration.

MRI using voxel-based morphology can provide precise maps of the areas of focal atrophy. Patients with bvFTD often have bilateral frontal atrophy, especially of the medial frontal cortex, the orbitofrontal lobe and anterior temporal lobe. These cortical areas are very important for motivation, inhibitory control and emotional processing. Whitwell et al reported that FTD cases associated with motor neurone disease (FTD-MND) have more paracentral atrophy on MRI when compared to pure FTD cases (111).

Functional imaging modalities, particularly positron emission tomography (PET) and single-photon emission computed tomography (SPECT) detect focal lobar hypometabolism or hypoperfusion respectively with great sensitivity and are good diagnostic tools. Both modalities are inferior to MRI as regards to anatomical resolution but have the advantage of identifying pathological involvement in areas that appear structurally normal, i.e. enhancing diagnostic certainty where structural imaging is inconclusive.

While presence of focal atrophy has a high positive predictive value for clinical dementia, the absence of atrophy has been noticed increasingly in patients with FTD phenocopies. The presence of asymmetric lobar atrophy in the frontal regions with or without temporal atrophy is the best predictor of bv-FTD.

MRI findings in svPPA tend to be more consistent. Cases typically have focal atrophy of the anterior temporal pole with involvement of the inferior surface (especially the fusiform gyrus). This pattern is typically seen asymmetrically as either bilateral or as mainly left-sided atrophy. A variable amount of frontal atrophy is also not uncommon in svPPA.

Imaging findings in nvPPA are considered less reliable than in either bvFTD or svPPA. Nonetheless, patients with nvPPA tend to have left hemispheric frontal and temporal lobe atrophy involving the perisylvian area. The left inferior frontal lobe, anterior insula and basal ganglia atrophy is also commonly seen.

In the clinical setting, neuroimaging changes in FTD are best seen using volumetric structural MRI (with coronal T1 images being particularly useful in assessing asymmetry) or with functional imaging using either FDG-PET or HMPAO-SPECT. These imaging modalities show frontal and temporal atrophy, hypometabolism and hypoperfusion depending on modality used.

Classical patterns of atrophy in FTD subtypes that have been described in research can be summarised as follows (starting by changes seen in mild disease followed by those seen with progression):

- In bvFTD: mild bvFTD in clinically defined cohorts show involvement of frontal and paralimbic areas, namely the anterior cingulate cortex, frontal insula, medial frontal and orbitofrontal cortices, hippocampus, striatum and thalamus, right more than left (117). Atrophy in the same areas becomes more pronounced as the disease progresses and more diffuse atrophy is seen in more lateral frontal areas and subsequently more posterior temporal and anterior parietal cortices (118).
- In svPPA: studies utilising VBM showed asymmetric atrophy (left more than right) of anterior and inferior temporal lobes (119,120). Further research using ROI studies of the temporal lobe) supported the same and further identified

particular involvement of the temporal pole, anterior parts of the entorhinal cortex, fusiform gyrus, inferior temporal gyrus amygdala and hippocampus with relative sparing of the superior temporal gyrus (121,122).

- In nvPPA: imaging studies in nvPPA are relatively fewer and results are heterogeneous reflecting the clinical heterogeneity of this condition. Early stage findings are comparable to those of svPPA as there is asymmetric involvement with more atrophy of the left hemisphere's inferior frontal lobe and anterior insula (123-126). In more advanced disease, there is involvement of the left middle and superior frontal, superior temporal and anterior parietal lobes (124).

1.8 Histopathology of FTD

On macroscopic brain examination on autopsy, FTD patients show various degrees of selective, circumscribed, or sometimes extreme atrophy of frontal and anterior temporal lobe, with marked sparing of posterior brain regions until advanced stages of disease (45). Microscopic examination of the cerebral cortex reveals neuronal loss and microvacuolar degeneration in layers II and III of the frontal and temporal cortex, with a variable degree of cortical gliosis and spongiosis. White matter changes include loss of myelin, astrocytic gliosis, in addition to neuronal loss in the basal ganglia and substantia nigra in some cases (46).

FTLD, a broader spectrum term for various pathological conditions, is the common underlying pathology of clinical FTD subtypes. However, subtypes differ from one another by differences in protein deposition or biochemical signature and inclusion morphology and distribution.

Three major proteins are implicated in the pathogenesis of FTLD. About half of cases are characterized by depositions of inclusions of hyperphosphorylated microtubule associated protein tau in neurons and glial cells (FTLD-tau) (134). In most of the

remaining cases, deposits of the transactive response (TAR) DNA-binding protein with molecular weight (Mw) of 43 kDa, known as TDP-43 (FTLD-TDP), are seen. Lastly, about 5–10 % of cases are characterized by abnormal accumulations of a third protein, the fused in sarcoma (FTLD-FUS). FTLD-FUS cases are characterised by bvFTD, a young age of onset, negative family history, and caudate atrophy on MRI (107). Mutations of FUS gene on chromosome 16 play pathogenic role in FTD and ALS.

A fourth but less frequent pathological type is FTLD-UPS: Cases in this group have ubiquitin-positive but TDP-43 and FUS-negative inclusions, termed FTLD-UPS. Most of the FTLD-UPS cases carry the charged multivesicular body protein 2B (CHMP2B) mutations albeit a few sporadic cases exist.

Depending on the protein concerned, the signature accumulations can take the form of inclusion bodies (neuronal cytoplasmic inclusions and neuronal intranuclear inclusions) or dystrophic neurites, in the cerebral cortex, hippocampus, subcortex or glial cells. In motor neurone disease (MND), TDP-43 or FUS inclusions can present within motor neurons of the brain stem and spinal cord. Tau protein can accumulate in both nerve cells and glial cells. In sporadic disease, the signature accumulations take the form of neuronal Pick bodies (known as FTLD-tau PiD), tufted astrocytes (FTLD-tau PSP), or astrocytic plaques (FTLD-tau CBD), whereas in inherited cases, these can present as inclusions similar to any of these or with unique tau pathology, such cases being defined as FTLD- tau MAPT (133). Pathologically TDP-43 is seen as inclusion bodies [neuronal cytoplasmic inclusions (NCI) and neuronal intranuclear inclusions (NII)] or dystrophic neurites (DN) in the cerebral cortex, hippocampus and subcortex. In many instances, the relative proportions of NCI, NII and DN within the tissue permits subclassification into histological subtypes, A, B, C and D (133), which can aid diagnostic precision, but not all cases always show clear-cut distinctions. Lastly, about 5–10 % of cases of FTLD and a few familial MND cases are characterized by the abnormal accumulation, as cellular inclusions, of a third protein, fused in sarcoma

(FUS) (25). Other ubiquitinated, but as yet unidentified, target proteins characterize FTLD cases with CHMP2B mutations (135).

Using immunohistochemical techniques, most cases of FTLD can be subdivided into the following three subcategories, which are based on the presence of specific inclusion bodies: (i) FTLD with tau inclusions (FTLD-TAU); (ii) FTLD with TDP-43 inclusions (FTLD-TDP); and (iii) FTLD with FUS inclusions (FTLD-FUS) (6). Additionally a small number of FTLD cases show no pathological aggregates, and it is increasingly realized that other coexisting neurodegenerative diseases, most commonly AD and DLB, are present with microscopy, particularly in older patients.

Predicting the underlying histopathology following clinical diagnosis of FTD has been often challenging, although there are strong correlations between some clinical syndromes and the neuropathological subtype (47,48). The underlying histopathology in typical bvFTD is heterogeneous, showing no clear association with one specific pathological subtype. svPPA is associated with predominantly FTLD-TDP type C histopathology; FTD with motor neuron disease (FTD-MND) was associated with predominantly FTLD-TDP type B histopathology. nfvPPA is associated with FTLD-tau, or TDP type A histopathology.

1.8.1 Tau-Positive FTLD (FTLD-TAU)

Tau is a microtubule-associated protein (MAP). Tauopathies are classified according to the predominant species of tau that accumulates, with tau proteins containing three repeats (3R) or four repeats (4R) of amino acids in the microtubule-binding domain. Tauopathies in the FTLD spectrum include Pick's disease, FTD with MAPT mutations CBD, PSP and argyrophilic grain disease (46).

Pick's disease is the prototypical tauopathy of FTLD, and is characterized by the presence of Pick bodies, which are solitary, round, argyrophilic inclusions found in the

cytoplasm of neurons located in the limbic system, including the dentate fascia of the hippocampus, entorhinal cortex and amygdala, and in the superficial frontal and temporal neocortex (49). Pick inclusions are composed primarily of 3R tau. CBD, PSP, and argyrophilic grain disease are tauopathies with predominantly 4R tau inclusions. CBD and PSP are as common as Pick disease in patients presenting with FTD clinical syndrome. The distribution of pathology and pattern of atrophy can distinguish CBD from PSP, and from Pick disease. Patients with FTDP-17 share the common characteristic of filamentous, hyperphosphorylated tau aggregates (50). Patients with mutations in the MAPT gene may show the pathologic features of 3R, 4R or a combination of 3R and 4R tau (51).

1.8.2 TDP-43-Positive FTLD (FTLD-TDP)

TDP-43 is a ubiquitously expressed nuclear protein that may function as a transcription repressor, activator of exon skipping or as a transcription regulator. TDP-43 becomes hyperphosphorylated, ubiquitinated and cleaved into C-terminal fragments under pathologic conditions (52). TDP-43 pathology is found in sporadic and familial patients of FTLD involving progranulin (PGRN), C9ORF72 hexanucleotide repeat expansion, and valosin-containing protein (VCP) gene mutations (53,54).

FTD-TDP is now considered to be the most common neuropathology seen in FTLD. Pathological TDP-43 inclusions are present in the dentate gyrus of the hippocampus, layer II of the frontotemporal cortex and anterior horn of the spinal cord. These inclusions are ubiquitin and TDP-43-positive, tau-, α -synuclein-, amyloid- β and neuronal intermediate filament-negative by immunohistochemical method (46).

Four histological subtypes, A–D, have been recognized according to a revised classification scheme based upon the distribution and morphology of the inclusions

(52). Type A is associated with cortical pathology predominantly in layer II, with progranulin (GRN) mutations, and with some sporadic nvPPA cases. Type B is found in patients with clinical bvFTD and FTD-MND, and it shows linkage to the C9ORF72 hexanucleotide repeat expansion. Type C is localized primarily to cortical layer II in patients with svPPA and bvFTD. Type D histology is found in all cortical layers and is associated with patients with familial inclusion body myopathy and Paget disease of the bone with frontotemporal dementia and VCP gene mutations. It remains still unclear what differences in underlying pathophysiology determine the distinction between these TDP-43 subtypes.

1.8.3 FUS-Positive FTLD (FTLD-FUS)

The majority of tau-negative/TDP-43-negative, ubiquitin-positive FTLD cases have positive immunohistochemical staining to the FUS protein, thus distinguishing a third category of FTLD neuropathology. The FUS protein contains 526 amino acids. It is as a nuclear protein involved in DNA repair and the regulation of RNA splicing. Mutations in the FUS gene on chromosome 16 emphasize its pathogenetic role in the clinicopathological spectrum of FTD and ALS (55,56). Clinically, most FTLD-FUS cases are characterized by early-onset FTD (age <50years), a negative family history, prominent psychiatric features such as delusions and hallucinations, and a caudate atrophy on brain imaging. These TDP- negative FTLD with ubiquitin pathology cases were formerly known as atypical FTLD with ubiquitin-positive inclusions (aFTLD-U), basophilic inclusion body disease (BIBD), and neuronal intermediate filament inclusion disease (NIFID) (57).

Of note here is that AD contamination is a particular problem when clinicopathological correlation of FTD is considered. Pathological diagnosis of AD, by definition, requires proof of the presence of neurofibrillary tangles in the brain, and often both extracellular

β -amyloid deposits and intraneuronal neurofibrillary changes (242). The term frontal AD is increasingly used when the ante-mortem clinical diagnosis is bvFTD while the pathological diagnosis is AD. Such cases occur, but rare, with AD pathology being reported in clinically diagnosed bvFTD cases (95).

1.9 Genetics of FTD

In the last fifteen years pathological investigations and genetic screening have contributed tremendously to understanding the common pathology and genetic variability associated with FTD. The likely polygenic inheritance of apparently sporadic FTD cases resulted in great difficulty in continuing to support the traditional disease dichotomy of sporadic versus genetic (11, 12).

It is certain that there is a strong genetic component underlying FTD and the tendency for the condition to cluster in families is well recognised as many as 40-50% of patients with FTD have an affected family member and an autosomal dominant mode of inheritance is seen in 10-27% of all FTD patients (96). . Furthermore, a positive family history of dementia was identified in 50% of patients with bv-FTD in the population-based initial Manchester study (116). bvFTD is familial in 30-50% of cases whereas patients with svPPA or nvPPA have a much lower frequency (58). A review by Seelaar et al found that 27% of cases of familial FTD had autosomal-dominant inheritance (12). Genes recognized to play an important role in autosomal dominant FTD include: 1) MAPT, encoding microtubule-associated protein tau, 2) PGRN, encoding the protein progranulin, and 3) C9ORF72, a recently identified hexanucleotide repeat expansion on chromosome 9 (11.12). These gene mutations explain the majority of autosomal dominant FTD cases as mutations in the remaining known genes; VCP, charged multivesicular body protein 2B (CHMP2B), TAR-DNA binding protein (TARDBP), and FUS genes are found in less than 5%.

1.9.1 Microtubule Associated Protein Tau (MAPT)

In 1998, Hutton et al (127) and Poorkaj et al (128) described mutations in the microtubule-associated protein tau gene, located on chromosome 17 (MAPT, 17q21.32). Tauopathies include FTD, ALS, progressive supranuclear palsy (PSP), Corticobasal degeneration (CBD), and Parkinson-dementia-complex of Guam.

By 2011, more than 40 mutations in the MAPT gene have been identified in families with FTD and parkinsonism linked to chromosome 17q (FTDP-17) (59), and as of 2012, more than 60 MAPT mutations had been identified. Most mutations are caused by missense mutations in exons 9–13 affecting the normal function of the tau protein to stabilize microtubules, or in the intronic regions, disproportionately influencing the splicing in of exon 10 at the mRNA level, resulting in a change in ratio of 3R to 4R tau isoforms. MAPT gene mutations account for approximately 20% of all familial FTD cases in some studies, although small numbers of cases have been reported in others (60,61).

The most common FTD clinical variant associated with MAPT mutations is bvFTD with prominent behavioral changes including disinhibition and obsessive–compulsive behaviour. svPPA and parkinsonian syndromes can also be seen in patients with MAPT mutations. The mean age at onset is 55 years, and the mean duration of illness is approximately 9 years. MAPT mutations are associated with more significant symmetrical anteromedial temporal and orbitofrontal atrophy (47,62).

In spite of the fact that the histopathological appearances of brains of patients with tau mutations are more or less consistent, the phenotypical patterns have varied considerably. This suggests that other factors are important in controlling the distribution of the pathological changes within the brain. It also means that much remains to be learnt.

1.9.2 PGRN

More than 60 mutations in the PGRN gene on chromosome 17 have been identified. PGRN mutation cases account for approximately 5–10% of all FTD patients, and up to 22% in familial FTD (12,47). Its frequency is similar to that of MAPT gene mutations as a cause for hereditary forms or FTLD (12). The mean age at onset is around 60 years; the mean duration is 8 years (12). GRN mutations have been most commonly associated with clinical diagnoses of bvFTD and nvPPA, although AD, Parkinson disease and CBS are also seen. The neuropathology of patients with GRN mutations is characterized by tau-negative, ubiquitin-positive, and TDP-43-positive inclusions. GRN mutations are associated with an asymmetrical frontoparietotemporal pattern of atrophy; the most common behavioral changes are apathy and social withdrawal. 25% of patients present with early isolated language dysfunction of an anomia nonfluent type. Hallucinations and delusions are often reported (64). Extrapyramidal features are frequently seen and can be associated with limb apraxia, and asymmetrical parkinsonism, but FTD-MND is a rare phenotype in patients carrying GRN mutations (65,66).

1.9.3 C9ORF72 Hexanucleotide Repeat Expansion

As early as 1991, linkage studies of ALS-FTD and FTD kindreds suggested a locus on chromosome 9p.21. In 2011 two research groups identified the genetic mutation at that locus as an expansion of GGGGCC hexanucleotide repeats in a non-coding region of the C9orf72 gene (54, 67). The gene is known to have three transcripts. The function of the final protein product is not confirmed but there is evidence to support a role in endocytic and autophagic pathways as well as motor function. Some evidence supports loss of function as the main pathomechanism underlying the C9orf72 gene

mutation, as it is associated with reduced expression of gene's three major transcripts. However, toxic gain of function is also possible in the context of the presence of brain aggregates of both aberrant protein and abnormal repeat RNA. The latter aggregates are termed RNA foci and form core of the 'toxic RNA' hypothesis.

C9ORF72 is the most frequent genetic mutation associated with familial cases of bvFTD and FTD-MND in families with strong clustering of either one or both conditions. C9ORF72 is also strongly associated with psychosis and it may show anticipation. It is particularly present in patients with FTD-MND overlap. Current data suggests that C9ORF72 mutation or repeat expansion accounts for approximately 20-25% of familial FTD and about 40-50% of familial ALS cases and 0-7% of sporadic cases in white Americans, Europeans and Australians (71, 129-131). This may raise the possibility of a single founder in Europe. Inheritance follows an autosomal dominant pattern with incomplete penetrance.

The cause of the chromosome 9-linked bvFTD, ALS and FTD-MND is an expansion of a GGGGCC hexanucleotide repeat in the chromosome 9 open reading frame 72 gene (C9ORF72) (54,67). This mutation is most often associated with bvFTD with or without MND and amyotrophic lateral sclerosis (ALS), while the mutation does not appear to be commonly associated with svPPA or nvPPA phenotypes. The GGGGCC hexanucleotide repeat is located between two 5'-noncoding exons of C9ORF (a gene involved in RNA metabolism). It remains unknown how many repeats are truly needed to cause disease and the significance of variable distribution of repeat expansion length has not been determined. Nonetheless, a repeat length of greater than 30 has arbitrary been defined as pathogenic in most studies.

Although C9ORF72 mutations are reported to pathologically associate with deposition of the FTLD-TDP type B (68), type A pathology also occurs. C9ORF is strongly associated with psychosis and in fact; besides family history, psychotic symptoms represent strong clues to look for this gene, in clinical practice, the presence of

psychiatric symptoms in the context of FTD-ALS should prompt consideration of a C9ORF72-repeat expansion. Such psychotic symptoms include visual and auditory hallucinations and delusions. Other common symptoms include anxiety, poor memory and poor outcome. Clinical observations predating the discovery of the C9orf72 gene mutation suggested the presence of delusional ideation in younger bvFTD patients with tau negative pathology, though the anatomic substrate was not fully understood. It is conceivable that these patients might have been positive for the C9orf72 repeat expansion.

On structural imaging, in addition to atrophy in dorsolateral frontal, medial orbitofrontal, atrophy in parietal, occipital, cerebellar, and thalamus regions are also observed (70,71).

C9orf72 associated bvFTD is characterized by a distinct radiological and pathological signature. There is symmetrical frontotemporal atrophy but changes often extend to involve the parieto-occipital cortex and the cerebellum. The pathological hallmark of *C9orf72* related disease is TDP-43 inclusions and ubiquitin-binding protein p62/sequestosome 1 inclusions. The latter inclusions are considered highly specific to the *C9orf72* gene mutation as they are rare in non-*C9orf72* FTD. The p62/sequestosome 1 inclusions can occur with and without co-existing TDP-43 and have been observed in the frontal neocortex, cerebellum and hippocampus. It is important to mention that *C9orf72* gene mutations have been reported in patients presenting with the clinical picture of a number of other neurodegenerative disorders including AD (*C9orf72* is associated with AD at low frequency, less than 1%, but notably more often than healthy controls) (235), corticobasal syndrome (CBS) (235), dementia with lewy bodies (DLB) (236) and Huntington disease (HD) (237).

1.9.4 Other Hereditary Forms

The genetic heterogeneity of FTD is further emphasized by the rare occurrence of mutations in the VCP, CHMP2B, TARDP, and FUS genes (59). This is in addition to less frequent genetic mutations as discussed below.

The valosin-containing protein (VCP, 9p13.3) gene was identified in 2004 and has been linked to a syndrome comprising inclusion body myopathy (90%), Paget disease of the bone (45%), and bvFTD (38%) or sometimes ALS, presenting between the ages of 40 and 60 years.

Mutations of charged multivesicular body protein 2B gene on chromosome 3 (CHMP2B, 3p11.2) was reported in a Danish family with FTD in 1984. This gene was identified in a very large family in Jutland in Denmark with an unusual dementia. There were over 27 affected individuals with a very wide range of clinical variability. In 1995 genetic linkage to chromosome 3 was established before the gene encoding the chromatin modifying protein 2B (CHMP2B) on chromosome 3p11.2 was identified in 2005. The CHMP2B gene protein product is a heteromeric ESCRT-III complex expressed by neurons and is believed to play an essential role in endosomal-lysosomal function, protein breakdown, and neuronal survival. Mutations usually result in aberrant splicing affecting the C-terminus of the protein. Up to date, CHMP2B mutations have only been reported in Danish families manifesting clinically as familial FTD with autosomal mode of inheritance. The pathological entity here is ubiquitin positive but tau and TDP-43 negative (FTLD-U). The clinical presentation of CHMP2B gene mutations consists of a frontal lobe syndrome and a more global cognitive impairment, with parkinsonism, dystonia, pyramidal signs, and myoclonus later in the course of the disease.

In 2008 mutation in the transactive response-DNA binding protein (*TARDBP*) gene on chromosome 1 was identified as a cause of ALS (usually familial but occasionally sporadic ALS), bvFTD and ALS-FTD. Since then more than 30 mutations in the *TARDBP* gene have been reported. Almost all reported *TARDBP* mutations are in exon 6 affecting the highly conserved C-terminus of the TDP-43 protein known to be involved in RNA recognition. TAR-DNA binding protein 43 [TDP-43, 17q21.32] or *TARDBP* gene mutations on chromosome 1 are found in 5% of familial ALS, and occasionally in FTD or FTD-MND cases.

Also, the fused in sarcoma gene (*FUS*) gene mutations are found in 5% of familial ALS cases; in such families bvFTD appears to be rare as *FUS* is often sporadic in ALS and cases are usually of young onset with no dementia.

There are other less frequent gene mutations that have been associated with FTD:

The sequestosome 1 gene (*SQSTM1*), which is located on chromosome 5 (5q35). It encodes p62 protein adapter protein, which is involved in multiple functions including autophagy, oxidative stress response, and cell signalling. Neuronal p62-positive inclusions have been shown to be abundant in both FTD and ALS patients, particularly disease associated with the *C9orf72* gene mutation. In addition, an increase in p62 immunoreactivity has also been reported in AD, DLB, Parkinson's disease and HD. *UBQLN2* (located on ChrXp11.21) gene: there is a single reported case of FTD related to *UBQLN2* gene mutation, which is a recognized but is a rare cause of ALS.

A few families with FTD have been shown to have mutations in *Presenilin1* a gene usually associated with familial AD. This finding confirms the notion of convergence amongst mechanisms of neurodegeneration and is reciprocal to recent finding of *MAPT* polymorphisms in large AD cohorts. The exact role of *presenilin* in FTD is unclear but the mutations appear to be novel.

Prion protein (PRNP) gene mutations have also recently been associated with clinical pictures resembling FTD.

1.10 Neurophysiology of FTD

EEG can help in differentiating FTD from AD as the background alpha rhythm tends to be preserved in FTD while general slowing with loss of background alpha organization is seen in the vast majority of AD cases. This becomes more evident as the disease progresses. The reasons for such preservation of normal alpha pattern in FTD are unclear but this observation may be related to the relatively rare association between FTD and seizures compared to AD.

Spectral EEG and Magnetoencephalography (MEG), Transcranial Magnetic Stimulation (TMS) are examples of new research tools used in studying connectivity network disruptions in FTD. TMS is a promising noninvasive neurophysiological tool that examines cortical networks by testing excitatory and inhibitory properties of the cortex, conduction in the corticospinal tracts, and functional integrity of cortical structures including the corpus callosum. Advances in TMS have enabled in vivo investigations of the cortical cholinergic, glutaminergic and GABAergic circuits.

Although TMS investigation in FTD is still in its early stages, the available limited data provide fascinating insights in certain disease aspects such as the presence of motor circuit abnormalities in the absence of clinical evidence of pyramidal involvement. Available data also suggest that TMS may have a potential therapeutic role as evidenced by improved language function in PPA patients following high frequency TMS over the dorsolateral prefrontal cortical region (241). Further research is needed to confirm or refute the applicability of TMS in FTD clinical care.

Small studies examined the role of Electromyography (EMG) and Motor Unit Number Estimation (MUNE) in studying lower motor neurone involvement in FTD. In view of co-

existence of ALS and FTD, EMG studies can serve as an important diagnostic tool in FTD patients who have associated motor or swallowing difficulties.

One of the main aims of this research was to investigate the role of the novel neurophysiology technique “Motor Unit Number Index Estimation” (MUNIX) in FTD by determining its role in monitoring disease progression and quantifying lower motor neuron dysfunction in FTD subtypes (please refer to specific MUNIX sections).

1.11 Cerebrospinal fluid (CSF) and blood biomarkers of FTD

Biomarkers are meaningful links between an event and a disease. They can be detected and measured in parts of the body to indicate either normal or disease process in the body. Biomarkers can be risk indicator or predictive (give an indication of whether there is a threat of the disease), diagnostic (if a disease already exists) or prognostic (give information on how a disease may develop in an individual case).

A key objective of most ongoing FTD studies is to develop biomarkers, which help identify the disease at its pre-symptomatic or earliest stages, as well as markers that allow disease progression and therapeutic response to be tracked. The eventual aim will be to use these markers in future clinical trials of drugs in FTD. A number of biomarkers have been used in the field of FTD both clinically and in research capacity. Such biomarkers include imaging, neurophysiological and biological markers.

Pathological heterogeneity and large variation in neurodegenerative severity of FTD make CSF biomarkers of limited values in identifying FTD reliably. Nonetheless, the identification of reliable protein biomarkers in the cerebrospinal fluid (CSF) or serum facilitates in depth investigation of disease proteomics during life (as opposed to neuropathological examinations on autopsy). CSF biomarkers have attracted more interest in neurodegenerative conditions, as they are more likely to mirror the pathological processes taking place in the CNS.

Levels of CSF Tau in FTD are decreased, normal or even increased while levels of phosphorylated tau is essentially normal (27). Levels of CSF amyloid β^{1-42} have been found to be either in the normal range or decreased (27).

It is not clear whether CSF or plasma level of TDP-43 is useful diagnostically or not. This is due to the fact that plasma level of phosphorylated TDP-43 correlate with the extent of TDP pathology in FTD (84,85).

Previous studies using ELISA concluded that decreased levels of progranulin levels are found in plasma, serum and CSF, and may differentiate GRN mutations carriers from non-carriers (86-90).

Ubiquitin is another major constituent of abnormal protein aggregates in FTLD-U. CSF ubiquitin levels in FTD patients have been reported to be significantly higher than those in AD patients, but not significantly different from that of healthy controls. This suggests a potential role for CSF ubiquitin in differentiating AD and FTD, but further research is needed to replicate these findings.

Neurofilaments, often in the phosphorylated isoforms, constitute an integral part of the axonal cytoskeleton. The high levels of neurofilaments in neurons have led to an interest in investigating their CSF levels in several neurodegenerative disorders as a surrogate marker of neuronal degeneration and loss. Several studies have shown remarkably high CSF levels of both light chain and hyperphosphorylated heavy chain neurofilaments in FTD. The degree of neurofilament phosphorylation is increased in FTD compared to both AD and controls. Of note, levels were normal in gene carriers with pre-manifest disease. The pathological significance of these neurofilaments remains to be determined.

Recently some neuropeptides have been discovered and may prove to be promising CSF biomarkers for FTD. This group of peptides includes Agouti-related peptide

(AgRP), adrenocorticotrophic hormone (ACTH), IL-17, IL-23 and Fas. Contradictory conclusions were drawn from the few studies conducted in this area (91,92).

Finally, some recent studies employed advanced mass-spectrometric techniques to simultaneously examine multiple analytes simultaneously (15 to more than 2000) in an attempt to identify a reliable biomarker in FTD. Candidate proteins proposed to date (alone or in combination) include the neurosecretory protein VGF, transthyretin, S-cysteinylated transthyretin, truncated cystatin C and a fragment of chromogranin B. However, more research needs to be done before translating these efforts into a biomarker or biomarkers of practical value in research or in the clinic.

1.12 Management and treatment of FTD

To date, there are no specific disease-modifying treatments for FTD. Instead, medications for other types of dementia and neurodegenerative diseases are frequently used as off-label symptomatic treatment of FTD. According to Hu et al, a similar percentage of AD and bvFTD patients have been reported to take AD medications (72). Current pharmacological strategies for FTD have focused on symptomatic neurotransmitter replacement and modulation for the treatment of behavioral symptoms. These medications include antidepressants, including selective serotonin reuptake inhibitors (SSRIs), atypical antipsychotics, acetylcholinesterase inhibitors (AChEIs) and N-methyl-D-aspartate (NMDA) glutamate receptor antagonists. Previous research (73, 97-99) reported defects in at least four brain neurotransmitter systems resulting in various symptoms in FTD:

1. The cholinergic system: dysfunction of this system results in the cognitive manifestations.
2. The dopaminergic system: resulting in motor and extrapyramidal signs.
3. The glutamatergic and GABAergic system; affecting cortical connectivity.

4. The serotonergic/ noradrenergic system; resulting in depression, anxiety and abnormal behaviour.

Treatments available and those under investigation can be divided into two categories: pharmacological and non-pharmacological treatments.

None of the currently available pharmacological treatments are proven for general use and must be considered investigational only. The trials are limited and many of standard measures of outcome are designed for Alzheimer diseases trials. A particular difficulty facing FTD drug trials is the heterogeneous nature of its population. This heterogeneity results from the complex and different clinical, pathological and genetic subtypes.

To date, implementation of drug trials has been challenging and most reports of FTD treatments are based on small case series, and only few large sample, double blind, randomized placebo controlled trials (73). A major caveat in this field is the lack of FTD-specific clinical rating scales. Clinical instruments used to measure efficacy in AD, such as the Mini Mental States Examination, Alzheimer Disease Assessment Scale Cognitive, or Clinical Dementia Rating Scale, emphasize memory loss and do not accommodate enough the executive and language deficits seen in FTD patients. Future pharmacological strategies are expected to shift from a symptomatic approach towards treatment of the underlying disease process, such as anti-tau protein compounds.

Nonpharmacological interventions are the mainstay of FTD treatment for now. Such interventions focus on behavioral management strategies, education, and caregiver support (74).

1.12.1 Nonpharmacological treatments

Non-pharmacological management includes education (of patients and care givers) about disease recognition, presentation and expected course. Sensitive communication of the diagnosis to patients, families and caregivers is very important. This should be done in a specialist environment with an understanding of the core features of FTD such as personality changes, loss of empathy and social cognition. The best management is that administered in a multidisciplinary setting. Practical steps include occupational and financial advice (because FTD strikes at the top wage-earning years of life, it is very devastating financially), genetic counselling where relevant, social and carer interventions and specific advice regarding driving. Cognitive, speech and language therapies to facilitate the use of spared functions may make the condition easier to bear for the patient, caregivers and family members. Patient's environment may need to be modified; this is by maintaining his/her immediate environment safe, constant, and familiar while avoiding sensory deprivation. Visual, hearing and mobility aids may be needed by some patients. Dietary improvement or modification, including food restriction may be essential. Appropriate behavioural interventions include communication aids, maintaining routines, reassurance and sleep hygiene.

The role of cognitive rehabilitation in FTD is not clear. Psychological interventions in FTD include psychological input to the diagnostic procedure, pre-and-post diagnostic counselling, supporting carers and families and behavioural management of patients.

Potential non-pharmacological approaches include reminiscence, pet and music therapy.

1.12.2 Selective Serotonin Reuptake Inhibitors

Of all neurotransmitter-based therapies for FTD, drugs that modify serotonergic neurons have the strongest biological basis, since there is strong evidence for

serotonergic deficit in this disorder (73). Furthermore, many of the behavioral symptoms of FTD, such as apathy, compulsions, repetitive behaviours, stereotypical movements, and eating abnormalities respond to SSRIs in patients with primary psychiatric disease.

To date, there are some open-label studies, but only two randomized, double-blind, placebo-controlled studies have been conducted. The first of these was a double-blind, placebo-controlled, crossover study to evaluate the efficacy of paroxetine (40mg/day) to target the behavioral symptoms of FTD (75). The results of this study showed no significant differences between placebo and study groups after 6 weeks of treatment. The second was a randomized, double-blind, placebo-controlled trial conducted to determine the efficacy of trazodone (up to 300 mg per day) (76). Trazodone demonstrated a significant reduction in the behavioral score after 12 weeks of treatment, especially irritability, agitation, depression, and aberrant eating behaviour.

1.12.3 Atypical Antipsychotics

Treatment with atypical antipsychotics may be considered in patients with severe behavioral disturbances such as agitation or irritability when SSRIs are not effective. This approach is supported by a single open-label study of olanzapine, which showed similar efficacy in lowering the behavioral score to that reported with SSRIs (77). An important point here is that the decision to use antipsychotics in the treatment of FTD should be made with caution because of potential significant adverse effects (especially extrapyramidal adverse effects) (78). Additionally, a meta-analysis of randomized, placebo-controlled trials determined that treatment of elderly patients with dementia with atypical antipsychotics is associated with a 1.6–1.7-fold increase in mortality secondary to cardiac events or infection, thus prompting the US Food and Drug Administration to place a “black-box warning” on their use.

1.12.4 Acetylcholinesterase Inhibitors and NMDA Glutamate Receptor

Antagonists

Acetylcholinesterase inhibitors (AChEIs) have attracted large interest as a potential treatment in FTD patients after they have proven to be beneficial in improving the underlying cholinergic system deficits in AD and DLB and hence treating cognitive and behavioral symptoms of these neurodegenerative disorders. Thus, a few studies have investigated the efficacy of AChEIs in the treatment of FTD, however, in contrast to AD and DLB, there is no strong evidence for cholinergic system deficit in FTD patients (73). Therefore, the efficacy of AChEIs in the treatment of FTD has not been established. In this regard, one open-label study found that rivastigmine improved neuropsychiatric symptoms and caregiver burden but did not improve cognitive decline (79). Another study investigated the efficacy of galantamine in patients with bvFTD and PPA (80). No significant differences in language or behavioral symptoms were reported between placebo and both treatment groups (combined bvFTD and PPA). However, language functions remained stable in the PPA group compared with the placebo group. But this is may be because patients in the PPA group included the logopenic variant patients whose symptoms are associated with underlying Alzheimer's pathology. The third study was an open-label trial with donepezil in bvFTD patients (81). There was no significant difference in global cognitive function or dementia severity between groups; however, exacerbation of behavioral symptoms was reported after treatment.

Memantine, a non-competitive NMDA glutamate antagonist, providing neuronal protection against glutamate-mediated excitotoxicity, effectively treats agitation in moderate-to-severe AD (82). There are some reports suggesting that glutamate excitotoxicity may play a role in the pathogenesis of bvFTD; therefore, the therapeutic

effects of memantine were thought to be useful in treating the neuropsychiatric features of FTD. A recent randomized, parallel group, double blind, placebo-controlled trial of memantine in patients with bvFTD could find no benefit (83). Memantine treatment had no effect on either the neuropsychiatric symptoms or global cognition after 26 weeks of treatment. This study confirmed the absence of benefit of memantine for treatment of bvFTD.

1.12.5 Future FTD Treatments

Efforts to develop disease-modifying therapies for FTD are focused on eliminating the specific proteins that are implicated in pathogenesis. Patients with CBD, PSP and MAPT mutations would be preferred candidates for a tau-specific drug. Upregulation of progranulin is the ideal approach for patients with GRN mutations; and silencing the C9ORF72 gene is a focus for those individuals who carry this gene. The development of tau- and TDP-43-specific biomarkers will be necessary to further improve prediction of histopathology, particularly in patients with bvFTD, and the advent of tau imaging shows great promise for separating out these subtypes of FTD. The efficacy of candidate drugs should be tested by clinical outcome measures that are sensitive to the changes seen with disease progression in FTD, a tool that is still not available. Collaborative, multicentre trials are needed in order to recruit sufficient number of subjects to test the promising therapies that are emerging for FTD.

Currently there are no approved disease modifying therapies for FTD. However, preclinical and early clinical phase trials of true disease modifying therapies are underway. The main targets are protein pathways known to be integral to the pathological process in FTD, including tau, progranulin, and TDP-43. This approach has produced several promising candidates. However, the logistic difficulties intrinsic to a disease such as FTD are significant. The first clinical trial for a disease modifying

therapy in bvFTD was initiated in 2013. This involved TRx0237 (also called LMTX™). This agent acts by reducing levels of aggregated or misfolded tau protein. Many patients in this trial were excluded for various reasons including lack of supportive MRI changes and/or diagnostic uncertainty raising the possibility of “FTD phenocopies”, advanced cognitive impairment, lack of interest and/or inability to give informed consent, and reduced ability of bvFTD patients to tolerate MRI scanning. Of the first 275 potential subjects who were pre-screened, 55 progressed to formal screening, and only 20 patients proceeded to the randomization. The results of the trial are still pending.

Granulin, the product of the *GRN* gene, is a growth factor that displays low levels in FTD patients with this mutation. Granulin is believed to play a role in multiple essential biological processes like regulating inflammatory reactions, energy and protein homeostasis, neurite outgrowth, and neuronal survival. Several new therapies are being developed to increase granulin including PTC124—a new chemical entity that selectively induces ribosomal read through premature but not normal termination codons. Early trials have demonstrated safety in healthy volunteers as well as in preclinical trials for *GRN* related FTD.

Davunetide is an intranasal neuropeptide therapy derived from a growth factor called activity-dependent neurotrophic protein and is believed to have neuroprotective effects. Despite early promising Phase II trials in MCI and AD patients, a more recent trial in FTD with predicted tau pathology (which included CBS and PSP) was halted following a large multicenter trial involving PSP patients reporting negative results in all outcome measures.

Preclinical studies are also investigating the therapeutic value of immune therapy or efforts to block cleavage in removing abnormal TDP-43.

1.13 Lower motor neuron dysfunction in FTD

The overlap between FTD and MND as well as the shared clinical, genetic and pathological characteristics between the two conditions has been discussed in previous sections of this chapter. Notwithstanding the robust evidence for this overlap between FTD and MND (103-105, 108-110), only one study in the literature described the rate of development of MND in pure FTD patients. It was reported in this study which was conducted by Catherine Lomen-Hoerth et al that 36% of FTD patients subsequently developed EMG criteria for diagnosis of MND after one year follow up (107). Whether MND or subclinical motor neuron dysfunction develops in all FTD patients remains unknown.

1.14 Unanswered questions and future directions of FTD research

- Remarkable advances have been made in clinical recognition and understanding disease biology of FTD in the last decade. However, areas like early detection of the disease, development of reliable biomarkers that can predict the underlying pathology, clinicopathological correlation and unravelling the pathophysiology in order to develop therapeutic strategies preventing or delaying disease progression are far from ideal as there is a dearth of information in these fields.
- Whether the previously mentioned cases of isolated right frontal or temporal degeneration represent true phenocopies; or even a challenge to the currently known diagnostic criteria needs to be clarified: Researchers tried to explain the bvFTD-phenocopy syndrome. While some suggested that this syndrome represent an extremely slowly progressing neurodegenerative process, others thought of these cases as part of undiagnosed Asperger's/Autism spectrum. A third group considered this syndrome as mid-life regression in vulnerable personality as some have life-long

personality disorders. There is an increase in prevalence of major psychiatric disorders (schizophrenia, mania and depression) in bvFTD- phenocopy cases.

Therefore, and with our current knowledge of bvFTD-phenocopy syndrome, the author feels that it is not unreasonable to suggest that this is a mix match of different conditions that masquerade as FTD.

- Criteria for diagnosing familial FTD needs to be developed.

While no phenocopy cases were identified during this research period, a central aim of this study was to establish a biomarker repository of FTD via a multidimensional approach that included clinical and epidemiological characterization, imaging, genetics and neurophysiological studies. Such approach would help in better understanding of the FTD rubric and identifying disease subtypes.

- Resting state functional magnetic resonance imaging (fMRI) seems to be a promising neuroimaging technique that has emerged as a novel concept from recent FTD imaging studies in showing changes in salience network in FTD: this is based on the idea that FTD is caused by degeneration within specific intrinsic functional connectivity networks that are selectively vulnerable to FTD pathologies (93). Resting-state fMRI studies showed attenuated connectivity within the anterior salience network of dorsal anterior cingulate and frontoinsula cortices (a network that has connectivity to subcortical and limbic structures) (94). Furthermore, and in contrast to AD, there appears to be enhanced connectivity in the more posterior default network in FTD (94). Further work is needed to investigate whether specific pathological subtypes are linked to specific neural network degeneration.

- A major challenge is development of a functional rating scale. An ideal functional rating scale will correlate with disease burden, will be easy to administer, reliable, applicable across syndromes, sensitive to change and predictive of prognosis.

Moreover, the incidence, severity and functional significance of lower motor neuron dysfunction in FTD have not been fully determined. A recent cross-sectional study conducted by Burrell et al (203) showed reduced neurophysiological index recorded from the abductor pollicis brevis muscle of 15 bvFTD and 10 nvPPA patients following stimulation of the median nerve at the wrist indicating lower motor neuron dysfunction. Of note, the Neurophysiological Index (136), which is a sensitive marker of progressive lower motor neuron degeneration in ALS = (compound motor action potential amplitude/distal motor latency) x (F-wave frequency). This study detected lower motor neuron dysfunction in the FTD group in comparison to normal controls, albeit less severe than in the MND group (FTD: 1.1 ± 0.9 ; controls: 1.9 ± 0.8 ; MND: 0.7 ± 0.6 ; $P < 0.001$) (203). The neurophysiological index was normal in svPPA patients in this study (10 svPPA patients were studied).

The neurophysiology arm of our research tested this hypothesis of lower motor neuron dysfunction in the FTD by applying longitudinal MUNIX testing and comparing the rates of MUNIX decline in the FTD subtypes of bvFTD, nvPPA, svPPA and FTD-MND to the rate of MUNIX decline in patients with ALS without cognitive impairment. This MUNIX comparative study also included compiling a normative data repository from healthy volunteers and pure lower motor neuron repository from Poliomyelitis patients. Poliomyelitis represented a non-progressive disorder affecting the lower motor neuron.

Moreover, key aspects such as the role of cognitive clinics, the pattern of aggregation of neurodegenerative and neuropsychiatric conditions in kindreds of FTD patients, phenotypic patterns of grey matter atrophy, extra-motor imaging changes in ALS patients without cognitive impairment and imaging profile of C9orf72 negative FTD-ALS patients remain poorly evaluated.

This thesis, which is based on the central theme of biomarkers and progression in Neurodegeneration, aimed at addressing some of these gaps. The overall

hypothesis of this research is that FTD with and without motor neuron degeneration (often referred to as FTD-ALS or ALS-FTD spectrum) represents a continuum that manifests clinically as differing phenotypes resulting from focal brain pathology but those phenotypes share common epidemiological, genetic, imaging and neurophysiological biomarkers.

1.15 Motor Unit Number Index (MUNIX) Estimation

1.15.1 Introduction

Motor units (MUs) are the functional entities in which muscle fibers are organized. In degenerative diseases such as amyotrophic lateral sclerosis (ALS) and muscle diseases, the number of MUs is reduced. Objective measures of estimating the number of MUs and tracking loss over time in a muscle will be of great value in prognosis, follow-up, and also evaluation of drug trials in such diseases. The existing methods such as Motor Unit Estimation (MUNE) are very time consuming (20 min or more per muscle), may require use of intra-muscular needles or hundreds of stimuli. On the other hand, this novel method, called motor unit number index (MUNIX) estimation, significantly reduces patient discomfort and time of test procedure. MUNIX uses the surface recorded compound muscle action potential (CMAP) obtained after one supramaximal stimulation of the nerve, and surface electromyographic (EMG) interference pattern (SIP) recorded during voluntary contraction.

Motor neuron disease (MND) and related conditions are due to progressive degeneration of upper (UMN) and lower (LMN) motor neurons characterised by progressive loss of motor units. Clinically this is evident as wasting and weakness of muscles. The diagnosis of motor neuron disease is based on a number of strict criteria that require:

1. Signs of lower motor neuron (LMN) degeneration by clinical, electrophysiological or neuropathologic examination,
2. Signs of upper motor neuron (UMN) by clinical examination, and
3. Progressive spread of signs within a region or to other regions, together with the absence of:
4. Electrophysiological evidence of other disease processes that might explain the signs of LMN and/or UMN degenerations; and
5. Neuroimaging evidence of other disease processes that might explain the observed clinical and electrophysiological signs.

Using these criteria means in many cases diagnoses can be delayed, which may limit access of patients to treatment and valuable care. There is a clear need to evaluate diagnostic tests that allow earlier diagnosis of patients with this severely disabling and almost invariably fatal condition.

Currently there are no reliable objective tests that permit an early diagnosis of MND.

It is possible by using newer neurophysiological techniques to assess loss of motor neurons earlier in the disease course. This has the potential to diagnose patients with these conditions earlier than by clinical evaluation.

Studies suggest that changes in MUNIX values mirror disease progression in patients with Motor Neuron Disease and related conditions.

Motor Unit Number index (MUNIX) is a technique utilizing a noninvasive method that requires minimal electrical stimulation. The technique involves utilizing the surface-recorded compound muscle action potential (CMAP) and electromyographic (EMG) interference pattern to compute the motor unit number index (MUNIX). Motor Unit Index (MUNIX) is a novel technique based on standard nerve conduction studies and surface EMG protocol using standard techniques and equipment.

The procedure is safe, non-invasive and is not painful.

MUNIX is a 3-step procedure that involves:

- 1- Measurement of compound muscle action potential (CMAP). The CMAP is a standard recording made in all nerve conduction studies.
- 2- Determining the surface area at 10 grades of muscle power to record the surface interference pattern (SIP). This is obtained by asking the patient to activate their muscle.
- 3- Analyzing those two parameters on an excel sheet using specific software to determine the MUNIX value.

MUNIX was first described by Sanjeev Nandedkar in 2004 (140). Neuwirth et al conducted the first ALS pilot trial in 2010. In this study lower motor Neurone loss was tracked longitudinally for 15 months using MUNIX (141).

1.15.2 The mathematical model and computation of MUNIX

This description of MUNIX mathematical model is derived from the original description of the technique developer (Sanjeev Nandedkar) and his colleagues in their original paper from 2004 (140):

If we consider a muscle with N number of identical motor units (MUs). Moreover, the power and area of each motor unit potential (MUP) are P_m and R_m respectively. The CMAP is the sum of all MUPs. Hence, we get:

$$\text{CMAP power} = N^2 P_m$$

$$\text{CMAP area} = N R_m$$

The SIP contains discharges of the motor unit potentials (MUPs). Now let us also assume that all MUs are identical and firing at the same rate (Hz). Then, we get following equation (based on two above assumptions):

$$\text{ICMUC} = (\text{CMAP Power} \times \text{SIP Area}) \div (\text{CMAP Area} \times \text{SIP Power}).$$

The above computation is called “the ideal case motor unit count (ICMUC)”. It is based on the assumptions that all motor units are identical, they don’t superimpose and they fire at the same rate.

The ideal case motor unit count (ICMUC) estimated when SIP area is 20 mVms is called the “motor unit number index (MUNIX)” for the purpose of this model (140).

The study is performed as follows (140): The CMAP is recorded using standard nerve conduction procedures. Its negative peak is used for area and power measurements (figure 1.1). The same surface electrodes are used to record SIP signals at ten different isometric force levels (10 grades of muscle power) ranging from minimal to maximum (figure 1.2). Signal quality is visually and acoustically monitored by the operator to exclude excessive noise and tremor.

For each signal, the ICMUC and SIP area are computed. Their relationship is modeled and analyzed. A linear regression between logarithms of ICMUC and SIP area is obtained in which the ICMUC values are plotted against the SIP area (figure 1.3).

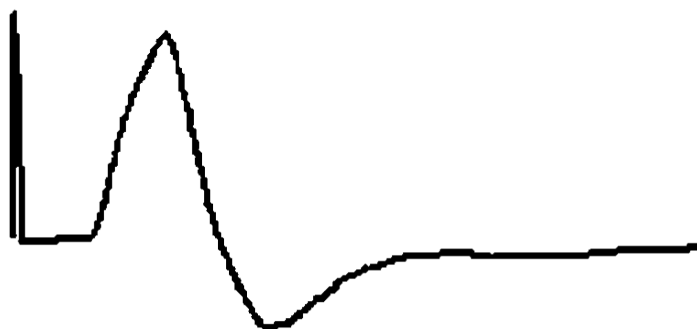


Figure 1.1: A diagram showing an example of Compound Muscle Action Potential (CMAP)

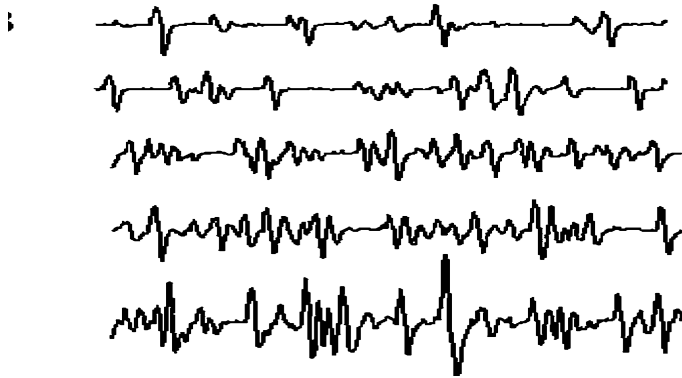


Figure 1.2: A diagram of Surface Interference Patterns (SIP) at five different grades of muscle contraction power. Each epoch is 300 ms long

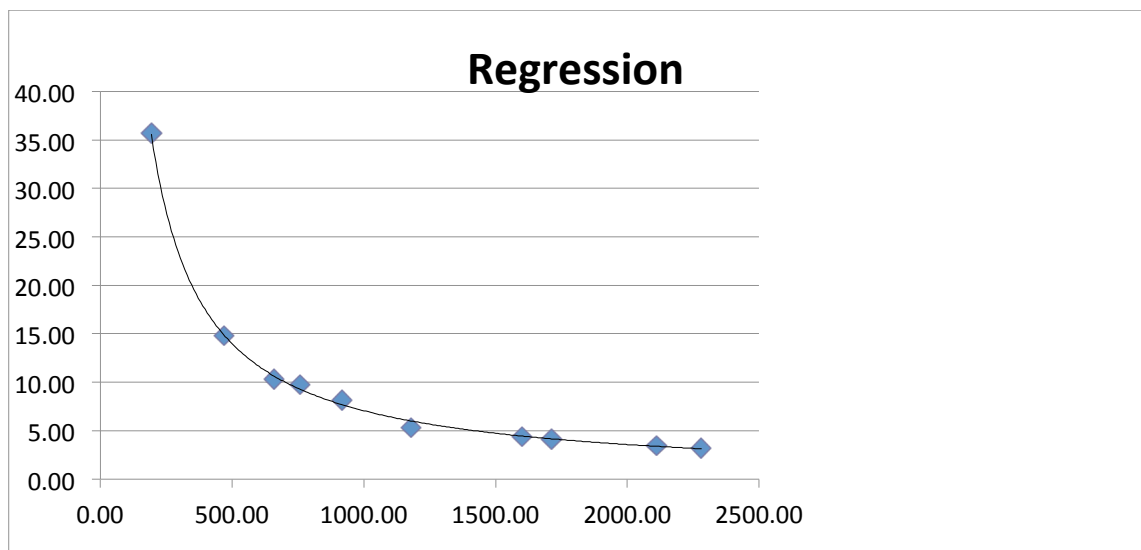


Figure 1.3: MUNIX regression line from one of the participants of this research showing the ICMUC values (Y axis) plotted against SIP area (X axis). The data from this participant is shown in filled squares. The solid curve through those data is the regression line.

MUNIX mathematical model is based on the following assumptions:

- Surface motor unit potentials (SMUPS) are identical
- There is no superimposition
- All have the same firing rate

MUNIX is calculated using an “ideal case motor unit count (ICMUC)” (140): On the assumption that in a given muscle there are “N” identical motor units (MU) and the power and area of each motor unit potential (MUP) are P_m and R_m , and the CMAP is the sum of all MUPs, one can assume that:

$$\text{CMAP power} = N^2 P_m \text{ and CMAP area} = N R_m$$

The surface electromyographic interference pattern (SIP) contains the discharges of the MUPs.

And hence, following this assumption, the ideal case motor unit is calculated according to the formula:

Ideal Case Motor Unit Count (ICMUC) =

$$\frac{\text{CMAP Power}}{\text{CMAP Area}} \times \frac{\text{SIP Area}}{\text{SIP Power}}$$

Using the negative peak of standard electrographic CMAP and the SIP at 10 different force levels of muscle contraction, for each signal SIP area and ICMU is computed.

The predicted value of ICMUC when SIP area is 20 mV/ms is the motor unit number index (MUNIX). The choice for 20 mV/ms SIP area is somewhat arbitrary. However, such SIP area will be recorded at slight contraction. At this force level, most activated MUs are small and should have similar size and due to a low firing rate, their SMUPs should not superimpose significantly. In this respect, the “ideal” conditions of the model are approached, if not perfectly satisfied. One could have used a slightly higher or lower value of SIP area to compute MUNIX. This will change the numerical values of MUNIX. Hence, it becomes obvious that this computation is an index, and not a direct estimation of the number of MUs (142).

The boundaries of the clustered data (obtained as a 2.25-SD band around the regression line) are shown as dotted lines. Such boundaries are called the ‘normal cloud’. The regression line, (which represents the data points), lies within the cloud and almost “parallel” to the cloud boundaries. This is referred to as the normal pattern.

The “theoretical” conditions above may not be satisfied in reality, where the SMUP size varies and the SMUPs are frequently superimposed, which significantly influences the ICMUC value. For instance, the superimposition of SMUPs results in less area and more power for the SIP signal than the sum of component SMUPs. Thus, with increasing force, the ICMUC value decreases significantly.

1.15.3 Principles and procedures (Technical aspects) of MUNIX

MUNIX is performed in a three-step procedure as follows:

First, the CMAP is recorded using standard motor nerve conduction techniques. The active electrode is placed over the motor point on the belly of the muscle while the reference electrode is placed distally. The position of the active recording electrode is

adjusted during the test to get the highest possible CMAP amplitude as suboptimal electrode placement can result in low CMAP and hence erroneously low MUNIX values. A ground electrode is used and is placed on a suitable point on the hand or leg depending on the muscle tested. The nerve is stimulated at supramaximal intensity at a distal site, such as the wrist for the ulnar nerve (EDB muscle) and the peroneal nerve at the neck of the fibula (TA muscle) . All efforts are made to obtain a clean baseline before the onset of the CMAP. The negative phase of the CMAP is used to compute its amplitude, area, and power.

In the second step the SIP is recorded. Each SIP epoch is 300 ms long. The patient is instructed to exert and maintain an isometric contraction at varying levels of effort. The force is not measured, but the operator offers manual resistance to help the patient produce different force levels. Five levels that would roughly correspond to 10%(or slight), 25%, 50%, submaximal (about 75%), and maximal voluntary effort are used. The progressive series of resistance is repeated again to obtain ten SIP epochs. The subject is given a short rest before the maximum contraction and the progressive series of resistance is repeated again to obtain ten SIP epochs.

In the third and final step, the CMAP and SIP signals are imported to analysis software (which was developed by Sanjeev Nandedkar). For each SIP, its area and power are measured. Together with the CMAP area and power, the “ideal case motor unit count”(ICMUC) is computed. CMAP and SIP relationship is modeled and MUNIX is computed as detailed in section 1.14.2. A regression curve characterizes the tested muscle (figure 1.3).

The motor unit size index (MUSIX) is obtained by dividing MUNIX into CMAP amplitude:

$$\text{MUSIX} = \text{CMAP amplitude} / \text{MUNIX}$$

MUNIX is measured in microvolts and reflects the average amplitude of the surface-recorded motor unit potential (SMUP). MUNIX is an index for the size of MUs.

Just before analysis, the operator views the SIP epochs to identify any artifacts, such as high-frequency noise, power line frequency interference, baseline shift, etc. When artifacts are significant, the epoch is rejected from analysis. When there is no apparent voluntary EMG activity, the SIP will have low but finite area and power values. This occurs from inherent noise and interference in the system, or from volume conduction from other muscles. Very low-amplitude signals give very high ICMUC values. To exclude this artifact, three criteria were built in the model assumption and they must be satisfied to accept an SIP epoch (142):

1. SIP area >20 mV/ms.
2. ICMUC <100.
3. SIP area/CMAP area >1.

Finally, tremor causes nearly synchronous firing of MUs. This generates high-amplitude bursts that give low ICMUCs. If tremor is best recognized at the time of study, such recording or recordings would be excluded from analysis.

1.15.3.1 MUNIX Quality control

The following quality control measures are adopted when measuring MUNIX:

- CMAP amplitude should be maximized.
- CMAP Amplitude should be > 0.5 mV. MUNIX is not measured when CMAP amplitude is below 0.5 mV.
- SIP Area should be > 20 mVms.
- SIP Area > CMAP Area.
- ICMUC < 100.

1.15.3.2 Advantages (Pros) of MUNIX technique

MUNIX has the advantages of being:

- Fast, as it requires only 3-5 minutes per muscles
- Non-invasive
- Minimal stimulation is needed therefore easily tolerated by patients.
- Reproducible
- Can be used to monitor changes in number of MUs over time

1.15.3.3 Disadvantages (Cons) of MUNIX technique

MUNIX has got the following caveats:

- It requires voluntary muscle activation. This may be difficult in severely weak muscles.
- Volume conduction may contribute to SIP.
- The test mathematical model discussed above is not intuitive.

1.15.4 Applications of MUNIX to date

Up to date, MUNIX has been applied in collecting normative data from healthy control subjects (142, 143, 145, 147).

The technique has also been used in studying the pattern of loss of lower motor neurones in the following diseases and conditions:

- 1- Motor neurone disease (141): In ALS patients minimal change in CMAP amplitude resulted in a significant drop in MUNIX and increase in MUSIX, reflecting the phenomenon of reinnervation as a compensatory mechanism for MU loss (142).

- 2- Tibialis anterior muscle in prior poliomyelitis patients (144).
- 3- Paretic muscles of stroke survivors (146)
- 4- Muscles paralyzed by spinal cord injury (148)
- 5- Chronic inflammatory demyelinating polyradiculopathy (CIDP) (151)

MUNIX technique has been applied on six limb muscles (APB, ADM, BB, TA, EDB and AH) and an extra-limb muscle (orbicularis oculi).

The feasibility of MUNIX as a measure that can be used in longitudinal tracking of loss of lower motor neurons in ALS patients, by testing multiple muscles, was first explored in a pilot study involving seven patients the study was conducted by Neuwirth et al in 2010 (141).

The reproducibility of MUNIX has been studied by researchers and those studies showed a good correlation for MUNIX between intra- and inter-operator results in both normal controls (143) and ALS patients (149).

1.15.5 Reasons for choosing MUNIX and comparison to MUNE

Motor Unit Index Estimation (MUNIX) and Motor Unit Estimation (MUNE) are neurophysiological tools that allow motor unit number and size to be evaluated. A study that compared the well established conventional MUNE technique with MUNIX showed that in patients with MND, MUNIX and MUNE are significantly correlated (150). MUNIX has an equivalent potential in detecting motor neuron loss compared to MUNE. MUNIX has a number of advantages over MUNE in that it is easier and quicker to acquire data on standard EMG machines. It is also non-invasive and relatively pain-free to patients.

1.15.6 The novelty of the MUNIX arm of this research

This study is novel in a number of ways: we collected normative data from 40 healthy controls. To our best knowledge, this is the biggest number of normative data that has ever been collected in one centre. This is also the first time that MUNIX has been applied to study FTD and to test the hypothesis of lower motor neuron dysfunction in FTD subtypes. Lastly, there is no prior published work in the literature, a part of one paper when tibialis anterior muscle was studied (144), describing lower motor neuronal loss in poliomyelitis using MUNIX. We propose to record MUNIX in a cohort of Irish patients with Motor Neuron disease and related conditions to determine the role, if any it has in managing and investigation of these patients. This will be the first time such a study has been performed on an Irish cohort.

1.16 Introduction to Amyotrophic lateral sclerosis (ALS)

Motor Neurone Disease (MND) is a progressive neurodegenerative condition characterised by degeneration of upper and lower motor neurons. The international convention is now to refer to the condition as ALS/MND to avoid confusion. ALS/MND is a heterogeneous condition; as there is clinical, genetic, imaging and pathological evidence for this heterogeneity. In general ALS/MND is characterized by progressive decline of all voluntary motor functions. Onset is conventionally divided into spinal onset (those with progressive decline in limb function), bulbar onset (progressive involvement of speech and/or swallowing), and cognitive onset (those who present with cognitive or behavioural impairment). As a rule, the condition progresses, spreading from one region to another and death is from respiratory failure in the majority of cases. Life expectancy is 3-5 years from the first symptom: 70% of incident cases die within 36 months of onset.). The peak age of onset of ALS is 62 years and the male to female ratio is 1.2:1. In Ireland, ALS is more common in males than females by a ratio of 1.4:1. Spinal ALS is more common in men while bulbar onset is more common in women. The overall lifetime risk is approximately 1:400. There is an unquestioned overlap between ALS and frontotemporal dementia (Chapter 1). Up to 15% of ALS is familial. Over 20 genes of major effect have been identified. In Ireland over 50% of all familial ALS is caused by a repeat expansion in the gene C9orf72. The causative gene(s) in the remaining 50% of familial ALS in Ireland remain to be determined as variants in other known genes are extremely rare in the Irish population.

1.17 Introduction to Poliomyelitis

Poliomyelitis is a very old disease that was mentioned even in the ancient Egyptian carvings and paintings. It is a highly infectious disease caused by a virus belonging to the Picornaviridae family. The clinical features range from mild asymptomatic illness (abortive poliomyelitis), aseptic meningitis (nonparalytic poliomyelitis), to paralytic poliomyelitis. The genomic structure of the Poliovirus and its pathogenesis were elucidated only in the 1990s (132). Global eradication of poliomyelitis was made possible after discovery of the Salk and Sabin oral polio vaccine (OPV). In 1994, the World Health Organization (WHO) Region of The Americas was certified polio free followed by the WHO Western Pacific Region in 2000 and the WHO European Region in June 2002 of the 3 types of wild poliovirus (types 1, 2, and 3). In 2013, only 3 countries remained polio endemic: Nigeria, Pakistan, and Afghanistan (132).

Poliomyelitis survivors often live with residual physical deficits associated with their condition. Many present with late effects of polio including reduced mobility, deformities, pain and deconditioning. Approximately 50 percent of Polio survivors develop a range of symptoms many years after the acute paralytic phase of the illness, known as Postpolio Syndrome (154). The constellation of Postpolio syndrome symptoms include deterioration of weakness in previously affected limb(s), new onset weakness, fatigue and psychiatric manifestations that can lead to functional disability and can worsen quality of life. There is an estimated 7,500 polio survivors in Ireland following epidemics of Poliomyelitis in the country in the 1940s and 1950s (153).

1.18 Conclusions and scope of this research

FTD is recognized as a leading cause of early-onset dementia. Important advances in research in areas like genetics and molecular mechanisms of FTD over the last two decades have led to increasing clinical recognition of this disease.

The clinical profile of FTD in Ireland is not known; therefore we have performed deep phenotyping of a clinic-based cohort of Irish patients with FTD by obtaining detailed clinical, epidemiological, neuroimaging, neurophysiological and genetic information. This is in addition to determining the pattern of aggregation of neurodegenerative and neuropsychiatric diseases in kindreds of those FTD patients and to compare this pattern with age-and-sex-matched healthy controls. This will aid in refining diagnosis, better clinicopathological prediction, laying the platform for future endophenotype studies, developing and testing future therapeutic interventions and to move towards primary prevention for FTD.

The neurophysiology arm of this research is a quality improvement and comparative study. This entailed collecting normative data as well as performing longitudinal Motor Unit Number index (MUNIX) Estimation studies on three groups of patients: FTD, MND and Poliomyelitis to determine the rate of decline of MUNIX over time. The two arms of FTD phenotyping and MUNIX are linked through the theme of neurodegeneration and progression.

Patients participating in this research were recruited from two clinics in Dublin: St James's Hospital cognitive clinic that primarily evaluated patients presented or referred with various cognitive symptoms and Beaumont Hospital neurodegenerative clinic for which patients suspected of having MND and/or other related disorders were referred and assessed. Chapter 3 below details the experience of the cognitive clinic through a descriptive analysis of the demographic characteristics and diagnoses (etiologic causes) of patients seen over the research period at this clinic.

2 Chapter 2: Aims

Despite recently rekindled interest in FTD and ALS-FTD spectrum following enormous discoveries that have been made in this field, key aspects such as the role of cognitive clinics, the pattern of aggregation of neurodegenerative and neuropsychiatric conditions in kindreds of FTD patients, phenotypic patterns of grey matter atrophy, extra-motor imaging changes in ALS patients without cognitive impairment, imaging profile of C9orf72 negative FTD-ALS patients, the degree and functional significance of lower motor neuron dysfunction in FTD, remain poorly evaluated.

This thesis is based on the central theme of biomarkers and progression in Neurodegeneration. The overall hypothesis of this research is that FTD with and without motor neuron degeneration (often referred to as FTD-ALS or ALS-FTD spectrum) represents a continuum that manifests clinically as differing phenotypes resulting from focal brain pathology but those phenotypes share common epidemiological, genetic, imaging and neurophysiological biomarkers.

The overall objective of this research was to perform multimodal biomarker profiling of FTD patients through clinical characterization, and the generation of a DNA, neuroimaging and neurophysiological repository. This would permit categorizing patients at subgroup level and discovering disease indices.

Specific objectives of studies performed as part of this research are detailed in the following sections:

2.1 Study 1: The cognitive clinic experience: descriptive analysis of the referral sources, demographics and etiological causes of 193 consecutive patients

Participants of this study were recruited from two clinics: the St James's hospital cognitive clinic and Beaumont hospital neurodegeneration clinic. To better characterize the role of the former we reviewed the experience of the cognitive clinic over the study period to more clearly delineate the demographic and etiologic characteristics of this population.

The specific objective of this study was to perform a descriptive analysis of the referral sources, demographics and diagnoses (etiologic causes) of patients seen over three years (the study period) at our specialist cognitive clinic of Saint James's Hospital, Dublin, Ireland. This was performed to identify the pathways to diagnostics and clinical features of FTD patients so as to suggest proposals for improvement.

2.2 Study 2: clinical phenotyping of FTD patients

The objective of this study was to perform deep phenotyping of a clinic-based FTD cohort in Ireland through performing detailed biomarker profiling. This was accomplished through clinical characterization, and the generation of a DNA, neuroimaging and neurophysiological repository.

2.3 Study 3: Family aggregation study of FTD patients

The main aim was to test the hypothesis that aggregation of neuropsychiatric diseases and neurodegenerative disorders occur to a higher degree among family members of people with frontotemporal dementia compared to relatives of controls.

A primary role of aggregation studies is to determine the commonality of risk; first by determining the recurrence rate of the proband's condition and then by determining whether other conditions are biologically linked.

Specific aims:

- 1- To compare the rates for neurodegenerative diseases (ALS, Alzheimer Dementia, Parkinson disease) and neuropsychiatric disorders (schizophrenia, depression, learning disability and suicide) among relatives of FTD patients with the rates of the same conditions among relatives of healthy controls.
2. To stratify this risk according to the absence or presence of any genetic mutation in the proband.
3. To use information collected to direct future genetic studies.

These aims were achieved by designing and carrying out a family aggregation study. A clinic-based cohort of FTD patients were identified during the study period from the two clinics mentioned above and a detailed family history taking technique was applied to identify clustering of neurodegenerative and neuropsychiatric conditions in their kindreds. The same data was collected from age and sex matched controls. Comparisons were made between cases and controls including family history, epidemiological data, family structure and size.

The information generated by this family aggregation study will answer question about the rate of aggregation of those conditions in kindreds of our FTD cohort. Findings from this study will direct future genetic studies in the field of FTD, and will also be clinically useful in provision of information to relatives of FTD patients attending for genetic counselling.

2.4 Study 4: Genetics of Irish FTD patients

Genetic analysis was performed on high quality whole blood DNA samples collected from 51 FTD patients for the purpose of detecting the presence of any mutation or mutations for known FTD genes. Repeat-primed PCR technique was used for the purpose of screening for the presence of a GGGGCC repeat expansion while targeted enrichment next-generation sequencing technique was used to sequence the exons of other genes known to be involved in FTD. We did not intend to identify novel mutations as such approach would require matched population of controls, which is beyond the scope of this work.

Specific aims:

- 1- To use blood samples (whole blood) collected from participants to identify patients carrying the C9orf72 repeat expansion
- 2- To examine the phenotype and radiological features associated with the C9orf72 repeat expansion, if detected, in FTD patients
- 3- To use whole blood samples of participants to identify the presence of any one or more of the known FTD gene mutations. Genes specifically tested for were: MAPT, GRN, FUS, CHMP2B, PSEN1, PSEN2 and TBK1.

2.5 Study 5: Neuroimaging of FTD patients

The objective of the imaging study was to perform a prospective quantitative neuroimaging study to evaluate phenotype-specific patterns of grey matter atrophy among FTD-ALS spectrum using both whole-brain voxel-wise statistics as well as region-of-interest analyses. White matter alterations were explored using multiple diffusivity indices; radial diffusivity, axial diffusivity and fractional anisotropy.

The hypothesis of the study is that FTD-ALS is a spectrum disorder, where the main clinical phenotypes are manifestations of focal brain pathology, a continuum where various degrees of motor cortex pathology can be identified in patients without ALS and extra-motor changes in ALS patients without cognitive deficits.

Such approach would characterise the imaging signatures of FTD phenotypes along the FTD-ALS spectrum using multiple complementary imaging techniques.

Specific aims:

1- To comprehensively characterize in vivo pathological changes in FTD phenotypes.

2- To specifically evaluate the neuroimaging profile of C9orf72 positive and negative FTD-ALS patients. This was to be achieved via:

- a- Evaluating motor cortex and corticospinal tract alterations in FTD cohorts without ALS.
- b- Exploring unilateral and bilateral patterns of neurodegeneration along the FTD-ALS continuum.
- c- Specifically evaluating grey matter pathology in key cortical regions (such as Broca's and Wernicke's area, orbitofrontal cortex, pre- and post-central gyrus).
- d- Assessing unaffected brain regions using both whole-brain and region of interest (ROI) statistics.

2.6 Study 6: Motor Unit Number Index (MUNIX) Estimation in FTD patients: a comparative study of FTD subtypes, ALS and Poliomyelitis

MUNIX is a noninvasive neurophysiologic method that can be applied to both proximal and distal muscles. MUNIX techniques utilize a compound muscle action potential (CMAP) obtained after one supramaximal stimulation of the nerve, and surface

electromyographic (EMG) interference pattern (SIP) recorded during voluntary muscle contraction. MUNIX uses a mathematical model based on the CMAP and the surface interference pattern following their import into analysis software created by Nandedkar et al (142). The result is presented as a plot and a numeric value reflecting the number and size of motor units recruited at various force levels. The result of the examination is directly related to the number of functioning motor neurons in a given muscle.

The overall objective of our MUNIX studies was to perform a quality improvement and comparative study by applying this technique in proximal and distal limb muscles to a) collect normative data; and b) to perform longitudinal studies on three groups of patients with conditions either causing lower motor neuron dysfunction (FTD) or primarily affecting the lower motor neuron (ALS and Poliomyelitis).

Specific aims:

- 1- To develop a repository of normative MUNIX values (lower motor neuron-LMN repository) from healthy volunteers so that this technique can be applied in patients with neurological diseases, where appropriate. This normative data profile will also be used for the purpose of improving the quality of reports issued by the neurophysiology department of our hospital (Beaumont Hospital Dublin).
- 2- We also aimed to determine the rate of decline in MUNIX value over time in patients with the three diseases of: FTD, MND and poliomyelitis. FTD is was studied as a condition that causes lower motor neuron dysfunction, MND as an examples of a progressive conditions primarily affecting the lower motor neurones while Poliomyelitis was chosen as an example of a non-progressive lower motor neuron disorder. Polio cohort also served the function of establishing pure LMN repository (in addition to the normative data). This would help in better understanding of disease processes to identify patients with

above conditions earlier so that any potentially disease modifying therapies can be offered as early as possible.

- 3- To compare the rate of MUNIX decline over time in ALS patients with that of the well established marker of diseases progression, ALSFRS-R with the possibility of establishing MUNIX as another equality reliable marker for the same purpose (monitoring disease progression).
- 4- To compare the rates of longitudinal MUNIX decline in FTD and ALS subgroups.
- 5- Despite the fact that the incidence, severity, pattern and functional significance of upper motor system involvement in FTD have been studied systematically using the neurophysiological methods of transcranial magnetic stimulation (234), the functional significance of lower motor neuron involvement in FTD remains unknown. As such, the present study aimed to identify the presence of any neurophysiologic evidence for lower motor neuron system dysfunction across FTD subtypes by applying MUNIX longitudinally to study consecutive patients with FTD. In addition, clinical parameters and MUNIX study findings were compared across FTD subgroups to explore relationships between phenotypes and lower motor neuron system dysfunction.

3 Chapter 3: Methodology

This chapter details the methodologies applied in different studies performed as part of this research.

3.1 Study 1: The cognitive clinic experience: descriptive analysis of the referral sources, demographics and etiological causes of patients reviewed during study period (193 consecutive patients)

3.1.1 Clinic structure and patient Identification

The neurology unit in St James's Hospital in Dublin (a teaching hospital affiliated to Trinity College and a tertiary neuroscience centre) provides a monthly cognitive clinic since 2010. Since its establishment in 2010, patients have been seen in this clinic from different parts of the country. There is no unified referring pathway/system followed but referrals are received from all specialists, primarily General Practitioners, Medical consultants, The Memory Clinic (which is run by Geriatricians), Psychiatrists and other Neurologists. The clinic is run by two consultant neurologists, two to three registrars and part-time allied health professionals (a social worker, a nurse, a physiotherapist and an occupational therapist).

3.1.2 Patient assessment

Patients underwent a standard procedure of clinical assessment that comprised detailed history taking and thorough neurological examination. Patients with neurodegenerative disorders had neuropsychological assessment, structural brain imaging (MRI/CT) and/or functional imaging (PET) to ascertain the diagnosis and to exclude mimics.

3.1.3 Inclusion criteria

All patients reviewed at St James's Hospital cognitive clinic within the specified study period (January 2013 through December 2015) were included.

3.1.4 Exclusion criteria

There were no exclusion criteria.

3.1.5 Data collection

Data were collected from patients attending the cognitive clinic of St James's Hospital during the specified time period: January 2013 - December 2015 (3 years). All patients were consecutive, new referrals to the cognitive clinic.

All available paper and electronic medical records were reviewed and relevant data were extracted. Patients were identified from hard-copy clinic templates and electronically cross-referred by checking all clinic codes active during that period. Data were compiled and information was located from reviewing the paper notes, clinic letters, imaging result reports in the electronic medical record, picture archiving and communication system.

The search identified 193 individuals who met the established inclusion criteria.

Symptom onset time was defined as the time at which cognitive changes were apparent to the patient, family or close associates.

3.1.6 Outcome measures

Patient characteristics, referring sources, referral diagnoses, final primary diagnoses and data from the diagnostic evaluations were reviewed and registered consecutively in a database.

3.1.7 Ethical approval

The study protocol was approved by the local hospital ethics committee.

3.1.8 Statistics

Statistical analysis was performed using SPSS (IBM SPSS version 24). Associations between categorical variables were hypothesised using Chi-square test. Associations between independent categorical variables and dependent scale variables were hypothesised using either Independent-Samples t-Test or ANOVA. Where required, correlation analyses were employed to analyse relationships between scale variables. Significance level of 5% was used for all statistical tests. These statistical methods were applied to analyse results of overall data as well as the three subgroups of neurodegenerative disorders (ND), early onset presentation (EOP) and early onset neurodegenerative disorders (EO-ND).

3.2 Study 2: Clinical phenotyping of FTD patients study

3.2.1 Identification of cases

Incident and prevalent patients in Ireland with a diagnosis of Frontotemporal dementia were identified from the following sources:

1. Sending a Request Referral Letter to Specialists. The following specialists were informed about the study by personal letters and information and were asked to notify the researcher about newly diagnosed patients fulfilling the inclusion criteria: All consultant Neurologists, Geriatricians, Psychiatrists, Neuropsychologists and General Practitioners working in Ireland at the time of the study.
2. Chart review and surveillance of patients attended either St James's hospital cognitive clinic or Beaumont Hospital neurodegeneration clinic.
3. Regular Clinic Surveillance.

A high degree of diagnostic accuracy was sought as each patient received his/her final diagnosis after evaluation at the clinic by both a neurologist with special interest in

cognitive neurology and/or neurodegeneration and a neuropsychologist. Such a diagnosis was based on through clinical evaluation, reliable informant interview (in most cases this was either the closest family member or carer), a neuropsychological evaluation, laboratory screening and brain imaging (MRI ± PET scans).

3.2.2 Study Procedures and recruitment of patients

Patient recruitment was done in a number of ways:

1. Potentially eligible patients were informed of the study by their specialist (who would have had received a Request Referral Letter). The specialist would then notify the researcher of potentially eligible patients who consented to being contacted. After they had consented to receiving an approach, the researcher then telephoned these patients and an appointment was arranged at a specialist Neurodegeneration and Cognitive Clinics at either Beaumont Hospital or St James's Hospital.
2. Having been informed of the research by their hospital specialist or general practitioner, patients were referred directly to the specialist clinics in neurodegeneration or cognition mentioned above.
3. Having been identified by clinical surveillance and chart review as a current patient, the relevant clinicians were notified and these patients were informed of this study at their following hospital visit by the clinical team, and invited to participate.

Patients were seen and recruited either at St James's hospital cognitive clinic or Beaumont hospital neurodegeneration clinic. Once patients of the neurodegeneration or cognitive clinic, initial assessments were carried out as part of standard care. High level of diagnostic certainty was sought as the final diagnosis of FTD was made in all cases after through clinical, neuropsychological and imaging evaluation. The diagnosis was made in all cases by a consultant neurologist with special interest in cognitive neurology or neurodegeneration.

Those consented to inclusion (or after next of kin assented for those who did not have capacity to consent) underwent evaluation as per research protocol. Such evaluation included:

1. Detailed Medical history, general and neurological examination.
2. Detailed family history to assess the pattern of aggregation of neurodegenerative and neuropsychiatric conditions in their kindreds.
3. Blood sample for genetic analysis.
4. 3T MRI brain scanning.
5. Longitudinal MUNIX testing.

MUNIX testing was performed every three months whenever that was possible.

Patients were informed that all of the above are optional, and that they could opt out of any or all of the research protocols at any time. The Patient Information Leaflets and Consent Forms had been designed in order that patients might choose to take part in some aspects of the study and not others.

3.2.3 Inclusion criteria

All incident and prevalent individuals above the age of 18, residing in Ireland with evidence of progressive neurodegeneration consistent with either:

1. Raskovsky's Revised Diagnostic Criteria for behavioural variant Frontotemporal Dementia, or
2. Progressive language difficulties consistent with diagnosis of any of Primary Progressive Aphasia subtypes: svPPA, nvPPA, lvPPA, or
3. Features consistent with the diagnosis of FTD-MND, FTD-PSP or FTD-CBS.
4. Additional ALS patients were recruited for the imaging study (details in imaging sections).

5. ALS and Poliomyelitis patients were also recruited for the neurophysiology arm of the research (MUNIX longitudinal comparative study). See MUNIX sections for details.

3.2.4 Exclusion criteria

1. Evidence of extensive cerebrovascular disease accounting for the cognitive impairment.
2. Evidence of significant head trauma/ acquired brain injury.
3. Other structural/ metabolic causes of reversible neurodegeneration (e.g. space occupying lesion, organ failure etc.)
4. Parkinson's disease.
5. Huntington's disease.

3.2.5 Register dataset

The database was password-protected and encrypted and located in on a password-protected computer housed in a locked facility in the Academic Unit of Neurology at Trinity College Dublin.

3.2.6 Bio bank

Serum DNA was stored in a locked fridge at -80C in the Academic Unit of Neurology, Trinity College Dublin. All samples were encrypted before leaving the Hospital.

3.2.7 Statistical analysis

SPSS version 24 (IBM SPSS Inc. Chicago, IL) was used to analyse clinical, epidemiology and family history data.

MUNIX statistical analysis was performed in MATLAB (Mathworks Inc., Natick, MA, USA).

3.3 Study 3: Family aggregation study of FTD patients

3.3.1 Study design

A family history method rather than family study one was adopted in this study.

In the **Family History Method**, the proband answers questions about the health of their relatives (139). An abbreviated family history may be taken by enquiring about the presence of specific diseases in relatives, without clarifying further details of the illness or demographic factors. A detailed family history, where the researcher collects as much information on as many family members as possible, including specifics relating to any disorders identified (e.g age at onset, demographic details etc), may also be taken. Clarification of the history from more than one family member is advisable, particularly in FTD patients as probands here have cognitive impairment. This method makes collection of information on large numbers of relatives possible.

The **Family Study Method** differs from the family history method in that, in this case each member of the proband's family is contacted directly to enquire about health status and disease conditions (139). This method ensures that the information collected is accurate as each person reports his or her own medical history directly to the researcher. If features of the condition under study (e.g stiffness in the study of aggregation of PD) are reported then the researcher arranges to review the person and verifies diagnosis by clinical examination. This method is very labour intensive and expensive to perform compared to the family history method. Patients may also be reluctant to enter into a study if every living member of their family is to be contacted.

A case control study design was chosen for this family aggregation study. The presence of neurodegeneration clinic and cognitive clinic permitted recruitment of

patients diagnosed in the three-year period 2013-2015. A detailed family history approach was used to collect data on all first-and second-degree relatives of cases and age-and sex-matched controls. Factors such as cost, manpower, study-length and ethical restrictions made using the ideal family study method impossible. As this study focused on FTD, a disease with a short survival time, the family study method would not yield the volume of information that a detailed family history study would over the same time period. Recruitment, data collection and subsequent analysis were carried out from January 2013 – December 2015.

3.3.2 Data collection

As described previously, incident and prevalent patients in Ireland with a diagnosis of Frontotemporal dementia were identified from the multiple sources:

1. A request referral letter was sent to relevant specialists (All consultant Neurologists, Geriatricians, Psychiatrists, Neuropsychologists and General Practitioners working in Ireland at the time of the study). Those specialists were asked to notify the researcher about newly diagnosed patients fulfilling the inclusion criteria.
2. Surveillance of patients attended either St James's hospital cognitive clinic or Beaumont Hospital neurodegeneration clinic was made and subsequent charts were reviewed.
3. Regular Clinic Surveillance was made.

All patients fulfilling the inclusion criteria were seen at either Beaumont hospital neurodegeneration clinic or St James's hospital cognitive clinic and were approached and recruited subsequently.

3.3.3 Design of questionnaire

A previously used 22-page family history self-administration questionnaire by our research group to collect family history from ALS patients was used as a template to

design a data collection questionnaire for this study. The original questionnaire was reformatted and was made significantly shorter using a guide to survey design by Dillman (1972). The final questionnaire was reduced in length to 13 pages, was printed in back and front (thus reducing the volume of paper) and the language was simplified. It was structured to collect information on first-, second-, and third-degree relatives (first-degree: children, parents, siblings, second-degree: grandparents, aunts/uncles, nieces/nephews, and third-degree: first cousins). The information collected from each person includes age, vital status, gender, place of birth, place and date of death when relevant. A general question was also asked inquiring about the possibility of more distant relatives, not specifically mentioned on the questionnaire.

This final questionnaire used in the full study is in **appendix C**.

3.3.4 Inclusion and exclusion criteria for recruitment and selection of participants

FTD Patients: All patients with the diagnosis of behavioural variant Frontotemporal dementia (bvFTD), Non-fluent variant primary progressive aphasia (nfvPPA), Semantic variant primary progressive aphasia (svPPA), Frontotemporal dementia-Motor neurone disease (FTD-MND), Frontotemporal Dementia-Progressive supranuclear palsy (FTD-PSP), and Frontotemporal Dementia-Corticobasal syndrome (FTD-CBS) in Ireland referred to either the cognitive clinic in St James's Hospital Dublin or the neurodegeneration clinic in Beaumont Hospital Dublin between January 1st 2013 and December 31st 2015 were included. Patients were excluded if the diagnostic criteria for FTD were not met or the patient did not qualify for inclusion in the study.

The aim was to include and to collect data from all prevalent and as many incident cases as possible within the period of the study.

Controls: for each patient, an age- (+/- 1 year) and sex-matched control was collected. Control participants were recruited through a network that included a general

newspaper advertisement campaign, which did not mention the focus of the study, and primary care providers.

Patients and controls willing to participate were consented using standard consent methods.

3.3.5 Family history data collection

Initial contact: All identified patients have previously consented to inclusion of their details on an electronic database for research purposes. Patients diagnosed with FTD within the study period (next of kin or spouse if the patient was unable to speak) were contacted outlining the nature of the study in non-scientific language.

Face-to-face meeting: patients willing to participate in research and at least one family member were initially met face-to-face. The purpose of the initial contact was to explain the purpose of the research and the nature of the information being collected. Information leaflets and consents/ascents were not collected at this time, but rather the participating individual was informed that an information leaflet, consent/ascent form and the family history questionnaire would be mailed to their home.

Questionnaire: Immediately after this face-to-face contact, the consent/ascent form and family history questionnaire were mailed in a stamped-addressed envelope for ease of postal return. The participant was encouraged to seek help from at least one other close relative.

The questionnaire asks participants about medical conditions in all first, second and third degree relatives. The following information was also collected for each family member:

- Full name
- Date and county of birth
- Date, place and cause of death if deceased

Data collection primarily concentrated on the participating individual and his/her children, siblings, parents, grandparents, maternal and paternal uncles, aunts and cousins. Medical data was not collected for children below age of 18, however demographic data was collected on all life offspring (including those less than 18 years). An identical dataset was collected from controls.

Telephone call: within two weeks of receipt of the completed questionnaire and signed consent form, we contacted the participant by telephone to clarify information provided in the family history questionnaire. This initial telephone call was followed by a second call or a face-to-face interview to further clarify information provided in the questionnaire as detailed below.

To increase validity of the family history data and power of the study, information contained in the completed questionnaire was verified by at least one other relative within each family in the process of completion of the family history questionnaire. This was important, as most patients were cognitively impaired.

3.3.6 Family history questionnaire processing procedures:

After the participant returned the questionnaire, a follow-up phone call or visit was made to all participants to further clarify the family history information.

Family history of Neuropsychiatric diseases: A family history of neuropsychiatric disorders was ascertained and verified using a combination of information from the questionnaire and a structured telephone or face-to-face interview. The interview started by the following open-ended question: “have you or any family member ever had any psychiatric disorder or spent any time in a psychiatric hospital?”

In order to establish and clarify a diagnosis of a neuropsychiatric disorder the following semi-structured interview was carried out where appropriate:

- Schizophrenia:
- Diagnosed by a clinician?
 - Paranoid delusional disorder requiring psychiatric intervention?

- Age at onset

Depression:

- Diagnosed by a clinician?
- Severity of depression- mild/moderate or severe and requiring hospitalisation or ECT?
- Any episodes of mania?

Learning disability (including Asperger's syndrome/Autism):

- Diagnosed by a clinician?
- Severity of the condition?
- Affected school performance/ work/ social life?

Suicide/attempted suicide:

- Age at suicide/attempted suicide?

A condition was classified as an unspecific psychiatric disorder when a relative of the proband has a psychiatric disorder but the proband/informant was unaware of definitive psychiatric diagnosis. Attempts were made to clarify if the psychiatric disorder was predominantly a disturbance of mood or primarily characterized by psychotic feature but these cases were not included in the analysis.

Family history of Neurodegenerative disorders: where a family history was positive for any neurodegenerative condition, further questioning thought to clarify the diagnosis. At the beginning of the interview the following open-ended question was asked: "have you or any family member had any neurological disease?" Then in order

to clarify a diagnosis of a neurodegenerative disease the following semi-structured interview was carried out when appropriate:

- Alzheimer dementia:
- Diagnosed by a clinician?
 - Predominately amnesic or behavioural/personality change?
 - Age at onset?
 - Severity- Able to live independently (mild) or requiring supervised housing (moderate/severe)?

Frontotemporal dementia:

- Diagnosed by a clinician?
- Predominately behavioural or language change?
- Age at onset?
- Able to live independently or requiring supervised housing?

Motor neurone disease

- Diagnosed by a clinician?
- Age at onset?
- Motor, bulbar symptoms or both?
- Duration of illness?
- Any associated behavioural or personality changes?

Parkinson diseases

- Diagnosed by a clinician?

- Age at onset?
- Bradykinesia, rigidity or tremor?
- Unilateral at beginning?
- Related to other medications?
- Any related dementia?

3.3.7 Database

A database was created in SPSS to record data on all of the kindreds included in the study. Each family was given a unique code. The database was split in two files; the first file contained information on the proband and their family structure and the second file contained information on every relative within a family. For each relative age, gender and relationship to proband was recorded. If the relative was living any comorbidities were recorded. If the person was deceased then the cause of death was noted.

3.3.8 Data storage and confidentiality

All of the information obtained from participating families was stored on an encrypted password-protected database. Each individual included in the database was assigned a unique identifier to ensure further encryption.

3.3.9 Statistical analysis

Baseline characteristics were tested for difference using the Chi square test for independence and the independent sample t test. Non-parametric tests were used if the distribution was not normal. All FTD patients were included in the analysis.

Relative risk of the disease in question, lambda (λ), was calculated by dividing the rate of disease among relatives of patients with FTD by the rate of the diseases among

relatives of controls. Cox regression hazards analysis is used to calculate the hazard rate ratio and 95% confidence interval for each disorder in relatives of FTD patients compared to relatives of controls, after weighting for proportion of relative type (e.g. parent, uncle, etc) and correcting for other factors such as degree of relatedness, sex etc.

Because some relatives may not have reached the age of risk for the disease, the estimated hazard ratios (HRs) and 95% CIs were calculated using Cox proportional models with age as the time scale. This method also accounts for any difference in the proportion between first- and second-degree relatives among the case and control groups.

Statistical analysis was carried out using SPSS V24 (SPSS Inc. Chicago, IL). All statistical testing was performed at the conventional 2-tailed α level of 0.05.

The relative risk and hazard are calculated for:

- Rate of FTD in relatives of FTD patients compared to controls.
- Rate of each of the neuropsychiatric and neurodegenerative conditions studied in this research in relatives of FTD patients compared to rate among relatives of controls.

Cluster analysis included direct observations of histograms showing the frequency in number of affected relatives between cases and controls. Binary logistic regression in a generalized linear model was used to assess for clustering within kindreds. This method has the advantage of taking into account the family size and number of affected individuals within kindreds.

Statistical analysis was carried out using SPSS 24. All statistical testing was performed at the conventional 2-tailed α level of 0.05.

3.4 Study 4: Genetics of Irish FTD patients

3.4.1 Sample selection

51 high quality patient blood samples were collected from participants and were stored in a DNA bank. Samples were screened for the presence of C9orf72 mutation and for any of the known FTD genes MAPT, GRN, FUS, CHMP2B, PSEN1, PSEN2 and TBK1. Of the 54 patients, 16 patients were diagnosed as bvFTD, 13, 2, 13, 4 and 3 patients carried the diagnosis of nvPPA, svPPA, FTD-MND, FTD-PSP and FTD-CBS respectively.

3.4.2 Genetic testing for hexanucleotide repeat expansion in C9orf72

51 DNA samples were screened using repeat-primed PCR for the presence of a GGGGCC repeat expansion. Primer sequences for the PCR stage were:

Forward: 6-FAM / AGTCGCTAGAGGCGAAAGC

Reverse: TACGCATCCCAGTTTGAGACGGGGGCCGGGGCCGGGGGCCGGG

Anchor: TACGCATCCCAGTTTGAGACG

The PCR assay was performed in a reaction volume of 38 ul, containing 9 ul DNA (5nm), 14 ul Faststart PCR Marker Mix (Roche), 5 ul 7-Deaza-dGTP (5nm) (Roche), 1 x Q solution (Qiagen), 7% DMSO (Sigma-Aldrich), 0.9mM MgCl₂ (Qiagen), 0.7 uM reverse primer consisting of 4 'GGGGCC' repeats with an anchor tail, 1.4 uM 6-FAM-flourescent labelled forward primer. A touchdown PCR cycling programme was used, with the annealing temperature gradually lowered from 70 degrees Celsius to 56 degrees Celsius in 2 degrees Celsius increments, and a 3 minute extension time for each cycle. PCR products were analysed on an applied Biosystems 3130x1 genetic analyzer and visualized using GeneMapper software (version 4.0). Patients with the characteristic appearance of the expanded hexanucleotide repeat on repeat-primed

PCR consisting of a decaying series of 24 or more peaks were regarded as having a positive repeat expansion, as described previously in the paper by DeJesus-Hernandez and colleagues (54). All results were reproducible.

3.4.3 Mutation screening for other known FTD genes (excluding C9orf72)

For screening of known FTD gene mutations target-enrichment sequencing libraries were prepared from genomic DNA using a protocol described below and sequenced in an Illumina Genome MiSeq analyzer. Sequence data were aligned and processed using BWA, SAMtools, Picard and GATK to generate variant calls and known FTD mutations were extracted from the resulting variant calls. A concise description of the method and steps used is given below (**sections 6.3.3.1 – 6.3.3.7**).

3.4.3.1 Target enrichment strategy used

An in-solution target enrichment kit was purchased from Integrated DNA Technologies Ltd (IDT x Gen Lockdown probes).

The kit enriches a DNA sample for a number of both FTD and ALS genes, including some overlapping genes (7 FTD and more than 30 ALS genes). The 7 genes with entries for FTD in the AD & FTD Mutation Database that were tested for were: MAPT, GRN, FUS, CHMP2B, PSEN1, PSEN2 and TBK1.

3.4.3.2 DNA sequencing library preparation

DNA was extracted from whole blood commercially by Trinity Biobank in the first step.

Then Dual-indexed sequencing libraries were prepared for each DNA sample following the KAPA HyperPlus KR1145-v3.16 protocol as follows:

- DNA was quantified using either a Nanodrop ND-1000 spectrophotometer or a Qubit 2.0 fluorometer with dsDNA BR assay Kit
- 300 ng of DNA was initially purified to remove any EDTA from the buffer using Agencourt Ampure XP beads and eluted in Tris-HCl

- Resulting purified DNA samples were fragmented to a target size of 400 base pairs (bp) for 8 minutes using Kapa HyperPlus fragmentation enzyme
- Kapa HyperPlus library preparation kit was used for end-repair and A-tailing of DNA fragments
- NEBNext hairpin adapters were ligated onto the resulting DNA fragments (60 minute ligation time) and the adapter-ligated libraries were treated with USER enzyme (60 minute incubation) to remove uracil in the adapters
- Resulting libraries were amplified by polymerase chain reaction (PCR) (8 cycles: 98°C 45 sec, 8x(98°C 15 sec, 60°C 30 sec, 72°C 30 sec), 72°C 1 min, 4°C ∞) using unique i5 and i7 adapters to index each individual sample with a unique identifier and to generate sequencer-ready libraries
- Samples were assessed for quality (concentration, fragment size distribution) on an Agilent TapeStation

3.4.3.3 DNA Size Selection

In order to obtain libraries of the correct length for sequencing, size selection was carried out using gel extract size selection as follows

- A 1.5% low weight molecular agarose gel was prepared with the addition of SYBR to a final concentration of 1/5000
- After running the gel, SYBR stained DNA was visualised with a UV screen
- DNA was excised between 500bp-600bp relative to the 100bp reference DNA ladder
- Size selected, library prepared DNA was extracted from the gel cut following the Qiagen MinElute Gel Extraction Protocol

Samples were assessed for quality (concentration, fragment size distribution) on an Agilent TapeStation and a Nanodrop ND-1000 spectrophotometer

3.4.3.4 Target enrichment

Target enrichment was carried out following library preparation. Samples were pooled in equimolar quantities and subjected to target enrichment using a specifically designed kit: An in-solution target enrichment kit was designed by our team and was purchased from Integrated DNA technologies Ltd (IDT xGen Lockdown Probes).

The kit enriches exons for:

- 7 genes with entries for FTD in the AD and FTD Mutation Database (FUS, CHMP2B, GRN, MAPT, PSEN1, PSEN2, TBK1) --> 2 of these overlap with the ALSod genes, and

- 30 genes listed as “major” ALS genes in the ALS online genetics database (SOD1, ALS2, SETX, SPG11, FUS, VAPB, ANG, TARDBP, FIG4, OPTN, ATXN2, VCP, UBQLN2, SIGMAR1, CHMP2B, PFN1, ERBB4, HNRNPA1, MATR3, CHCHD10, UNC13A, DAO, DCTN1, NEFH, PRPH, SQSTM1, TAF15, SPAST, ELP3, LMNB1)

3 further genes recently implicated in ALS by GWAS or NGS (SARM1, C21orf2, NEK1).

Samples were pooled to equal concentration.

A pooled mass of 66ng of DNA was target enriched using the IDT Hybridization capture of DNA libraries using xGen Lockdown Probes protocol:

- Blocking oligos, Cot-1 DNA and the pooled FTD library were combined and liquid was evaporated using a Savant DNA110 DNA SpeedVac Concentrator

- Biotinylated capture probes were hybridized to the library (incubation 65°C 4 hours)

- Biotinylated probes and hybridized DNA were pulled down using streptavidin coated beads and a magnetic rack

- Enriched DNA was amplified with PCR (98°C 45 sec, 18x(98°C 15 sec, 60°C 30 sec, 72°C 30 sec), 72°C 1 min, 4°C ∞).

3.4.3.5 DNA Sequencing

Target enriched, size selected library was assessed for quality (concentration, fragment size distribution) on an Agilent TapeStation, Nanodrop ND-1000 spectrophotometer and Qubit 2.0 fluorometer with dsDNA BR assay Kit.

Library diluted to 4nM in 5uL

The pooled, target enriched, size selected libraries were sequenced on an Illumina MiSeq at the TrinSeq facility at St. James's Hospital with 300bp single end sequencing.

3.4.3.6 Alignment

Alignment: Sequenced data were aligned to the GRCh37 build of the human genome (utilised as a reference genome) using the Burrows Wheeler Aligner (BWA) software package.

3.4.3.7 Post alignment data processing

Sequencing generated 21,294,341 300 bp single end reads of which 19,165,787 passed initial Illumina filtering.

Table 6.1 outlines the number of reads attributed to each sample

Sequencing adapters were trimmed from reads with Cutadapt v1.11 (176).

Enrichment statistics were determined using the R package TEQC on R v 3.2.3.

Enrichment specificity (the proportion of reads falling within targeted regions) was 24.39% (SD 6.59) averaged across all samples.

The relative enrichment of sequencing within targeted regions was 8641 fold (SD 2334), averaged across all samples.

The average coverage in targeted regions across all samples was 46.54X (SD 31.36).

This is broken down per sample in Table 6.1.

Data processing was carried out in accordance with GATK best practices pipeline.

Alignment to Reference Genome: Reads were aligned to the hg19 build of the human reference genome using Burrows-Wheeler Aligner (BWA-MEM v 0.7.13-r1126) and Aligned reads were converted to BAM format using SAMtools v 1.3.1, (177).

Removal of Duplicate Reads: Duplicate reads (primarily arising from PCR) were removed using Picard v 2.8.2

GATK: GATK version 3.5-0-g36282e4 was used to recalibrate base quality scores assigned during DNA sequencing. The GATK tool HaplotypeCaller was used to identify reads which had variants relative to the hg19 build of the human reference genome. Variants included both single nucleotide polymorphisms (SNPs) and insertions/deletions (INDELs). Bedtools v 2.25.0 was used to remove SNPs and INDELs outside of targeted regions. The number of variants remaining at this point for each sample was determined using RTG Tools v 3.7.1 and are outlined in Table 3.1.

177 unique SNPs were identified.

SNP Quality Filtering: GATK hard filtering was carried out with the following parameters: QualByDepth (QD) 2.0, FisherStrand (FS) 60.0, RMSMappingQuality (MQ) 40.0, MQRankSum -12.5 and ReadPosRankSumTest -8.

9 out of the 177 unique SNPs failed filtering. Many of the 168 remaining SNPs were common among samples (likely non-pathogenic and common in the population).

21 unique INDELs were called.

INDEL Quality Filtering: GATK hard filtering was carried out with the following parameters: QualByDepth (QD) 2.0, FisherStrand (FS) 200.0 and ReadPosRankSumTest 20.0.

5 out of 21 unique INDELs failed filtering. Many of the 21 INDELs were common among samples (likely non-pathogenic and common in the population). The number of SNPs and INDELs retained for each sample before and after filtering are outlined in Table 3.1.

Table 3.1: Details of SNPs and ENDELS retained for each of the DNA samples before and after filtering.

SNP=single nucleotide polymorphism, **ENDEL**= insertion or deletion of bases.

Sample	Assigned Reads	Reads Retained After Duplicate Removal	Average Coverage in Target Regions	On Target SNPs Called Before Filtering	On Target SNPs Retained After Filtering	On Target INDELS Called Before Filtering	On Target INDELS Retained After Filtering
FTD001	1081194	393640	53.71	36	35	9	7
FTD002	897849	312674	56.5	47	44	8	8
FTD003	188573	88634	38.08	47	46	11	9
FTD004	278152	119423	51.61	49	49	10	8
FTD005	942142	345300	128.27	38	36	8	8
845_FTD 006	95577	35560	15.5	43	40	8	7
FTD007	991889	365907	128.46	40	37	8	8
FTD008	188666	78416	33.49	37	36	8	8
FTD009	105613	40302	17.68	46	44	8	8
FTD010	459951	173752	70.04	44	42	8	8
FTD011	485733	180262	75.05	39	39	11	8
FTD012	359108	141025	57.54	43	38	11	8
FTD013	231298	87581	26.33	45	43	12	9
901_FTD 014	249416	89940	39.03	42	41	10	8
FTD015	181401	77755	35.1	48	47	8	7
832_FTD 016	73482	31391	14.65	40	38	7	7
900_FTD 017	393718	138440	51.47	38	38	10	9
FTD018	109317	46028	20.41	44	42	10	9
FTD019	118303	47508	19.82	45	45	10	9
FTD020	265695	92797	41.69	39	38	9	9
FTD021	68959	24057	10.62	40	39	10	8
FTD022	977842	377292	144.74	42	42	8	8

955_FTD 026	283776	105590	45.72	42	42	7	7
816_FTD 027	683981	222219	86.93	36	35	13	10
FTD028	328248	123271	53.01	45	43	9	8
FTD029	120516	44909	29.16	42	41	10	8
1079_FT D030	255427	71924	32.63	39	38	10	9
970_FTD 031	184459	57316	25.73	41	40	8	8
954_FTD 032	1049831	316640	108.39	40	39	11	8
FTD033	216983	71624	31.55	43	43	11	9
FTD034	166626	55665	14.79	35	32	8	8
FTD035	594554	176843	73.15	35	33	7	7
981_FTD 036	269968	83621	34.87	30	30	10	9
FTD037	249412	80132	34.45	39	39	8	7
FTD038	243422	86792	36.78	37	37	11	8
FTD039	210596	63402	20.32	42	42	7	8
1013_FT D040	392715	135824	33.23	41	41	9	7
FTD041	548663	209929	84.34	40	39	11	9
FTD042	115949	41953	19.73	40	39	7	7
1014_FT D043	410070	149075	61.66	47	46	10	7
FTD045	262894	111479	48.08	38	37	11	8
FTD046	193211	88271	38.99	42	39	8	8
FTD047	410118	171189	70.36	39	37	8	8
FTD048	144976	43059	8.29	34	34	8	7
FTD049	327282	136862	57.29	44	40	8	8
FTD050	402908	152047	21.73	47	46	8	8
FTD051	368629	145608	62.94	39	38	10	9
FTD052	444167	149786	11.17	49	48	8	8
FTD053	1246869	411213	47.78	42	38	9	7
TO- 21012016 -I	257443	105512	46.08	42	41	8	8
TO-	38216	14176	4.62	27	27	8	8

3.5 Study 5: Neuroimaging of FTD patients

3.5.1 Participants

A total of 100 participants were included in this study: Seven patients with behavioural variant FTD (bvFTD), 11 patients with non-fluent-variant primary progressive aphasia (nfvPPA), 2 patients with semantic-variant primary progressive aphasia (svPPA), 10 patients with amyotrophic lateral sclerosis and FTD carrying the C9orf72 hexanucleotide repeat (FTD-ALS C9+), 10 patients with FTD-ALS without hexanucleotide repeats (FTD-ALS C9-), 20 ALS patients without behavioural or cognitive deficits (ALSnci) and 40 healthy controls (HC) were included in a prospective quantitative neuroimaging study.

All participants provided informed consent in accordance with the Medical Ethics Approval of the research project (Ethics (Medical Research) Committee - Beaumont Hospital, Dublin, Ireland). Exclusion criteria included cerebrovascular disease, traumatic brain injury, and coexisting psychiatric illness. Inclusion criteria included the ability to lie supine in the scanner for the duration of data acquisition.

BvFTD was diagnosed according to Raskovsky's criteria (43) while participating ALS patients had probable or definite ALS according to the El Escorial criteria (204). ALSnci patients had no cognitive impairment based on a large neuropsychological battery including tests for executive function, letter fluency, category fluency, attention, memory, language, visuo-spatial skills, and behavioural domains. The neuropsychological battery has been previously described in detail and reference

psychometric values were provided by a large population-based, age and education matched cohort (n=110) of healthy controls (202, 205).

Participants' demographic characteristics are shown in table 3.2.

Table 3.2: Demographic profile of patients participating subjects. SD: Standard deviation, nfvPPA: non-fluent-variant PPA, svPPA: semantic-variant PPA, C9+: carriers of the C9orf72 hexanucleotide repeat expansions, C9-: tested negative for the C9orf72 hexanucleotide repeat expansions, ALSnci: ALS patients without cognitive or behavioural impairment

	C9- bvFTD n = 7	C9- nfvPPA n = 11	C9- svPPA n = 2	C9- ALS-FTD n = 10	C9+ ALS-FTD n = 10	ALSnci n = 20	Controls n = 40
Gender (Male/Female)	4/3	3/8	1/1	6/4	7/3	8/12	20/20
Age - years (mean/SD)	64.7/ 6.9	71.7/ 8.4	64.1/ 8.0	67.5/ 9.0	55.6/ 10.9	61.5/ 8.7	61.6/ 9.0
Handedness (Right/left)	7/0	9/2	2/0	9/1	7/3	19/1	37/3

3.5.2 Magnetic Resonance Imaging

Magnetic Resonance (MR) data were acquired on a 3 Tesla Philips Achieva system with a gradient strength 80 mT/m and slew rate 100 T/m/s using an 8-channel receive-only head coil. T1-weighted images were acquired using a 3D Inversion Recovery prepared Spoiled Gradient Recalled echo (IR- SPGR) sequence, with a field-of-view (FOV) of 256 x 256 x 160 mm, spatial resolution of 1 mm³, TR/TE = 8.5/3.9 ms, TI = 1060 ms, flip angle = 8°, SENSE factor = 1.5, and an acquisition time of 7 min 30 s. DTI images were acquired using a spin-echo planar imaging (SE-EPI) sequence with a 32- direction Stejskal-Tanner diffusion encoding scheme. FOV = 245 x 245 x 150 mm,

spatial resolution = 2.5 mm³, 60 slices were acquired with no interslice gap, TR/TE = 7639 / 59 ms, SENSE factor = 2.5, b- values = 0, 1100 s/mm², with SPIR fat suppression and dynamic stabilisation in a total acquisition time of 5 min 41 s.

3.5.3 Genetic testing

DNA samples from patients were tested for the presence of a GGGGCC hexanucleotide repeat expansion in C9orf72 by repeat-primed PCR as described previously (197). An Applied Biosystems (Foster City, CA, USA) 3130xl genetic analyser was used and visualised using the GeneMapper software version 4.0. Patients carrying more than 30 hexanucleotide repeats were considered positive for the expansion. ALS patients were also screened and tested negative for mutations in genes previously implicated in ALS including FUS, OPTN, SOD1, TARDBP, GRN, ANG, and ATXN2. The FTD cohort was screened and tested negative with targeted enrichment next-generation sequencing for the following genes known to be involved in FTD: MAPT, GRN, FUS, CHMP2B, PSEN1, PSEN2 and TBK1 (Full details in the genetics section of this thesis).

3.5.4 Grey matter analyses

Cortical grey matter morphometry analyses were conducted using FSL-VBM (206, 207). Following brain extraction, motion-correction and tissue-type segmentation, the resulting grey-matter partial volume images were aligned to MNI152 standard space using affine registration. A study specific template was created to which the grey matter images from each subject were non-linearly coregistered. Permutation based non-parametric inference and the threshold-free cluster enhancement (TFCE) approach was utilised to test for differences between study groups controlling for age. Affected cortical regions were mapped using a statistical significance threshold of $p < 0.05$.

3.5.5 Region of interest grey matter analyses

In addition to whole-brain voxel-wise statistics, supplementary region of interest (ROI) grey matter analyses were also carried out using atlas-based cortical segmentation. Based on the key, phenotype-defining clinical features the following nine cortical ROIs were defined: the bilateral precentral gyrus, the bilateral postcentral gyrus, Broca's area, Wernicke's area, bilateral orbitofrontal cortex, and bilateral cortical ROIs for the occipital, parietal, frontal and temporal lobes. Cortical masks for the above ROIs were defined based on the Harvard-Oxford cortical (HO) and the Montreal Neurological Institute (MNI) atlases. Cortical ROIs for the occipital, parietal, frontal and temporal lobes were defined based on the labels of the MNI atlas (208). The precentral and postcentral gyrus ROIs were defined based on the labels of Harvard-Oxford (HO) probabilistic cortical atlas, which was created based on the individual segmentation and affine-registration of T1-weighted images of 21 healthy male and 16 healthy female subjects to MNI152 space (209, 210).

Wernicke's area was defined based on the HO label for the posterior aspect of the left superior temporal gyrus and Broca's area defined by merging the left pars opercularis and left pars triangularis labels of the HO cortical atlas. For each ROI the 2mm 25% threshold maps were utilised of the original atlas labels. The resulting ROI masks are shown in Figure 3.1 overlaid the study specific grey matter template. Average T1-signal intensity values were subsequently retrieved from the above nine ROI mask in each study participant. To illustrate phenotype-specific grey matter alterations in these cortical regions, average T1 intensity values were plotted in boxplots for each study group. Finally, in an exploratory descriptive analysis, group specific mean and standard deviation values were calculated for each structure and Analyses of Covariance (ANCOVA) were carried out correcting for age.

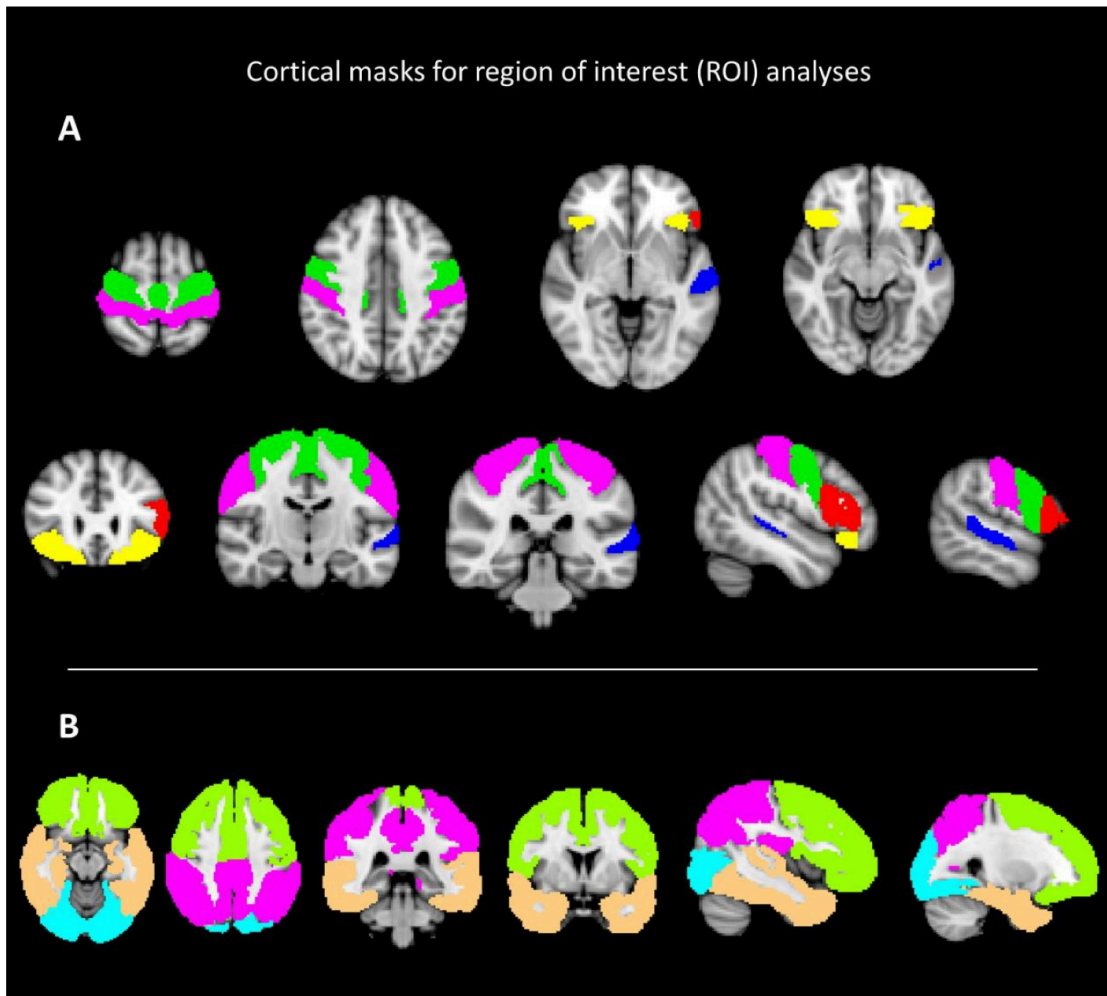


Figure 3.1: Atlas-based, cortical, region of interest mask. A: Regional cortical masks for the precentral gyrus (green), postcentral gyrus (purple), orbitofrontal cortex (yellow), Wernicke's area (blue) and Broca's area (red). B: Cortical grey matter masks for the frontal lobe (green), occipital lobe (turquoise), temporal lobe (copper) and parietal lobe (purple).

3.5.6 White matter analyses

Diffusion tensor imaging (DTI) analyses were carried out as previously described (211). Briefly, fractional anisotropy (FA), axial diffusivity (AD), mean diffusivity (MD) and radial diffusivity (RD) images were created by fitting a tensor model to the raw diffusion data following Eddy current corrections and brain extraction. Following non-linear

registration and skeletonisation, each subject's diffusivity image was merged into a single 4D image file and a mean FA mask was created. Permutation-based nonparametric inference was used for the voxelwise comparison of diffusion parameters between study groups controlling for age, and applying the threshold-free cluster enhancement (TFCE) method (212). Affected regions were explored for AD, MD, RD and FA and statistical significance defined at $p < 0.05$.

3.6 MUNIX in FTD patients: a comparative study of FTD subtypes, ALS and Poliomyelitis.

3.6.1 Normative data

In the first stage of this study we collected normative data from 40 healthy controls for service quality improvement and to serve as controls for the study groups in this and in future studies. We recorded locally acquired normal control values for Motor Unit Index Estimation (MUNIX) test as populations are heterogeneous and this gave us a greater insight into the role of MUNIX in a number of other disease states.

3.6.2 Patient cohorts

The second stage of the study was a comparative study in which MUNIX data was collected longitudinally from 3 groups of patients with neurological diseases either known to affect the lower motor neurone: Poliomyelitis and Motor Neurone Disease (MND) or known to cause lower motor neuron dysfunction: Frontotemporal Dementia (FTD).

MUNIX was repeated in participants every 3 months for two years. The rate of decline in MUNIX was measured and was compared to the rate of decline in other previously validated parameters of disease progression as well as other disease metrics.

3.6.3 Inclusion criteria:

1. For normative data: Healthy volunteers above the age of 18 and residing in Ireland, with no evidence of nerve or muscle disease were recruited for the control group.
2. For the disease groups: Patients who have been diagnosed with FTD, MND or Poliomyelitis and are attending specific specialist clinics in Beaumont Hospital. ALS patients had to fulfil the category for possible, probable-lab supported, probable or definite ALS regarding to the revised El Escorial criteria. Patients with pure upper motor neuron signs or “suspected ALS” were excluded from the study. ALS symptom onset, defined as onset of weakness, muscle wasting, fasciculations, dysarthria, dysphagia, dyspnoea, falls or disturbance of fine finger movements of less than 18 months from test 1 was also an inclusion criterion.

3.6.4 Exclusion criteria:

1. For normative data: Evidence of any neurological or primary muscle disease like neuropathy, myositis or metabolic myopathy.
2. For disease groups: Alternative neurological diagnoses that could explain their clinical symptoms such as but not limited to Myelopathy, radiculopathy, immune-mediated neuropathies, plexopathies, etc.
3. Inability to give informed consent.

3.6.5 Research protocol and clinical investigation plan:

The research protocol described here was applied to all MUNIX studies performed as part of this research.

3.6.5.1 Identification of Patients and controls

Healthy volunteers were recruited from different sources while patients were recruited from specialist clinics. Patients with FTD, MND and Poliomyelitis were informed of the study when they attended their routine appointments at their specialist clinics. They were given an information leaflet explaining the purpose of the study and they were asked to decide to participate. Once a patient declared his/her intention to participate then he/she would get a phone call from the research fellow to organise testing times after obtaining his/her consent.

3.6.5.2 Study Procedures

Patients who have been telephoned by the researcher were reviewed by him in a specialist neurophysiology clinic at Beaumont Hospital. This interview was followed by the following procedures:

- 1, Standard Medical History and neurological examination was undertaken as required.
- 2, All normal control subjects and patients were asked to consent to the inclusion of their codified clinical data on the research database.
- 3, Normal control subjects who agreed to participate in the study and who had signed the informed consent form underwent MUNIX testing once.
- 4, All patients who agreed to participate in the study and who signed the informed consent underwent MUNIX testing every 3 months for two years.
- 5, MUNIX studies was performed by the researcher, who is trained and experienced in neurophysiology in the neurophysiology department of Beaumont Hospital.

The method was described in the information leaflets. Patients were informed that all of the above were optional, and that they could opt out of the research at any time. The Patient Information Leaflets and Consent Forms had been designed accordingly.

For ALS patients the following clinical data was collected: gender, age, bulbar upper or lower or limb onset, duration of symptoms, level of certainty according to the revised El Escorial Criteria and disease duration till death if ALS patients deceased. The revised ALS Functional Rating Scale (ALSFRS-R) was measured by the same experienced physiotherapist at each visit and just before MUNIX testing.

3.6.5.3 Muscles and nerves tested

The right side was chosen for MUNIX testing in normal controls while MUNIX was recorded on the clinically less affected side of patients' groups from the abductor pollicis brevis, abductor digiti minimi, biceps brachii, tibialis anterior, extensor digitorum brevis and abductor hallucis muscles after supramaximal distal stimulation of the median, ulnar, musculocutaneous, peroneal and tibial nerves. If both sides were affected symmetrically, the right side was chosen and the chosen side in each individual was kept throughout all follow-up examinations.

3.6.5.4 Data recording

All subjects were free of any neurological condition or conditions that might have affected MUNIX measurements such as peripheral neuropathy, neuronopathy, radiculopathy or neuromuscular disease. Subjects were positioned on a supine comfortable position during testing and all measurements were performed by the same examiner, who was also the research fellow and the author of this thesis (T.O).

A commercially available electromyography (EMG) machine (Dantec Keypoint®, Natus Medical Inc., Pleasanton, CA, USA) and software (Keypoint.NET®) was used for all recordings. Analysis time for sweeps was 300 msec. The high pass filter was set to 2 Hz and the low pass filter was set to 10 kHz. Motor nerves were stimulated supramaximally using conventional motor nerve conduction recording methods (158).

3.6.5.5 Recording and maximizing CMAP

Particular attention was paid to electrode position and temperature (not less than 30 degrees on the dorsum of hands and not less than 27 degrees on the dorsum of feet). To ensure consistency between repeated measurements the placement of stimulation and recording electrodes and distance between the active and reference electrode were standardized. The compound muscle action potential (CMAP) of the muscle supplied by that particular nerve was recorded using disposable, self-adhesive surface ground, 15mm disc recording electrodes (Ref 019-415200, CareFusion, Middleton, WI, USA) placed over the belly of the muscle studied. Using routine motor nerve conduction recording techniques, the CMAP (baseline to negative peak) was obtained after appropriate adjustment of the recording electrode to achieve maximum amplitude. Particular attention to shape of the CMAP was observed to ensure a sharp take off with minimal rise (A flat baseline was obtained just before CMAP). The position of the recording electrode position was adjusted several times to achieve maximal amplitude and rise time and a sharp negative take-off of the CMAP. This was one of the most critical steps and was performed carefully. To perform this critical step of maximizing the CMAP, and after positioning the stimulating electrode, the current intensity was gradually increased until CMAP reached a plateau. Then the current intensity was further increased by additional 20% to ensure supramaximal stimulation. After this supramaximal stimulation had been achieved, the recording electrode was placed several times until maximal CMAP amplitude recorded. When CMAP amplitude reached less than 0.5 mV in any particular muscles, this muscle was excluded and MUNIX was rated as zero.

3.6.5.6 Generating and recording appropriate SIPs

To accurately calculate MUNIX for each muscle it was important to capture an appropriate range of force during collection of SIPs. The patient was asked to gradually increase force, while the investigator provided resistance, receiving visual and acoustic

feedback (signal quality was visually and acoustically monitored to exclude noise, interference or tremor and SIP recordings with interference, noise, artefact or tremor were rejected). Motor unit potentials gradually increased as a result. The contraction was isometric and any limb movement was avoided. Care was taken by the examiner not to touch the recording and reference electrodes. SIPs with a quality index below 1.0, ICMUC > 100 and SIP area < 20 mVms were not included in MUNIX calculation as per protocol. SIPs with baseline shift, tremor or bimodal pattern with very low volume conducted activity were deleted/excluded manually.

3.6.6 Frequency of MUNIX tests performed

- Normative data for MUNIX values was obtained (data from normal objects was collected only once).
- Initial MUNIX values, as well as rate of decline with time was determined from 3 groups of patients: patients with FTD, MND and Poliomyelitis. Patients were tested every 3 months 21 months (8 measurements). CMAP and MUSIX values were also recorded on each visit.

3.6.7 Power

Based on the data from a previous MUNIX pilot study conducted by Neuwirth et al in 2011, power analysis indicated the need for a minimum of 30 recruited patients to allow reliable statistical analysis with a relative error below 5% (145).

3.6.8 Procedures for processing MUNIX data

3.6.8.1 Data storage and confidentiality

Research Database: The database was password protected and encrypted and located on a password-protected computer housed in a locked facility in the neurophysiology department in Beaumont Hospital.

Data Interpretation: Normative data was determined and analysed before being stored in the department for future clinical use and maybe used in future studies, subject to appropriate approval from the Research Ethics Committee.

Data from patients was stored and analysed for each of the studied disease states and changes over time was plotted (details are presented in MUNIX results sections).

3.6.8.2 Statistical analysis

The statistical analysis was performed in MATLAB (Mathworks Inc., Natick, MA, USA).

3.6.9 Details of electrode placement and counter resistance during SIP recording for individual muscles

Subjects placed lying comfortably on an examination table during MUNIX measurements. This was especially relevant for positioning of the arm for the biceps measurements.

3.6.9.1 Abductor Pollicis Brevis (APB)

- The hand was placed upon flat surface, with the palm facing up.
- The recording electrode was placed on thenar eminence just lateral to mid-point of first metacarpal, aligned with first metacarpal.
- The reference electrode was placed distally at the thumb.
- The ground electrode was placed on the dorsum of the hand.
- The stimulating electrode was placed at the wrist between flexor carpi radialis and Palmaris longus tendons.
- Partial abduction of the thumb and pronation of the forearm was particularly avoided.
- Counter resistance during SIP recording: the examiner's hand was placed over the patient's hand, with the examiner's thumb giving resistance to the patient's thumb (Figure 3.2).

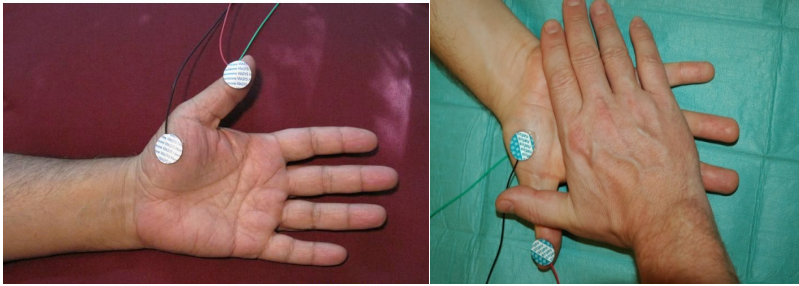


Figure 3.2: Electrode placement and counter resistance provided by the examiner's hand during SIP recording for Abductor Pollicis Brevis (APB) muscle.

3.6.9.2 Abductor Digiti Minimi (ADM)

- The hand was placed on flat surface, with palm facing up.
- The recording electrode was placed on ADM at midpoint of the fifth metacarpal.
- The reference electrode was placed distally at the little finger.
- The ground electrode was placed on the dorsum of the hand.
- The stimulating electrode was placed at the wrist adjacent to flexor carpi ulnaris tendon.
- Counter resistance during SIP recording: Fingers and hand were stabilized by examiner's fingers/thumb, preventing abduction of digit V (Figure 3.4).

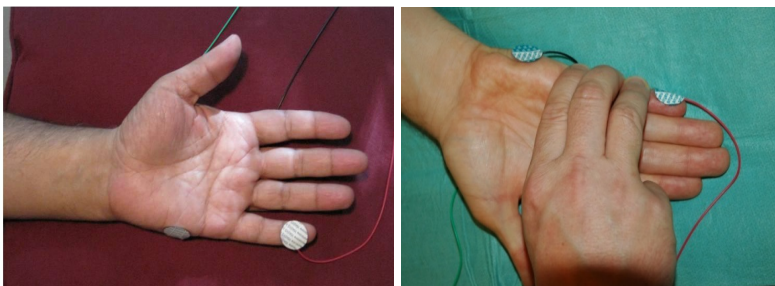


Figure 3.4: Electrode placement and counter resistance provided by the examiner's hand during SIP recording for Abductor Digiti Minimi (ADM) muscle.

3.6.9.3 Biceps brachii

- The arm was placed so that biceps tendon and attachment to the coracoid was easily palpated, with the elbow flexed at about 90 degrees. A flat positioning of the subject with arm abduction and elbow flexion (forearm on a pillow) was the most comfortable position.
- The recording electrode was placed on the bulk of biceps muscle between antecubital fossa and acromion, 1/3rd way up from antecubital fossa.
- The reference electrode was placed over the medial epicondyle of the elbow.
- The stimulating electrode was placed just inferior to the tendon of the short head of the biceps (The musculocutaneous nerve point of emergence from the axilla).
- Isolated stimulation of the musculocutaneous nerve was challenging at times, therefore, it was verified visually that there was no additional wrist flexion by median nerve co-stimulation nor double peak shape of the CMAP.
- Counter resistance during SIP recording: the examiner's hand, forearm or elbow was placed at the participant's wrist/distal forearm to avoid elbow flexion (Figure 3.5).

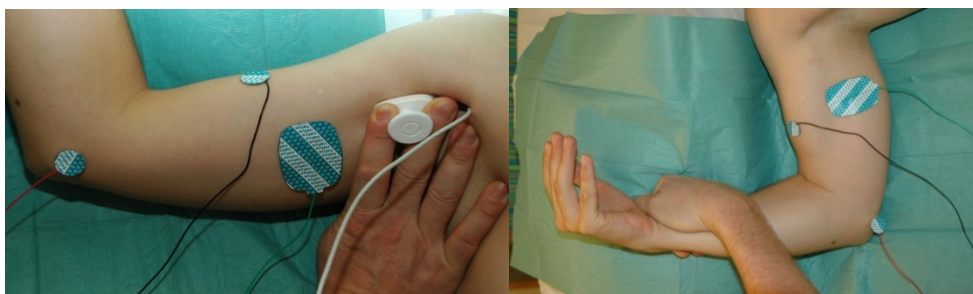




Figure 3.5: Electrode placement and counter resistance provided by the examiner's hand during SIP recording for Biceps Brachii (BB) muscle.

3.6.9.4 Tibialis Anterior

- The lower limb was positioned straight on the table with knee flexed at approximately 90 degrees.
- The recording electrode was placed lateral to the tibial crest, at a point of the junction between the proximal third and the distal two-thirds of the distance between the ankle and the knee (closer to the knee).
- The reference electrode was placed over the patellar tendon.
- The ground electrode was placed just above the fibular head.
- The stimulating electrode was placed one to two fingerbreadths inferior to the head of the fibular.
- Counter resistance: the examiner's hand was used to give resistance with the foot positioned at 90 degrees, avoiding pronation/supination of the foot (Figure 3.6).



Figure 3.6: Electrode placement and counter resistance provided by the examiner's hand during SIP recording for Tibialis Anterior (TA) muscle.

3.6.9.5 Extensor Digitorum Brevis (EDB)

- The lower limb was positioned comfortably straight on the bed.
- The recording electrode was placed on the dorsum of the foot two to three fingerbreadths away from the lateral malleolus.
- The reference electrode was placed distally over the little toe.
- The ground electrode was placed on the dorsum of the ankle.
- The stimulating electrode was placed at the ankle, just lateral to the tibialis anterior tendon.
- Counter resistance: the examiner's ulnar side of the hand was placed on proximal phalanxes of the toes, with the subject's foot at 90 degrees position (Figure 3.7).



Figure 3.7: Electrode placement and counter resistance provided by the examiner's hand during SIP recording for Extensor Digitorum Brevis (EDB) muscle.

3.6.9.6 Abductor Hallucis (AH)

Lower limb position and electrodes placement are shown in Figure 3.8.

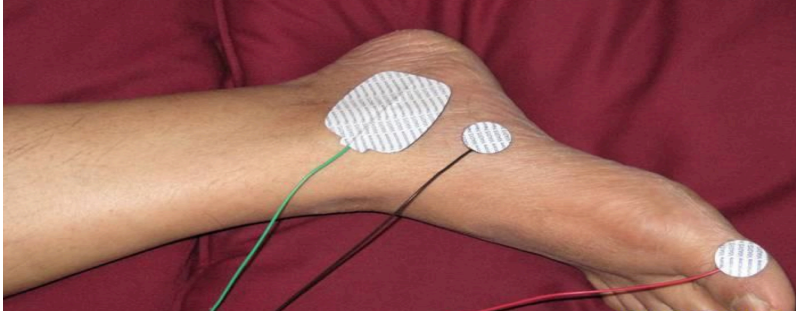


Figure 3.8: Electrode placement and counter resistance provided by the examiner's hand during SIP recording for Abductor Hallucis (AH) muscle.

3.7 Normative data MUNIX study

3.7.1 Methods and Materials

We have performed MUNIX in a cohort of 40 healthy controls to determine the normative range, variance and effects of gender and ageing on our population.

3.7.2 Participants

40 Healthy subjects were recruited and studied prospectively at the Neurophysiology Department of Beaumont Hospital; Dublin, Ireland following approval by Beaumont Hospital Ethics and Medical Research committee. Written informed consent was obtained from all subjects. Only subjects without a history on peripheral nerve dysfunction, neuromuscular disorder or chronic neurological diseases qualified for the study. All patients were studied once except for 4 patients in whom intra-rater reliability was studied. Those 4 subjects were studied twice.

3.7.3 Data Recording and technical aspects:

All subjects were free of any neurological condition or conditions that might have affected MUNIX measurements such as peripheral neuropathy, neuronopathy, radiculopathy or neuromuscular disease. Subjects were positioned on a supine comfortable position during testing and all measurements were performed by the same examiner (T.O).

Details of MUNIX technical aspects, mathematical model and computation were described in chapter 1 and the practical steps of data recording and processing were detailed in sections 3.6.5, 3.6.8 and 3.6.9 of this thesis.

Briefly, MUNIX was measured in 40 healthy subjects aged 23 - 80 years. The test was performed by an experienced qualified clinician trained in MUNIX acquisition and analysis. When subjects were tested bilaterally or on multiple occasions, only the first MUNIX measurement from the right side was used for defining the lower normal limit. Subjects were separated into two age groups (<60 and \geq 60 years) to reflect the known phenomenon of loss of motoneurons in aging (170). In this study, a total of six muscles were tested per participant: three muscles in the upper limb and three muscles in the lower limb. Muscles investigated were: the right abductor pollicis brevis (APB), abductor digiti minimi (ADM), biceps brachii (BB), tibialis anterior (TA), extensor digitorum brevis (EDB) and abductor hallucis (AH). Three measurements, namely MUNIX, The "Motor Unit Size Index" (MUSIX); an index that reflects the average size of the motor units and CMAP were calculated for each muscle. Of note, the MUNIX technique calculates MUSIX as a secondary computation by dividing the CMAP amplitude by the MUNIX value and is expressed in microvolts.

The intra-rater reliability was validated by repeating MUNIX in 4 normal subjects by the lead investigator. 4 subjects were also studied twice in each of the disease subgroups for the same purpose. When performing intra-rater reproducibility tests, electrodes were removed after the first measurement and all marks were removed that would

indicate the electrode position. The second study was performed 30 min to 7 days after the first measurement. Inter-rater variability was not studied in this population. Of note here is that neither determining inter-rater or intra-rater variability was an aim of this study.

3.7.4 Statistical analysis

The statistical analysis was performed in MATLAB (Mathworks Inc., Natick, MA, USA).

Descriptive statistics: The measures that were calculated for each muscle individually, a individually, as well as for the pool of muscles included: Mean, Standard deviation, Median, Inter-quartile range (IQR), Range (min, max), 95% Confidence interval of Mean (using 2000-iteration boot strapping), Coefficient of Variation and its non-parametric counterpart (IQR/Median).

ANOVA: A 3-way ANOVA was used for each measure with 3 main effects: a 6-level muscle effect, a 2-level age effect, a 2-level gender effect, as well as all the interactions. The 3 main effects, 3 interaction effects, as well as post-hoc comparisons for the effects within each muscle (using Tukey's method) are reported as p-values.

Correlations: Spearman's Correlation Coefficient and its significance level (p-value) were calculated between MUNIX and MUSIX (MUSIX refers to the motor unit size index: this parameter is obtained by dividing CMAP amplitude by MUNIX value and is expressed in microvolts), as well as between MUNIX and CMAP. Descriptive statistics and ANOVA results were individually calculated for each measure of MUNIX, MUSIX and CMAP. A significance level of 0.05 was used for inference. Subjects were separated into two age groups (<60 and \geq 60 years) to reflect the known phenomenon of loss of motoneurons in aging (170).

3.8 Poliomyelitis MUNIX study

This was a longitudinal study in which MUNIX data was collected from 41 subjects with prior poliomyelitis. All participants were recruited from a specialist poliomyelitis clinic, had documented history of paralytic poliomyelitis and were diagnosed by a consultant neurologist with special interest in this condition.

Data were obtained from six different upper and lower limb muscles: abductor pollicis brevis, abductor digiti minimi, biceps brachii, tibialis anterior, extensor digitorum brevis and abductor hallucis, as per protocol. MUNIX was repeated in participants every 3 months \pm 1 week for twenty-one months (eight visits/tests per patient). The least affected side clinically was chosen for longitudinal MUNIX testing. When three limbs were clinically then the side with the clinically not affected fourth limb was chosen.

Data collected from each of the participants also included:

- Demographics, including age at disease onset and disease duration
- Type of poliomyelitis (spinal or spinobulbar)
- Acute paralytic polio (APP) duration and age at onset
- Type of recovery from APP (complete/partial)
- Post polio syndrome (PPS): age at onset, symptoms, duration and time interval between APP to PPS.

3.9 FTD MUNIX study

3.9.1 Methods

A total of 39 patients were recruited in this study: 22 bvFTD, 9 nvPPA, 6 FTD-MND and 2 svPPA patients. The right side was chosen for testing and all participants did not show clinical evidence for lower motor neuron involvement as they had no muscle weakness (as indicated by MRC power score of 5), fasciculations or wasting.

3.10 ALS MUNIX study

3.10.1 Methods and participants

This was a longitudinal study in which MUNIX data was collected from 43 patients who carried the diagnosis of ALS. Six different muscles: abductor pollicis brevis, abductor digiti minimi, biceps brachii, tibialis anterior, extensor digitorum brevis and abductor hallucis, were measured on the less affected side. MUNIX was repeated in participants every 3 months for twenty-one months (eight visits/tests per patient). The rate of decline in MUNIX was measured and was compared to the rate of decline ALS FRS-R. All patients were diagnosed by an experienced ALS neurologist and fulfilled the diagnostic categories of possible, probable or definite ALS according to the revised El Escorial criteria (204). Only patients with no other diseases that could have influenced results (e.g. polyneuropathy, focal nerve entrapment, etc) were included in the study. A specific criterion was added to avoid potential bias towards either slowly progressing patients or patients with advanced disease stage and wasted muscles at study entry: symptom onset (defined as weakness, dysarthria, dysphagia, dyspnoea, gait impairment or disturbance of fine finger movements for ALS patients), had to be shorter than 12 months in duration. Assessments were performed every 3-months \pm 1 week.

MUNIX was performed in the APB, ADM, BB, TA, EDB and AH muscles after supramaximal distal stimulation of the corresponding nerves. The position of the recording electrode was adjusted several times to achieve maximal CMAP amplitude with minimum rise time and sharp negative rake-off. Measurements were performed on the same side of the body throughout the study. The clinically stronger side was selected in order to avoid measurements in wasted muscles and hence the risk of an early “ floor or basement effect” with resultant difficulty in detecting changes in lower

motor neuron pool. The right side was chosen for participants with symmetric strength on both sides.

At each visit, the ALS FRS-R score was assessed by the same ALS physiotherapist. ALS FRS-R rater and MUNIX rater were blinded to each other's results.

The rate of decline of MUNIX per months was calculated. This rate of decline was defined as the percentage changes from baseline-defined as 100%. This was calculated for all muscles combined and for each muscle individually. The two rates of decline in MUNIX and ALSFRS-R were compared.

Subgroup analysis was carried out and the pattern of decline of the parameters of MUNIX, CMAP and ALS FRS-R in the 4 subgroups of: 1-bulbar onset, 2-upper limb onset, 3-lower limb onset and 4-FTD-MND was determined.

NB: Testing was stopped if the patient died, was too weak to offer resistance (muscle power was low; as defined by MRC < grade 3). MUNIX testing was also ceased when CMAP value dropped to <0.5 mV, if the patient withdrew from the study or there was advanced cognitive impairment.

Results of this research and discussion of findings are presented in next chapters (chapters 4-9) below.

4 Chapter 4: Results and Discussion of study 1: The cognitive clinic experience: descriptive analysis of the referral sources, demographics and etiological causes of patients reviewed during study period (193 consecutive patients)

4.1 Introduction

Neurodegenerative and neuropsychiatric conditions that affect cognition present difficult clinical circumstance. The differential diagnosis of conditions that present with cognitive symptoms is broad and they pose numerous psychological implications for the patient and the family, therefore, caring for those patients at specialist cognitive clinics that have the capacity to deal with memory and non-memory related conditions results in better outcome for patients, families and carers. Few data exist regarding evaluating demographic, phenotype and outcome of such specialist clinics. The available literature on this field is derived from studies evaluating the role of standard memory clinics: two direct randomized control trials compared specialist memory clinics (as a diagnostic setting) with alternatives. The first study conducted by Wolfs et al (173). This study reported that 'in comparison with usual care an integrated multidisciplinary approach to dementia diagnosis in a memory clinic setting increases health-related quality of life of the dementia patients, adds very useful information and is affordable'. The second study was a pilot study conducted by Logiudice et al (174). The aim of this study conducted by Logiudice et el was to determine the effects of attendance at a memory clinic on the psychosocial health of carers and it showed significant improvement in psychosocial health related quality of life of carers (as a result of attending memory clinics).

Standard memory clinics have spread around the world since they were first introduced in the US in the mid 1970s (175). Such clinics classically serve the role of identifying and managing patients with memory impairments and dementia. However, the scope of diagnostic aetiologies, assessment tools and emerging therapies of conditions that present with amnesic and non-amnesic cognitive symptoms has become wider and more complex. This is especially relevant in those below 65 years of age and hence highlights the importance of assessing young and atypical cases in cognitive clinics that operate in a neurology setting.

Furthermore, the differential diagnosis of patients presenting with cognitive symptoms is extensive, including a number of neurodegenerative disorders, neuropsychiatric diseases, non-progressive static brain insults, hereditary aetiologies and other syndromic diagnoses. In some individuals, the aetiology remains indeterminate even after brain biopsy (156).

Moreover, there is increasing appreciation of the requirement to reconfigure neurological services and to have specialist cognitive clinics to meet this need, but the challenge is to provide a service for those who most need it with an under-resourced speciality. We propose in this study that timely involvement of a cognitive neurologist leads to refining and/or changing diagnosis and hence management (This was the case in 44.6% of cases in our cohort).

Patients participating in this research were recruited from two clinics in Dublin: St James's Hospital cognitive clinic that primarily evaluated patients presented or referred with various primarily amnesic and non-amnesic cognitive symptoms and Beaumont Hospital neurodegenerative clinic for which patients suspected of having MND and/or other non-amnesic related disorders were referred and assessed. This chapter describes the experience of the former clinic over three years (The study period).

To better characterize this cohort, we reviewed the experience of our cognitive clinic to more clearly delineate the demographic and etiologic characteristics of this population.

The specific objective of this study was to perform a descriptive analysis of the demographics and diagnoses (etiologic causes) of patients seen over three years at our specialist cognitive clinic of Saint James's Hospital, Dublin, Ireland.

4.2 Design

Observational, retrospective, clinic-based, descriptive study of a single-cohort of consecutive referrals to a cognitive clinic.

4.3 Setting

An outpatient multidisciplinary cognitive clinic of a tertiary referral university hospital in a neurological setting.

4.4 Results

4.4.1 Demographic data

193 patients fulfilling prespecified criteria were evaluated at the cognitive clinic during the specified study period. Summary demographics of the cohort are given in Table 4.1. The mean age of patients at symptom onset was 56.8 years (Range 18 – 89, 95% CI 54.9 - 58.7). 101 patients (52.3%) were males and 92 (47.7%) were females. The female-to-male ratio was 1.0: 1.1 and the cohort was 96.9% Caucasian. The mean educational attainment was 13.7 years (range 6-19, 95% CI 13.2-14.2).

Of the 193, 66 patients were seen in the first years while 60 patients and 67 patients were seen in the second and third years respectively.

Table 4.1: Demographic Data

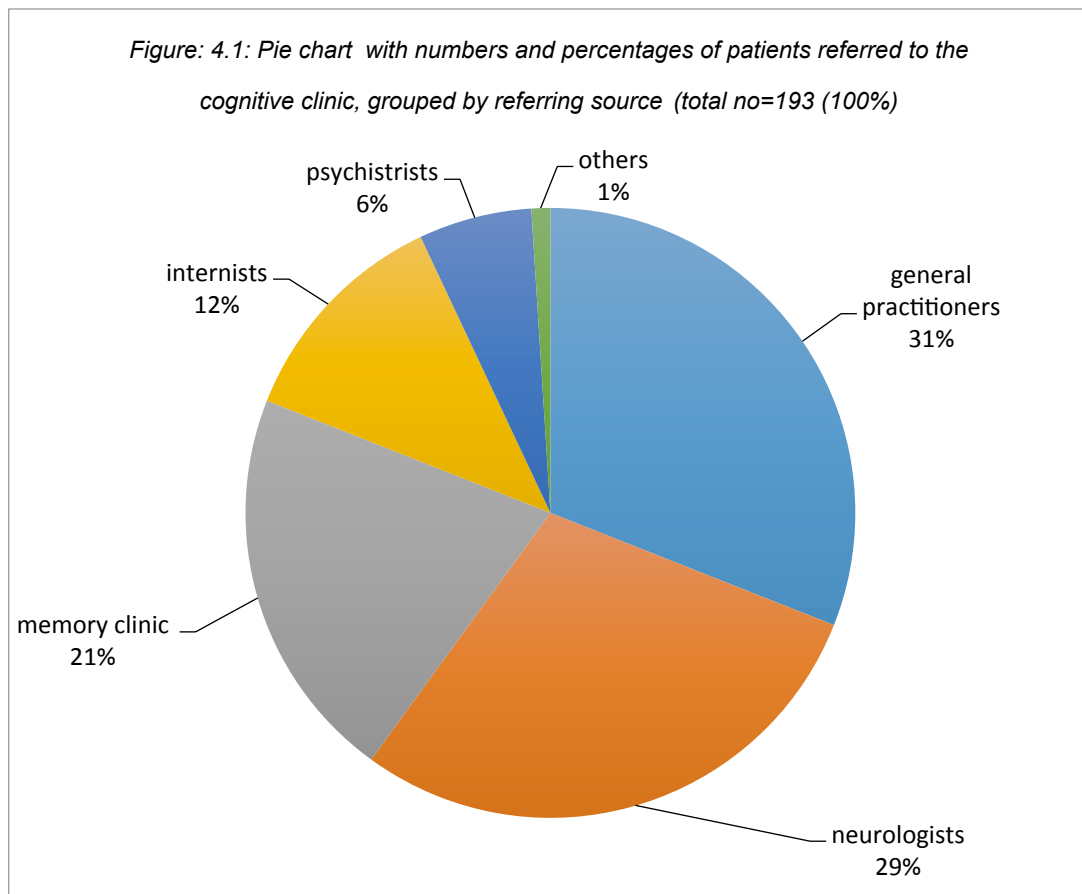
Variable	Value (N =193)
Gender	
Male	101
Female	92
Education attainment, mean (SD), year	13.7 (3.8)
Age at symptom onset, mean (SD), year	56.8 (13.5)
Age at presentation, mean (SD), year	58.7 (13.7)
Time from onset to presentation, mean (SD), months	23.2 (14.80)

4.4.2 Sources of referral

A total number of 193 patients were referred and assessed at the cognitive clinic during the study period. The clinic received referrals nationally from 6 different sources: The main ones were: General practitioners (GPs), Neurologists and the memory clinic. Patients were also referred by Internists, Psychiatrists and other specialists (Emergency and Surgery consultants). Table 4.2 and figure 4.1 details the numbers and percentages of referrals received from each of those sources.

Table 4.2: Numbers and Percentages of Patients referred to the Cognitive Clinic grouped by Referring Source

Referring source	Number referred	Percentage
General practitioners	60	31%
Neurologists	56	29%
Memory clinic	41	21%
Internists	23	12%
Psychiatrists	11	6%
Others (Emergency & Surgery specialists)	2 (1 from each)	1%
Total	193	100%



4.4.3 Etiological data

The final diagnoses of the total 193 patients belonged to several aetiologic categories. Table 4.3, figures 4.2 and 4.3 summarize these data and identify the number and percentage for each of the specific diagnoses identified.

Table 4.3: Etiologic data (primary underlying cause of presenting symptoms) among 193 consecutive patients referred to the cognitive clinic

Etiology (primary diagnosis)	Number of patients seen (Total=193)	Percentage of total seen (100%)
Neurodegenerative disorder	119	62%
Anxiety (worried well)	18	9.5%
Alcohol excess	14	7%
Unknown	7	4%
Epilepsy	6	3%
Depression	4	2%
Autoimmune/Inflammatory	6 Multiple sclerosis 4 HIV-related dementia	3% 2%
Head injury/frontal lobe trauma	2	1%
Fainting episodes/collapse	2	1%
Unclassified learning disability	2	1%
Bipolar affective disorder	2	1%
Subdural hemorrhage	2	1%
Metabolic (Cushing's syndrome)	1	0.5%
Chronic traumatic	1	0.5%

encephalopathy		
Ateriovenous malformation	1	0.5%
Brain infarct (stroke sequelae)	1	0.5%
Post-concussion syndrome	1	0.5%

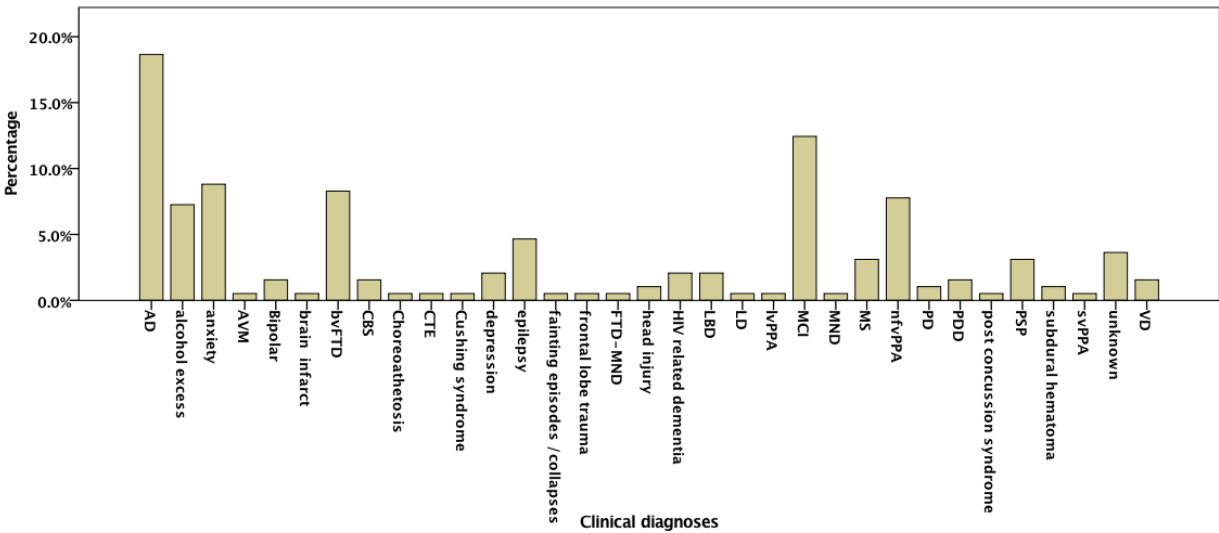


Figure 4.2: Distribution of all applied primary diagnoses, expressed as percentage of total patients.

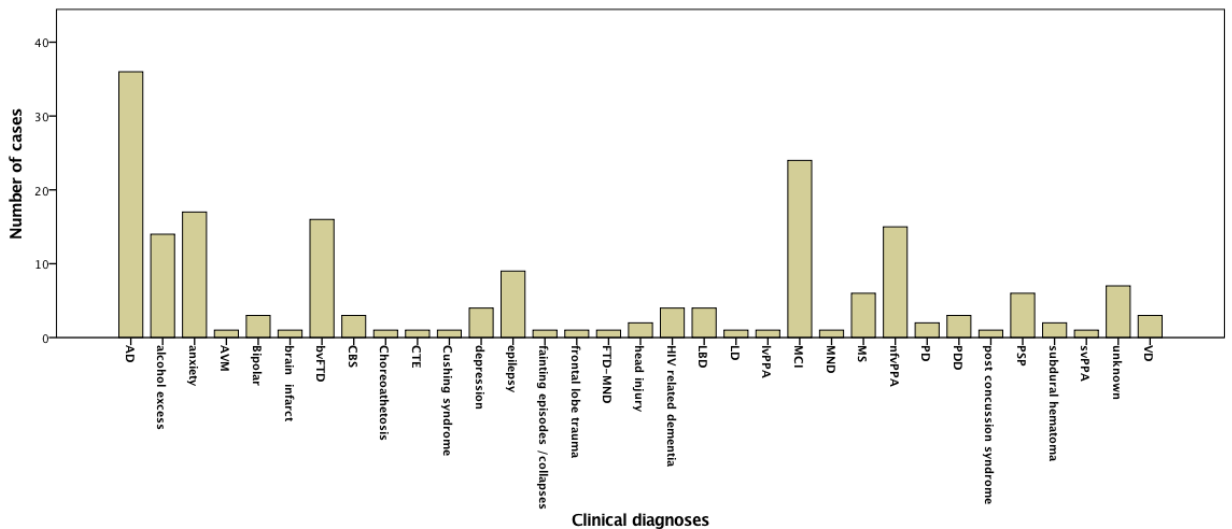


Figure 4.3: Distribution of all applied primary diagnoses, expressed in numbers.

Diagnoses varied but the most common aetiologic group by far was neurodegenerative, accounting for 62% of the cohort. Other less common causes included anxiety (9.5%) and alcohol excess (7%). At last follow up, 4% had an unknown aetiology, despite exhaustive evaluation. A metabolic cause was uncommon and was identified in one case only (0.8%).

To categorize young onset and early onset presentations participants were separated into three age groups: below 45, 45-64 and 65 and above and the contribution of each of the etiologic groups into each of those three age groups was analysed. Figures 4.4A and 4.4B show the relative percentage that each aetiologic group contributed to the specified age group ($\chi^2=64.98$, $df=12$, $p<0.001$).

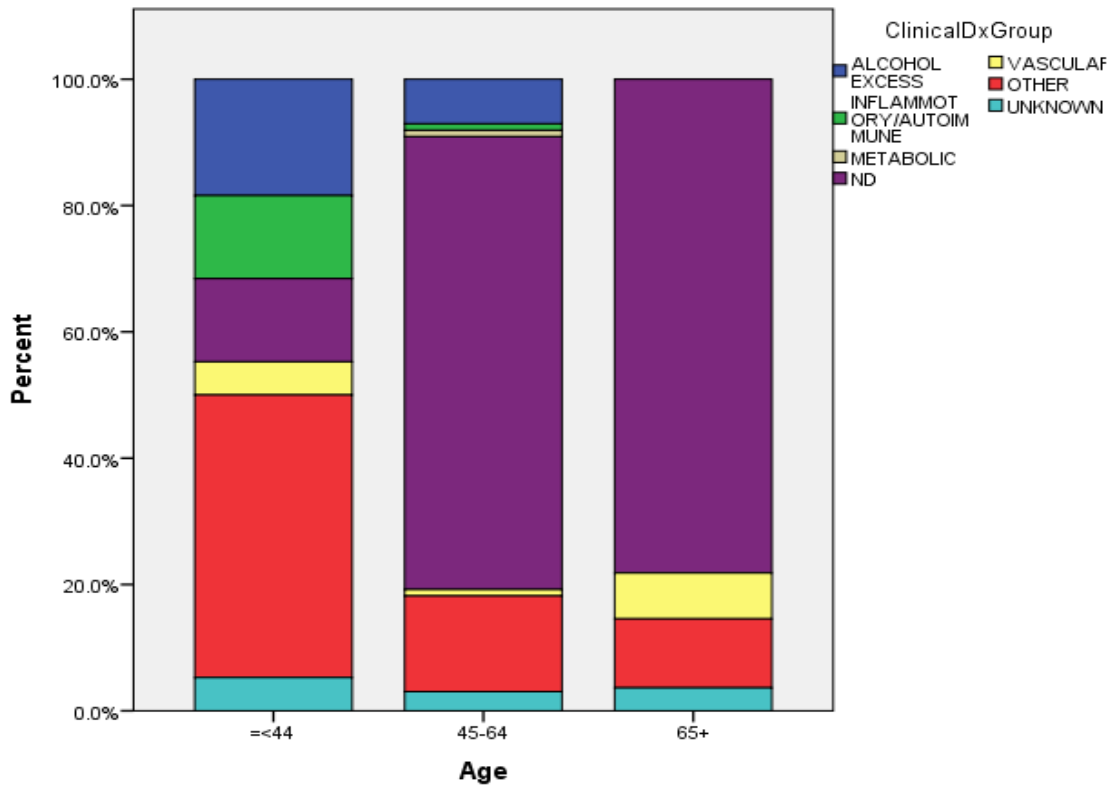


Figure 4.4A: Histogram of the percentage of patients within each etiologic group stratified by three age groups and the percentage of patients that each etiologic group contributed to each of the three age groups

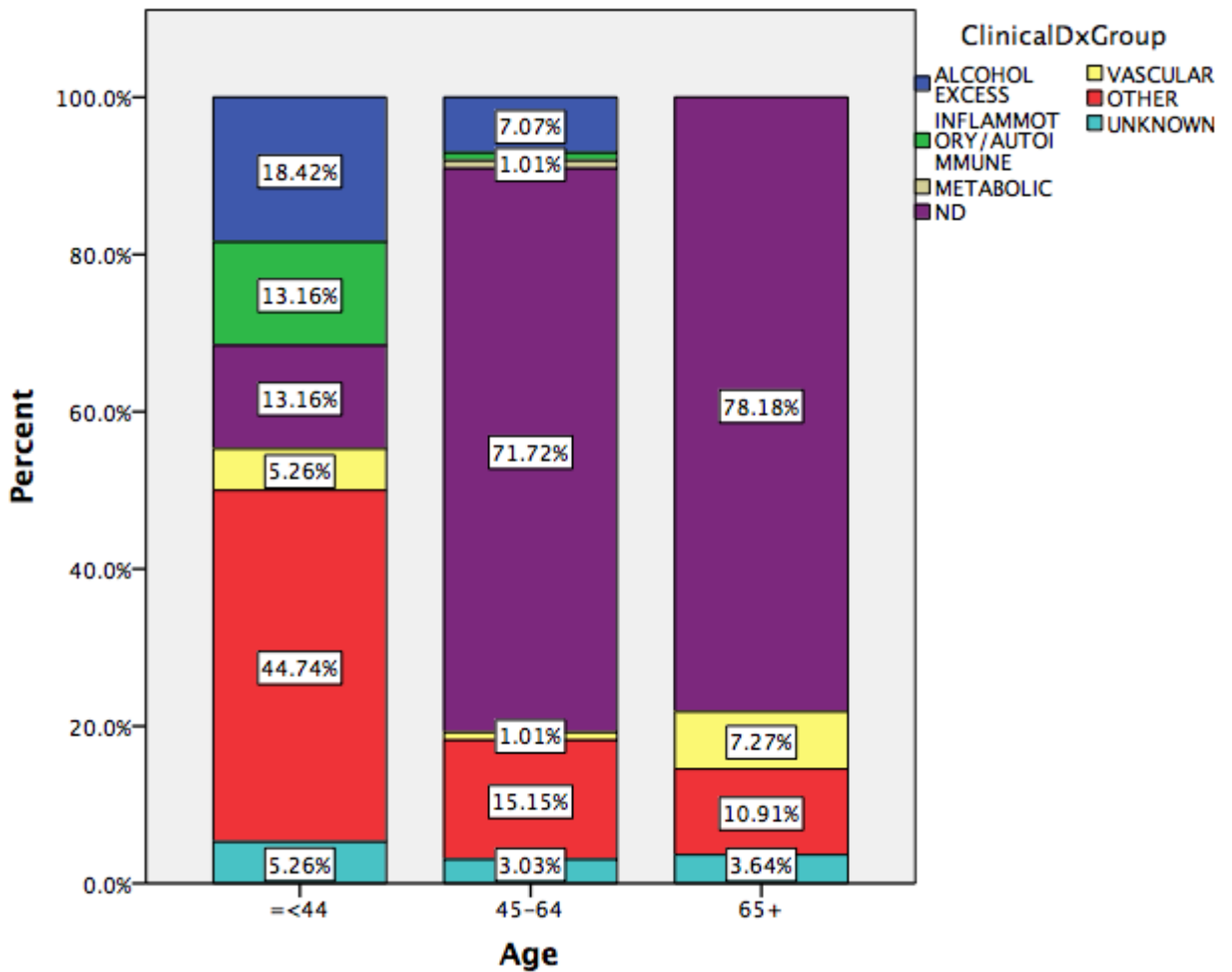


Figure 4.4B: Histogram of the percentage of patients within each etiologic group, stratified by three age groups and the percentage of patients that each etiologic group contributed to each of the three age groups.

Analysis of variance (ANOVA) showed significant difference in age of onset between etiological groups ($F=13.76$, $p<0.001$). Causes varied with age, with diagnoses like alcohol excess and autoimmune/inflammatory being more common before the age of 45 years and neurodegenerative disorders being more common after age of 65.

Of 193 patients, 46 (>24%) had potentially reversible/treatable conditions. Those potentially reversible conditions included for example epilepsy and autoimmune/inflammatory conditions.

After full assessment at the cognitive clinic, 80 out of 193 patients had their final diagnoses either changed or refined. This constituted 41% of all referrals. Details are given in table 4.4 below:

Table 4.4: Numbers and Percentages of Patients in whom Final Diagnosis was either Changed or Refined after Assessment at the Cognitive Clinic, stratified by Referring Source

Source of referral	Number of referral with diagnoses changed or refined	Percentage of referral with diagnoses changed or refined
General practitioners	43 (out of 60)	71%
Internists	13 (out of 23)	58%
Memory clinic	12 (out of 41)	29%
Psychiatrists	3 (out of 11)	24%
Neurologists	12 (out of 56)	21%
Others (Emergency & Surgery)	2 (out of 2)	100% (NB: 2 referrals only)
Overall	80 (out of 193)	41%

A certain pattern was noticed on examining the referral letters in this subgroup of patients for whom the final diagnosis was refined/changed as a result of clinic evaluation: neurology clinic letters tend to contain a detailed account of patient's history and clinical examination before providing a list of differential diagnoses, in which the primary diagnosis was included in most cases. These attempts to suggest a diagnosis or differential was seen to a less extent in letters received from general practice and psychiatry service despite providing a good account of presenting symptoms generally.

4.4.4 Demographic differences between aetiologic groups

Age at onset for all patients ranged from 18 to 89. There was significant difference in age at onset across the etiologic groups ($P < 0.001$). Educational attainment ranged

from 0 to 19 years. Significant difference in the number of years of education was identified among etiologic groups ($p < 0.001$). Overall, the male to female ratio was 1.1:10. No significant difference was noted in sex distribution among etiologic groups ($p = 0.35$).

A significant difference in disease duration before presentation was identified among etiologic groups ($p 0.025$). As summarized in Table 4.5, Patients having metabolic and autoimmune/inflammatory aetiologies had shorter times between onset and presentation (mean of 7 and 6 months respectively) compared to those having neurodegenerative or unknown aetiologies (mean of 24 and 27 months respectively).

Table 4.5: symptom onset to presentation stratified by diagnostic group

Diagnostic group	Symptom onset to presentation (months)	
	Mean	SD
Alcohol excess	21	10
Inflammatory/autoimmune	7	2
Metabolic	6	.
Neurodegenerative	24	12
Vascular	13	12
Other	25	22
Unknown	27	13

Analysis of referring sources showed that certain neurodegenerative conditions are more likely than others to be recognized by referring specialists. For instance, at initial presentation, 15/193 (7.8%), 11(5.7%) and 16(8.3%) of cases were referred with clinical diagnoses of bvFTD, nfvPPA and MCI respectively, but these numbers

increased to 19(9.8%), 14(7.3%) and 24(12.4%) respectively after evaluation at the cognitive clinic.

The three subgroups of: Neurodegenerative disorders (ND), Early onset presentation (EOP) and Early onset neurodegenerative disorders (EOP-ND) will be discussed in detail in sections 4.4.5, 4.4.6, and 4.4.7 below.

4.4.5 Neurodegenerative disorders subgroup

Neurodegenerative disorders (ND) accounted for 62% of patients in our cohort (119 patients). The number of individuals having a neurodegenerative disease increased with older age (Figures 4.4A and 4.4B). A neurodegenerative cause was uncommon before the age of 45 (only 5 patients or 2.6% of the total cohort) with the rate increased to account for 71(36.8%) and 43 (22.3%) of patients having onset at the age groups of 45-64 and 65 and above years respectively. The mean age of symptom onset was 62.7 years (SD 14.1). Figure 4.5 shows referral sources for patients in this group.

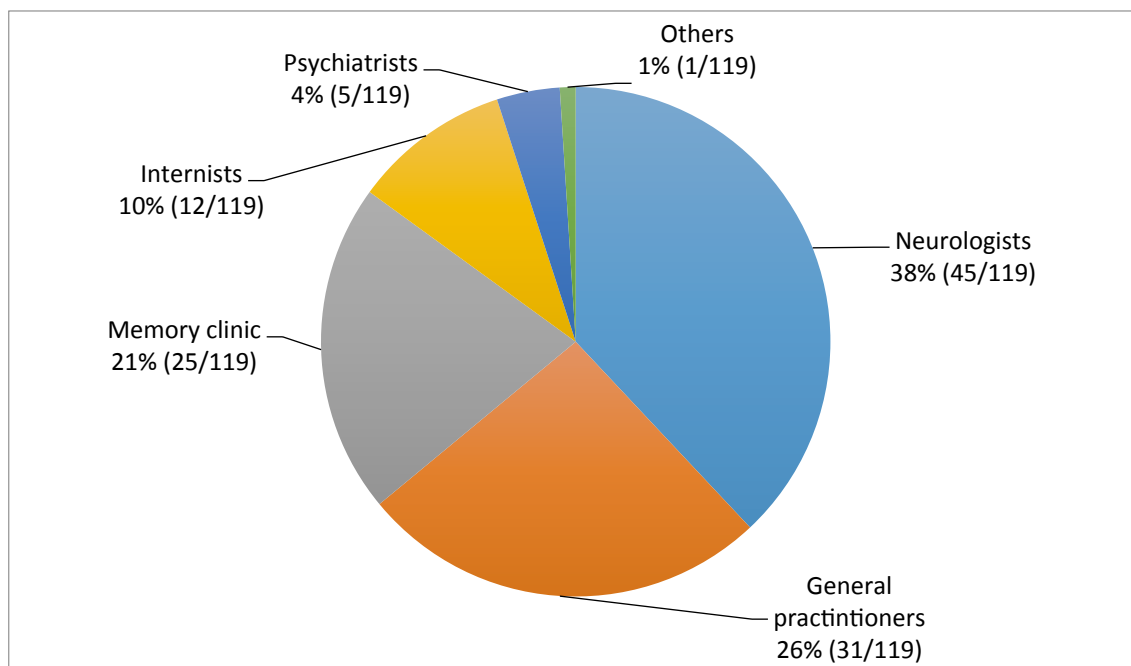


Figure 4.5: Pie chart showing numbers and percentages of patients diagnosed with neurodegenerative disorders, grouped by referring source (total no=119 (100%))

The primary etiologic diagnoses of patients presented with neurodegenerative disorders are presented in table 4.6 and diagrammatic breakdown of these disorders in both numbers and percentage are shown in figures 4.8A and 4.8B.

Table 4.6: Diagnostic Classification of 119 Patients presented with Neurodegenerative disorders

Neurodegenerative etiological category	Number of patients	Percentage
Amnesic syndrome (AD & MCI)	59	49.7%
Frontotemporal dementia (FTD)	39: Subtypes as follows: - bvFTD: 19 (16.1%) - nvPPA: 14 (11.8%) - svPPA: 2 (1.7%) - lvPPA: 1 (0.8%) - FTD-MND: 1 (0.8%) - nvPPA-PSP: 1 (0.8%) - bvFTD-PSP: 1 (0.8%)	32.8%

Progressive Supranuclear Palsy (PSP)	6	5%
Parkinson Disease Dementia (PDD)	5	4.2%
Lewy Body Dementia (LBD)	3	2.5%
Vascular Dementia (VD)	3	2.5%
Corticobasal Syndrome (CBS)	3	2.5%
Choreoathetosis	1	0.8%
Total	119	100%

The distribution of the 119 patients diagnosed with neurodegenerative disorders was different in different age groups as 60.1% and 37.3% belonged to the age groups 45-64 and 65 and above respectively. $P < .0001$, $t = -9.2$, $DF = 190$. (Figure 4.6).

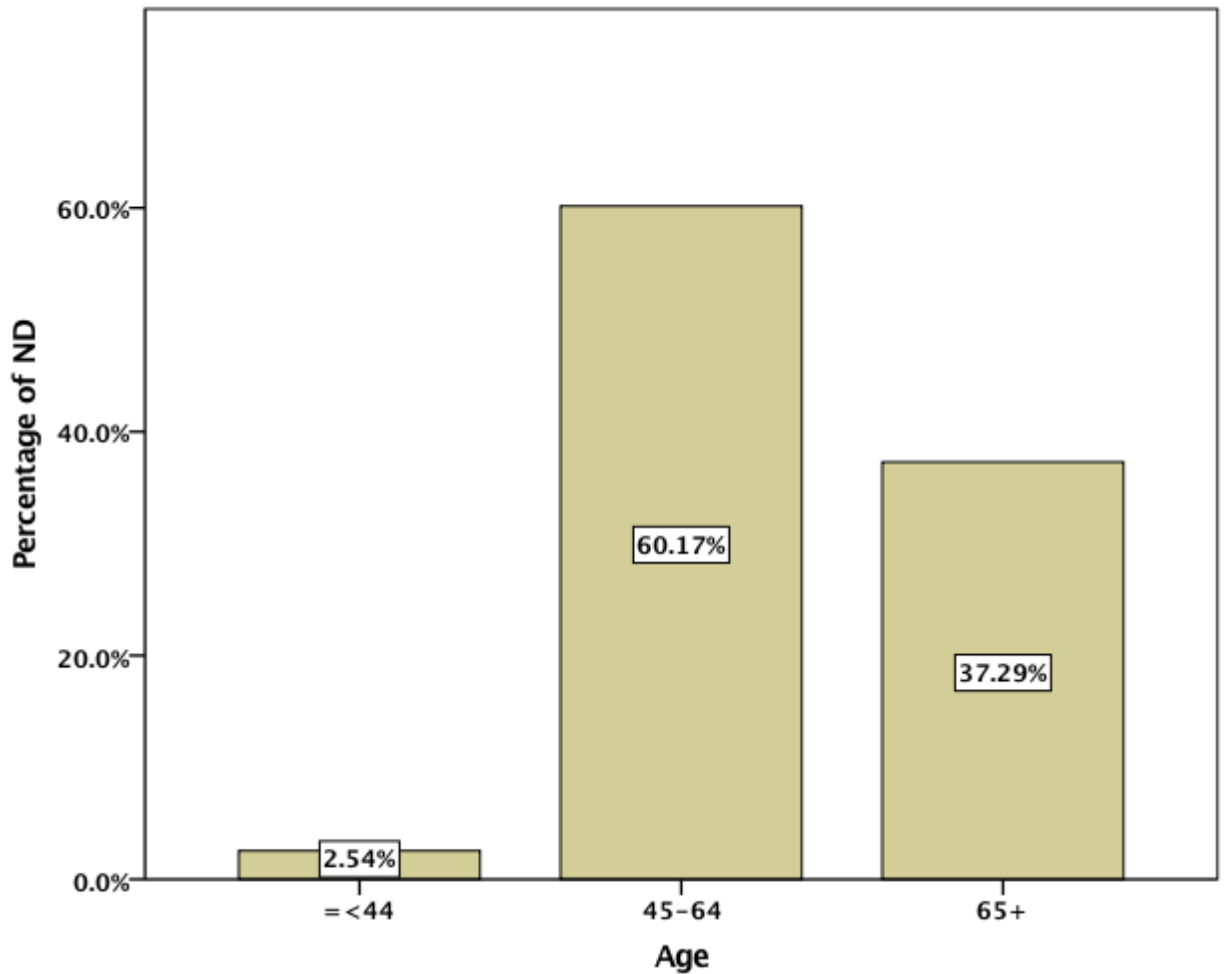


Figure 4.6: Percentage of Neurodegenerative disorders (Total 119) stratified by age group

Mean age at symptom onset was 62.7 years. This was significantly different for this group when compared to the rest of the cohort (47.3 years). $P < 0.0001$, $DF = 190$, $t = -9$. (Figure 4.7).

ND patients spent significantly less years in education (mean educational attainment was 12.9 years) when compared to participants in other diagnostic categories (mean educational attainment was 16.14 years). ($P < 0.0001$, $DF = 191$).

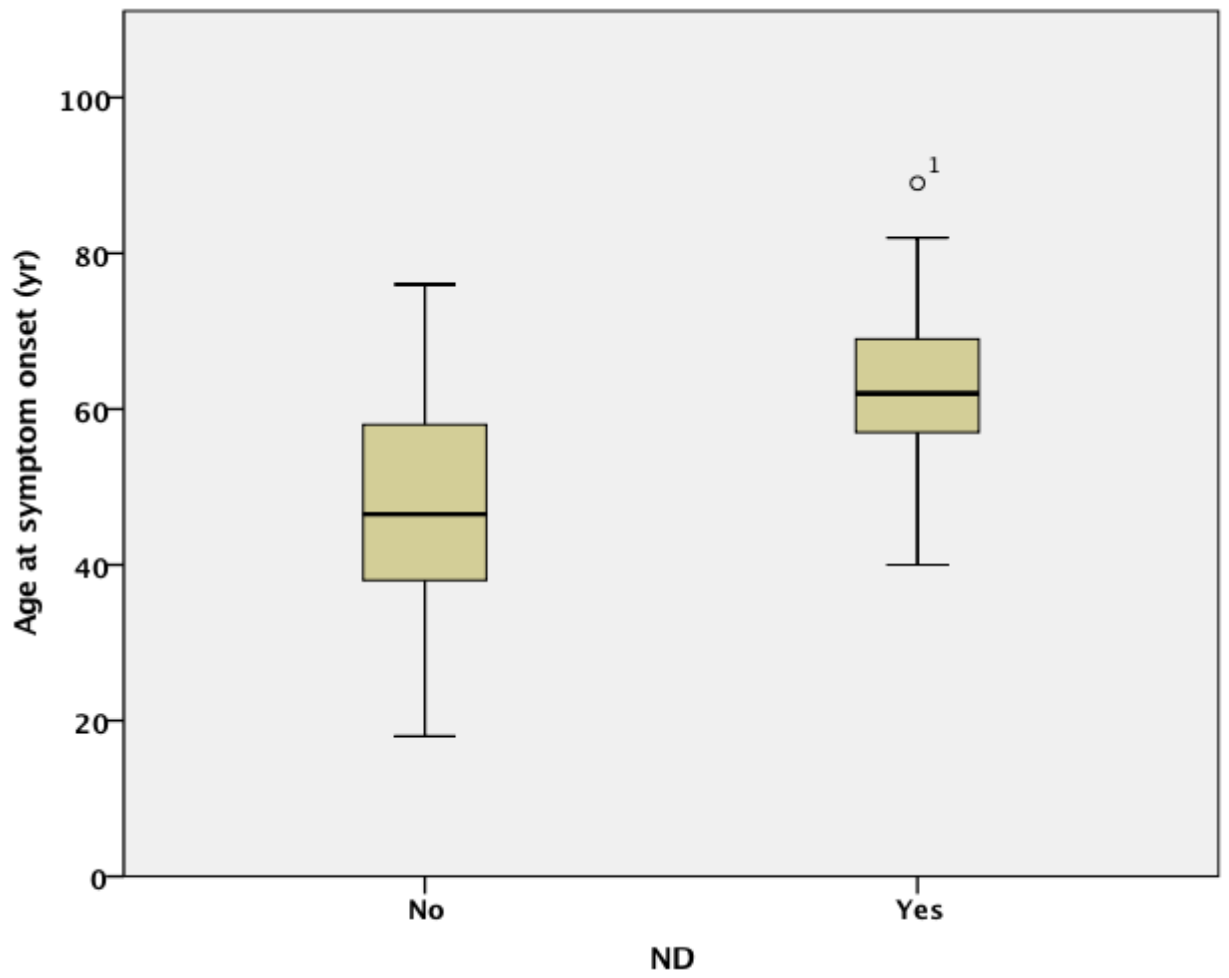


Figure 4.7: Showing significantly different age at symptom onset for ND cases in comparison to the rest of the cohort

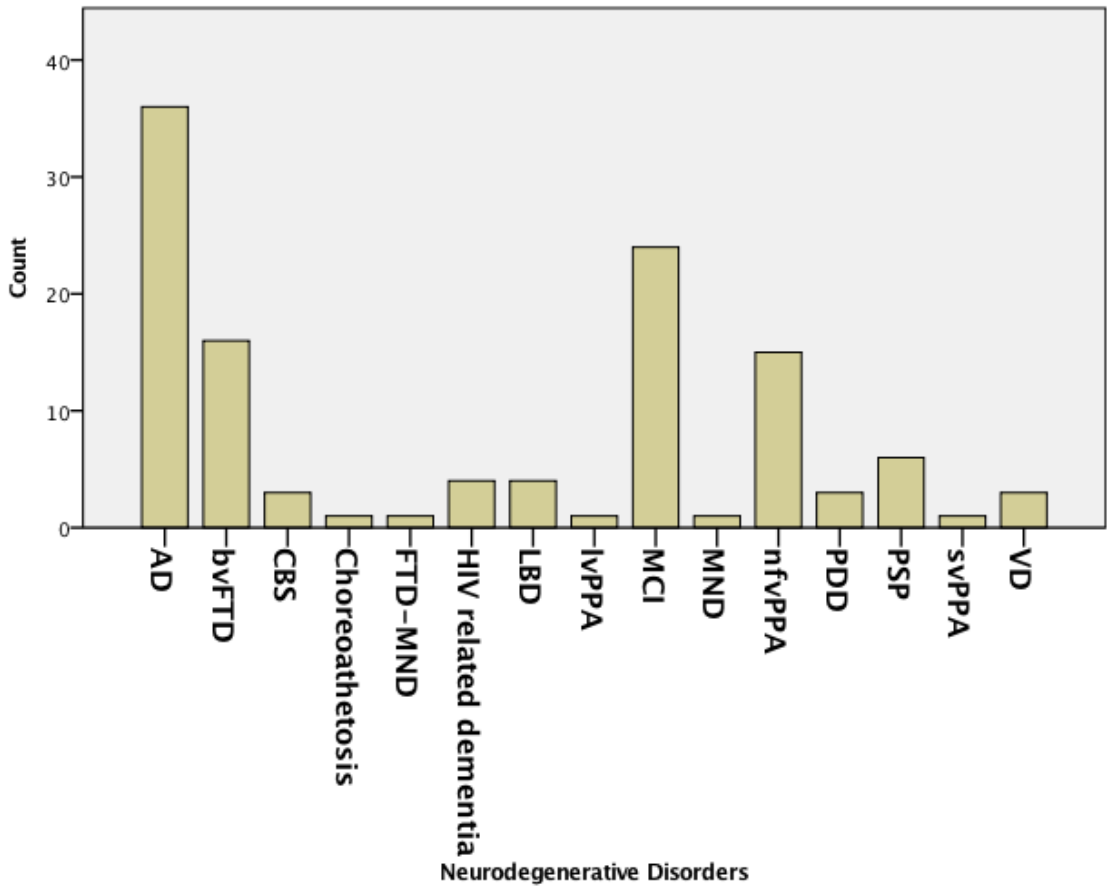


Figure 4.8A: Breakdown of all Neurodegenerative disorders by number (Total=119)

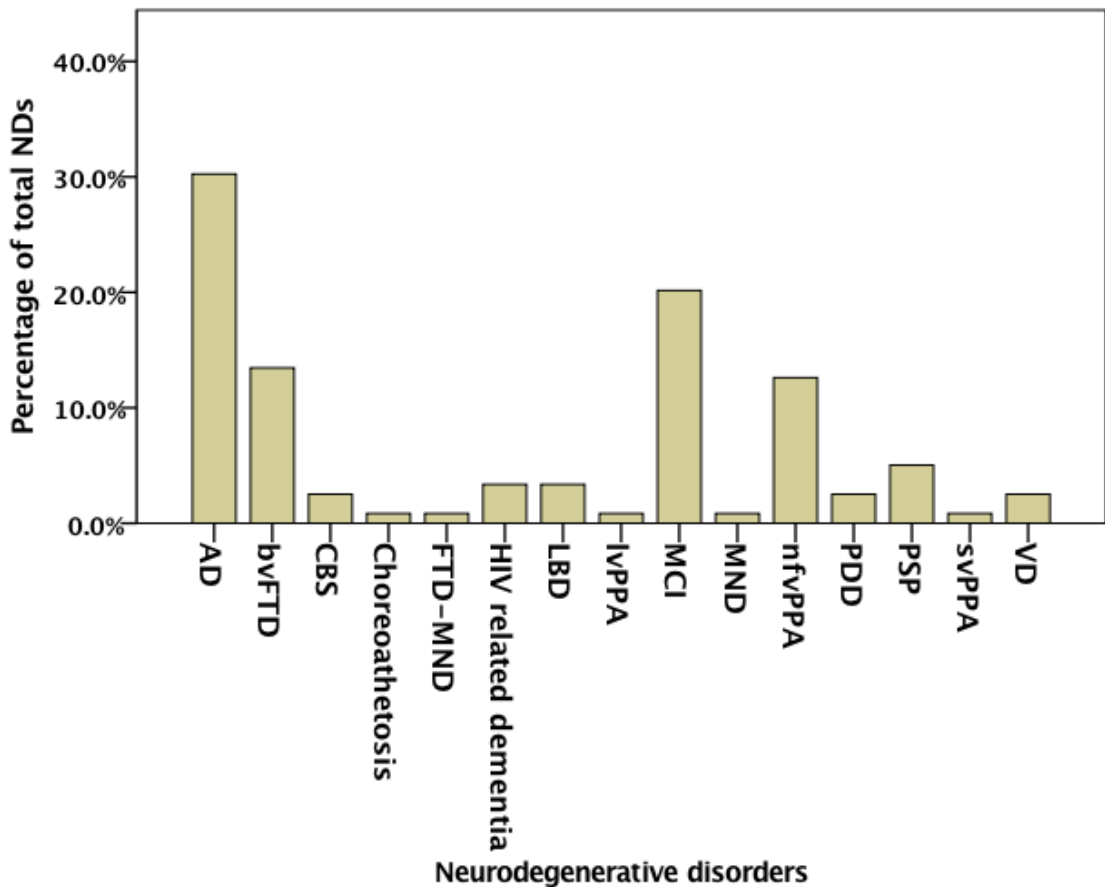


Figure 4.8B: Breakdown of all Neurodegenerative disorders by percentage (Total 119=100%).

Analysis of 109 patients determined to have dementia found that 32.8% (39 patients) had Frontotemporal dementia, 49.7% (59 patients) were diagnosed as having amnesic syndrome (AD and MCI), 2.5% (3 patients) had vascular dementia, 4.2% (5 patients) had Parkinson disease dementia and 2.5% (3 patients) had Lewy body dementia.

4.4.6 Early -onset presentation subgroup (EOP)

This refers to those below the age of 65 and it comprises early-onset dementia as well as other diagnoses. 68.4% (132 patients) in our cohort belonged to the younger age group. Etiologies in this group is detailed in table 4.7 below:

Table 4.7: Underlying Diagnoses in 132 Patients with Younger-onset (<65) of Cognitive Symptoms

Etiology	Number	Percentage
Amnestic (AD & MCI)	38	28.8%
Frontotemporal Dementia	22	16.8%
Anxiety (worried well)	16	12.1%
Excess alcohol	13	9.8%
Multiple Sclerosis (MS)	6	4.5%
Bipolar & Depression	6	4.5%
Unknown	5	3.7%
HIV-related dementia	4	3%
Epilepsy	4	3%
Head injury/Subdural hemorrhage	4	3%
Lewy Body Dementia	3	2.3%
Progressive Supranuclear Palsy	3	2.3%
Corticobasal Syndrome	2	1.5%
Learning Disability (Unclassified)	2	1.5%
Vascular Dementia	1	0.8%
Metabolic (Cushing's syndrome)	1	0.8%
Fainting/ collapse	1	0.8%
Chronic Traumatic Encephalopathy (CTE)	1	0.8%
Total	132	68.4% (of all referrals made to the clinic; no=193)

The majority (69.3%) of EOP cases belonged to the age group of 45- 64 years. The mean educational attainment for this group was 13.8 (SD=4). This was not significantly different statistically from the rest of patients (P= 0.31).

A detailed breakdown of final diagnoses for all patients in whom symptom onset was before the age of 65 is given in figure 4.9A and figure 4.9B.

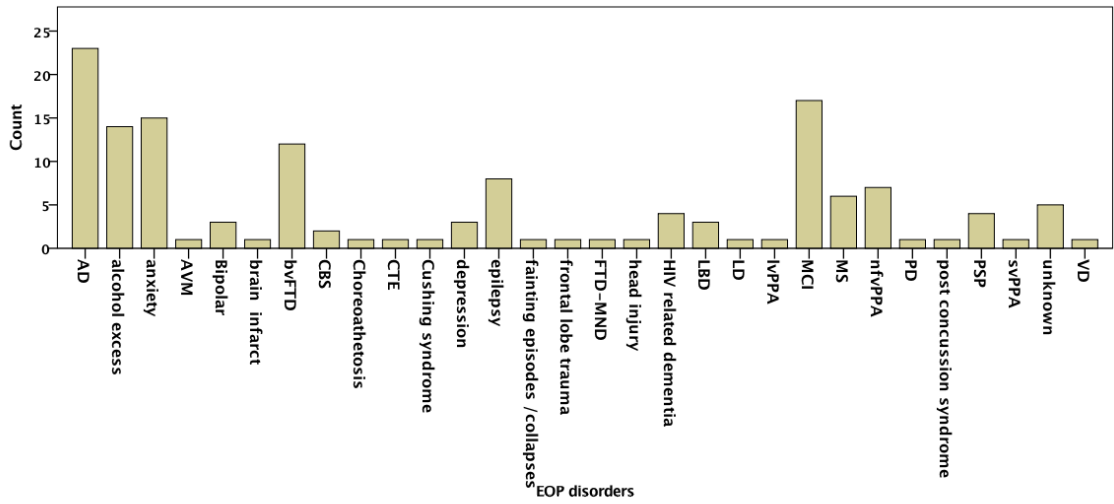


Figure 4.9A: Diagnostic aetiologies of all early onset presentation cases (below the age of 65 years)

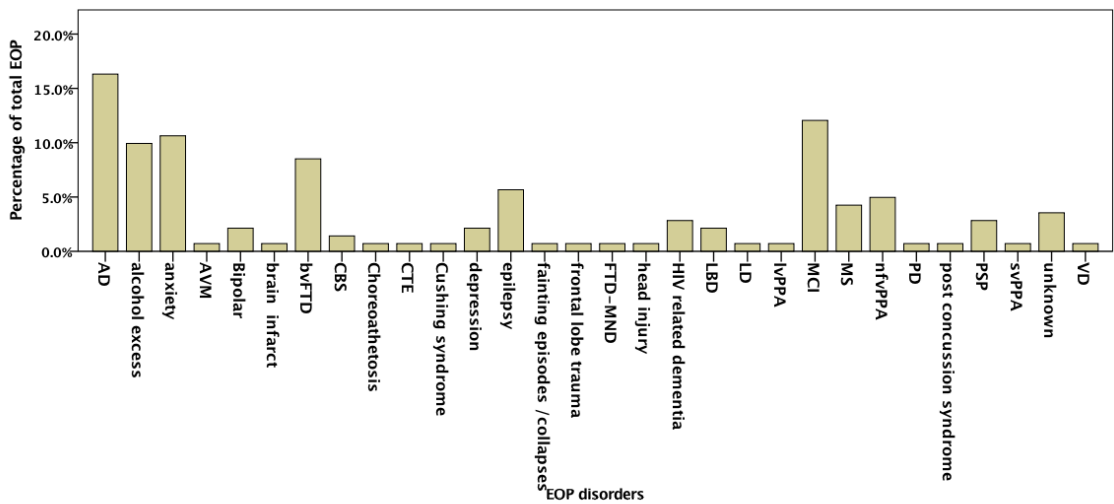


Figure 4.9B: Percentage of diagnostic aetiologies of all early onset presentation cases (below the age of 65 years)

4.4.7 Early-onset neurodegenerative disorders subgroup (EOP-ND)

Early onset neurodegenerative subgroup refers to those with neurodegeneration and symptom onset below the age of 65. This group constituted 39.9% of the total cohort (77 patients). Details of their diagnoses are given in figures 4.10A and 4.10B below.

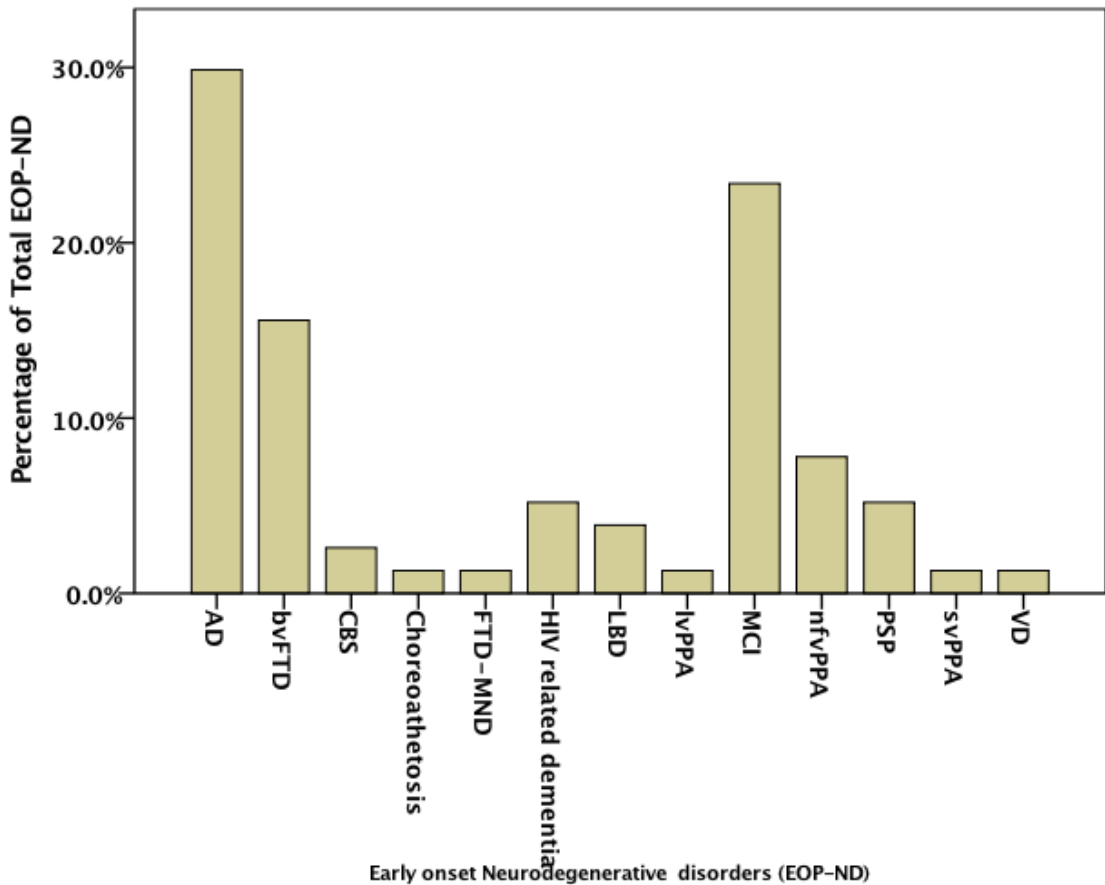


Figure 4.10A: Percentages of diagnostic aetiologies of patients diagnosed with early onset Neurodegenerative disorders (Total of 77 = 100%)

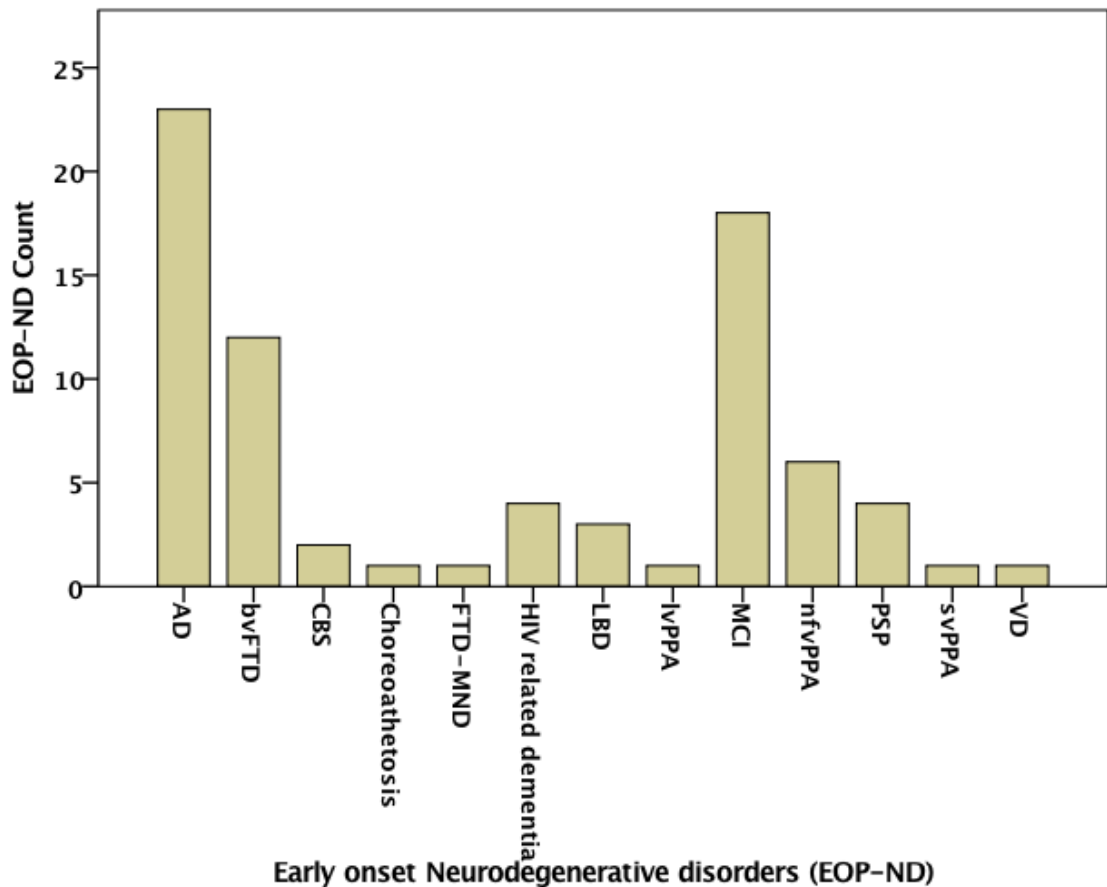


Figure 4.10B: Diagnostic aetiologies of patients diagnosed with early onset Neurodegenerative disorders in numbers (Total of 77)

4.4.8 Primarily amnesic syndrome (AD and MCI)

Alzheimer's disease (AD) and Minimal Cognitive Impairment (MCI) were the most commonly encountered diagnoses across all groups. Primary amnesic syndrome cases constituted 31% (59 cases), 49.7% (59 cases) and 28.8% (38 cases) of overall number of patients, neurodegenerative and early onset subgroups respectively.

4.4.9 Vascular etiologies

Individuals having these diseases did not have evidence of progressive cognitive decline, as one would expect. The relatively low number of patients with vascular dementia may be explained by the referral bias, as patients with cerebrovascular disease are more likely to be referred to either stroke or geriatric clinics.

4.4.10 Autoimmune and inflammatory conditions

Autoimmune and inflammatory conditions, being potentially reversible/treatable, were important causes in this population, with multiple sclerosis accounting for 3% and HIV-related dementia accounting for 2% of patents in our cohort (Table 4.3).

4.4.11 Unknown etiologies

It is of note that, and despite extensive evaluation, no etiologic diagnosis could be established in 4% of the cohort. This finding is not unique to similar groups of patients, as a study conducted by Warren et al in 2005 investigating diagnostic brain biopsy findings in the evaluation of dementia found that the most common biopsy finding for the series as whole in (37%) was nonspecific gliosis, and that 43% of biopsy specimens in the series were nondiagnostic (156).

4.4.12 Frontotemporal dementia

This clinic was one of the two sources of recruitment for this research as mentioned earlier. Findings are detailed in the rest of this thesis. Deep phenotyping of recruited FTD patients was performed by means of clinical (chapter 5), epidemiological (chapter

6), genetic (chapter 7), imaging (chapter 8) and neurophysiologic (chapter 9) characterization.

4.5 Discussion

The study demonstrates the multiplicity of underlying aetiologies in a cognitive clinic population. The information obtained from an accurate diagnostic assessment of the patient is essential to determine the appropriate immediate and/or long-term treatment, to plan further interventions (e.g psychological or social), to counsel the patient and family about possible heredity and to guide health care planning.

The main sources of referral were General Practitioners, Neurologists and the Memory Clinic with smaller proportions of referrals originated from Internists and Psychiatrists.

Our retrospective review identified 193 patients evaluated at the cognitive clinic between January 2013 and December 2015. Reviewing the diagnostic classification of patients attending the clinic, Neurodegeneration predominates, accounting for 62% of presentations.

Of particular importance that the anxious (worried well) and patients with non-progressive conditions were identified; reassured and hence unnecessary further investigations were prevented. A number of patients were diagnosed with potentially treatable disorders and were either given specific treatments or referred to other specialists as appropriate. This group includes those with alcohol excess, epilepsy, psychiatric illnesses and metabolic disorders.

A significant difference in the age of onset was found among the etiologic groups, likely driven by the younger mean age of onset among patients having diagnoses like alcohol excess and autoimmune/inflammatory aetiologies.

A previous population-based study of dementia below the age of 65 (Early Onset Dementia) conducted by Harvey et al estimated the prevalence of Alzheimer Disease and Frontotemporal Dementia at 34% and 12% respectively (157). In comparison (Bearing in mind the different methodologies scopes of the two studies), the figures for this age group in our study were 28.8% for primary amnesic syndrome (AD and MCI combined) and 16.8% for FTD. This relatively higher FTD percentage in our clinic is likely to be due to a referral bias (primary interest of clinic specialists).

The referral bias inherent to performing this study at a hospital setting (clinic-based cohort) precludes our ability to infer frequency analysis and obviously the validity of generalizations from this cohort to the more general population of all patients with cognitive symptoms. To accurately ascertain these figures, a population-based study would be required; however, many factors make such undertaking logistically difficult. Reasons behind this logistic difficulty include the rarity of most of these conditions, the absence of clear diagnostic pathways and the likelihood of missing the diagnosis.

Nevertheless, several important points can be made. Our study described the experience of our cognitive clinic over three years by detailing referral sources and etiological diagnoses of a clinic-based cohort of 193 patients that presented with differing cognitive symptoms. Although no incidence or prevalence estimates could be made because of reasons that we alluded to earlier, our cohort provides a resource examining a large population of patients having this sort of neurologic presentation.

There were some limitations to this study. Firstly, the clinic-based nature of the study precluded prevalence and incidence estimation of various conditions described. Secondly, there was referral bias towards certain conditions like Frontotemporal dementia in view of the primary interest of the clinic as well as the presence of memory clinics to which amnesic cognitive disorders were more likely to be referred. Thirdly, there was no clear long-term follow up plan, diagnoses (including neurodegenerative disorders) were made on clinical grounds and the 'gold standard' confirmation by

autopsy was not contemplated. There were also limitations related to other factors like ascertainment bias as well as the fact that diagnoses were based on chart review.

4.6 Future considerations

Data obtained from this retrospective cohort did not enable us to perform a frequency analysis of various diagnoses or to estimate the prevalence or incidence of various conditions for the reasons mentioned in section 4.5 above. We suggest that a national registry would provide a source by which this population could be better defined and characterized. Such registry could also better characterize the societal burden of such diseases and provide valuable insights into how to diagnose these conditions earlier and how to provide services for affected individuals, their families and carers. Furthermore, the existence of a registry could provide families of such individuals with a valuable support network to address the innumerable personal, social, spiritual, financial and other implications of the development of a progressive neurodegenerative or neuropsychiatric disorder.

We also recommend development of national guidelines and consensus statements on diagnostic evaluation, referral pathways and long-term follow up patients with cognitive disorders.

4.7 Conclusions

By analysing and our clinic's experience, as reported in this chapter, we have shown that cognitive symptoms can be due to a broad variety of aetiologies, with some patients having potentially treatable disorders. The experience presented here demonstrated the importance of such specialist clinic in assessing patients presenting with cognitive symptoms in order to make the correct diagnosis and to guide further

management. In our view, dedicated time, dedicated space, dedicated core team, committed expertise of other disciplines and effective links with other agencies are essential attributes to best outcome.

Data generated by this study can serve as a foundation for future population-based studies in order to gather accurate epidemiological data to support better health service planning and delivery (for instance; development of specialised support services, day care and care homes for this group of patients).

The FTD cohort constituted our primary interest in this research. A summary of results from clinical phenotyping and studies of biomarker profiling of frontotemporal dementia patients is given in the next chapter (chapter 5).

5 Chapter 5: Results and Discussion of study 2: Clinical phenotyping of FTD patients

5.1 Introduction

Frontotemporal Dementia (FTD) is a neurodegenerative disorder affecting the brain. It is the third most common type of dementia after Alzheimer Disease and Vascular Dementia. Recent advances that have been made in the field of FTD pathophysiology and disease mechanisms revived interest in this condition and prompted systematic research.

A succinct account outlining aims and summary of findings of FTD phenotyping studies carried out in this research is given in this chapter while in depth descriptions of specific studies are detailed in subsequent chapters.

5.2 Aims

The main aims of this study were:

1. To perform deep phenotyping of a clinic-based FTD cohort in Ireland.
2. To perform detailed biomarker profile of patients with FTD in Ireland. This is through performing clinical characterization, and the generation of a DNA, neuroimaging and neurophysiological repository.

Specific objectives:

1. To characterize the differing biomarker profiles of different subtypes of FTD, including FTD-ALS and to compare diagnostic breakdown, clinical and epidemiological characteristics, Montreal Cognitive Assessment scores and neurophysiologic characteristics of FTD subtypes.

2. To perform a detailed family aggregation study to assess for aggregation of neurodegenerative and neuropsychiatric conditions in kindreds of patients with FTD. The aim here is to determine whether neurodegenerative and neuropsychiatric conditions aggregate in kindreds of patients more than in relatives of healthy controls.
3. To perform genetic analysis of DNA samples collected from participants in order to detect the presence of any known FTD gene or genes mutations.
4. To conduct a multiparametric neuroimaging (MRI) study across the FTD-ALS spectrum with the view of characterizing imaging signatures or MRI patterns of FTD phenotypes along the FTD-ALS spectrum using multiple complementary imaging techniques and to correlate genetic status with advanced neuroimaging and to compare FTD with ALS-FTD.
5. To accomplish Motor Unit Number Index (MUNIX) quality improvement and comparative study. This is to be achieved through compiling normative data repository and collecting MUNIX longitudinal data from three cohorts of patients: FTD, ALS and Poliomyelitis so as to determine the rate of MUNIX decline over time and to compare this rate with established parameters of disease progression.

5.3 Results

5.3.1 FTD cohort clinical and epidemiological characteristics

A total of 55 FTD patients were enrolled in this research during the study period. The vast majority of patients were recruited either from either the neurodegeneration clinic in Beaumont Hospital (29 patients) or St James's Hospital cognitive clinic (23 participants). Only 3 patients were recruited from other sources outside those two clinics (2 participants from the Mater Hospital, Dublin and 1 participant from Northern Ireland). Analysis of the referral sources revealed that 35 patients were referred by

Neurologists while 10 patients were referred by Internists and the memory clinic (5 from each source). 7 and 3 cases were received from General practitioners and Psychiatrists respectively.

Behavioural-variant Frontotemporal Dementia (bvFTD) represented one third of all cases. Other FTD subgroups recruited included both (pure) FTD as well as overlap syndromes: Semantic-variant Primary Progressive aphasia (svPPA), Nonfluent-variant Primary Progressive Aphasia (nfvPPA), Frontotemporal Dementia-Motor Neurone Disease (FTD-MND), Progressive Supranuclear Palsy (FTD-PSP) and Corticobasal Syndrome (FTD-CBS). Number and percentages of participants in each diagnostic subgroup is shown in table 5.1.

Table 5.1: Breakdown of FTD participants showing numbers and percentages of patients recruited in each diagnostic subgroup

FTD diagnostic subgroup	Frequency	Percentage
Behavioural variant frontotemporal dementia (bvFTD)	18	32.7%
Semantic variant Primary Progressive Aphasia (svPPA)	2	3.6%
Nonfluent variant Primary Progressive Aphasia (nfvPPA)	13	23.6%
FTD-Motor Neurone Disease (FTD-MND)	15	27.3%
FTD-Progressive Supranuclear Palsy (FTD-PSP)	4	7.3%
FTD-Corticobasal Syndrome (FTD-CBS)	3	5.5%
Total	55	100%

Participants underwent

- Clinical and epidemiological evaluation (all participants).
- Detailed family history analysis (48 patients).
- Genetic analysis that included C9ORF status and testing for other known FTD genes (51 participants).
- Magnetic Resonance Imaging (MRI) scanning (30 participants from this cohort, 100 participants in total): 7 bvFTD, 11 nfvPPA, 2 svPPA, and 10 C9orf negative FTD-MND participants.

70 more participants were added (10 C9orf positive FTD-MND, 20 ALS without behavioural or cognitive deficits (ALSnci) and 40 healthy controls (HC) to complete 100 participants.

- Neurophysiological profiling by performing Motor Unit Number Index Estimation (MUNIX) longitudinal testing (39 patients).

Added to this number was 43 ALS, 41 Poliomyelitis and 40 healthy controls, i.e. a total number of 163 participants. Healthy controls were tested once while all three disease groups underwent longitudinal MUNIX testing 3 monthly for 2 years (8 visits in total).

5.3.2 Diagnostic subgroups

Table 5.1 details the breakdown of participants' diagnostic subgroups by numbers and percentages.

Patients diagnosed as having behavioural variant frontotemporal dementia (mean age of 59.5 years, SD 7.5) and semantic variant primary progressive aphasia (mean age of 56.5 years, SD 2.1) had an earlier age at onset than patients diagnosed as having nonfluent variant primary progressive aphasia (mean age of 67.5 years, SD 6.9). The final diagnoses were reached after thorough evaluation that comprised clinical assessment of symptoms and signs by a neurologist at the clinic, full neuropsychological assessment by a clinical neuropsychologist, laboratory and imaging investigations that were essential to exclude alternative diagnoses and/or FTD mimics. Patients underwent full neuropsychology assessment whenever possible as part of their clinical evaluation while MOCA testing was performed at least twice in all participants, at initial evaluation and at a later stage. Results of MOCA testing are discussed in section 5.3.8 and presented in table 5.4 below.

5.3.3 Age at symptom onset

Age at symptom onset for the cohort ranged from 47 to 89 years with the mean (\pm SD) of 64.5 years (\pm 8.3). Comparison of onset ages between (pure) FTD subgroups showed that nfvPPA patients were older at symptom onset (mean 67.5 \pm 6.9 years) than both bvFTD (mean 59.5 \pm 7.5 years) and svPPA (mean 56.5 \pm 2.1 years). Comparison of differences in age at symptom onset between the three (pure) FTD subgroups of bvFTD (32.7%), nfvPPA (23.6%) and svPPA yielded P value of 0.452 for bvFTD vs nfvPPA and P value of 0.096 for svPPA vs nfvPPA). There was no statistically significant difference between bvFTD vs. svPPA (P=0.096).

5.3.4 Symptom onset to diagnosis

Patients diagnosed as having FTD-MND presented earlier than other groups as they had the shortest symptom onset to diagnosis period (14 \pm 7.4 months) while bvFTD patients had the longest delay period (32 \pm 16.9 months). The delay times to diagnosis for the subgroups of svPPA, nfvPPA, FTD-PSP and FTD-CBS were 23 \pm 17.8 months, 23 \pm 12.4 months, 24 \pm 14.4 and 20 \pm 6.7 months respectively. The overall mean was 24 \pm 16.5 months.

5.3.5 Gender

A logistic regression analysis of proportion of males to females yielded no significant difference in gender by diagnostic subgroups (Pearson $X^2=76.124$, P=0.74). There were more males in the bvFTD subgroup (M: F = 11:7) in comparison to nfvPPA (4 males and 9 females). (Table 5.2).

Table 5.2: Gender distribution of FTD subgroups (Total=55 patients).

		Diagnostic subgroup						Total
		bvFTD	svPPA	nvPPA	FTD-MND	FTD-PSP	FTD-CBS	
Sex	Female	7	1	9	10	0	1	28
	Male	11	1	4	5	4	2	27
Total		18	2	13	15	4	3	55

5.3.6 Educational attainment

Education ranged from 8 to 20 years. 43.6% of patients in the cohort received primary education and 38.2% achieved secondary education. Only 16.4% reached tertiary level of education while one bvFTD patient was missing education.

There was no significant difference in educational attainment (educational level, years in education) per subgroups (Chi Square value of 76.306, DF=65, P=0.159).

Table 5.3 details the highest level of education reached by participants in each of the subgroups.

Table 5.3: Educational attainment of participants in each of the 6 FTD diagnostic subgroups

		FTD Diagnostic subgroup						Total
		bvFTD	svPPA	nfvPPA	FTD-MND	FTD-PSP	FTD-CBS	
education attainment: primary/secondary/ter tiary	No Education	1	0	0	0	0	0	1
	Primary	10	0	2	10	1	1	24
	Secondary	3	2	10	3	1	2	21
	Tertiary	4	0	1	2	2	0	9
Total		18	2	13	15	4	3	55

5.3.7 Occupation

Participants did a number of different occupations but as a general trend most males earned living by either working in multiple different jobs (mainly manual labour jobs) or were unemployed while most women were housewives.

5.3.8 Montreal Cognitive Assessment (MOCA) scores:

All participants underwent Montreal Cognitive Assessment (MOCA) testing at least twice, once at initial evaluation (at their first clinic visit) and further testing at later visit(s). Data of the first (at time of diagnosis) and last available MOCA was analysed and FTD subgroups were compared as regards to the following metrics: symptom onset to first MOCA/ scores at diagnosis/ last available MOCA scores/ time interval between MOCA1 to MOCA2 and monthly rate of MOCA decline).

Symptom onset to MOCA1 duration was significantly shorter for FTD-MND subgroup participants but otherwise analysis of variance did not suggest significant differences between subgroups i.e. none of above metrics reached the level of statistical significance when subgroups are compared ($P=0.59$).

Table 5.4 shows details of MOCA scores at diagnosis, symptom onset to first MOCA test, time intervals between first and last MOCA test, rate of decline of MOCA per

month; overall and for each of the 6 subgroups of: bvFTD, svPPA, nfvPPA, FTD-MND, FTD-PSP, FTD-CBS.

Table 5.4: Details of MOCA scores at diagnosis, symptom onset to first MOCA test, time intervals between first and last MOCA test, rate of decline of MOCA per month; overall and for each of the 6 subgroups of: behavioural variant frontotemporal dementia (bvFTD), semantic variant Primary Progressive Aphasia (svPPA), nonfluent variant PPA (nfvPPA), FTD-MND, FTD-PSP, FTD-CBS. MND=Motor neurone Disease. PSP=Progressive Supranuclear Palsy, CBS=Corticobasal syndrome.

FTD Diagnostic group	Symptom onset to MOCA1 (months): mean(SD)	MOCA points dropped per months: mean(SD)	MOCA1 score: Mean(SD)	MOCA2 score: Mean(SD)	MOCA1-MOCA2 duration (months): Mean(SD)
Overall	24 (16.5)	4 (1.4)	16 (4.9)	13 (4.7)	15 (3.7)
bvFTD	32 (16.9)	5 (1.6)	17 (5.0)	13 (4.4)	15 (4.5)
nfvPPA	23 (21.0)	4 (1.2)	16 (5.5)	12 (6.0)	16 (2.9)
svPPA	23 (17.7)	6 (0.0)	16 (0.7)	10 (0.7)	18 (0.0)
FTD-MND	14 (7.4)	3 (0.9)	14 (4.7)	12 (4.3)	11 (2.0)
FTD-PSP	24 (14.4)	4 (1.7)	16 (6.0)	12 (4.5)	14 (3.5)
FTD-CBS	20 (6.7)	4 (1.2)	20 (1.2)	16 (2.1)	14 (3.5)

5.3.9 Summary of results of studies performed as part of this research

5.3.9.1 Family aggregation study

A detailed family aggregation study was conducted on 45 kindreds of FTD cases and 71 families of sex and age-matched controls. Data were available from 996 first- and second-degree relatives of cases and 1741 first- and second-degree relatives of controls. The same methodology and protocol previously detailed in chapter 4 were followed for recruiting cases and controls and full results are presented in chapter 6 of the results section of this thesis. In summary, we found that 40% of cases reported a family history of FTD. This included 11% of cases that reported a family history consistent with an autosomal dominant mode of inheritance. Aggregation of Schizophrenia, Learning disability, Suicide and MND occurred significantly higher in kindreds of cases in comparison to the controls. Detailed description of results of the family aggregation study is written in chapter 6.

5.3.9.2 Genetic analysis results

Of the 51 banked DNA samples collected from research participants, 2 patients (4%) tested positive for C9orf72 mutation having the characteristic appearance of a GGGGCC hexanucleotide expansion (30 or more repeats) consisting of a decaying series of 24 or more peaks. Both patients were from the FTD-MND phenotype.

All samples were also tested for the presence of other known FTD genes and there were no significant pathogenic mutations in our cohort of 51 FTD patients, which are reported in the literature within the genes tested. See details in chapter 7.

5.3.9.3 Imaging (MRI) results

Phenotype-specific patterns of grey matter atrophy were evaluated using both whole-brain voxel-wise statistics as well as region-of-interest analyses. White matter

alterations were explored using multiple diffusivity indices, radial diffusivity, axial diffusivity and fractional anisotropy.

Details of this comparative imaging study are presented in chapter 7 but in a summary, we demonstrated in this study that ALS-FTD patients who tested negative for hexanucleotide repeats in C9orf72 had considerable extra-motor frontotemporal pathology when compared to C9orf72 positive patients. We also showed that the clinical manifestations of FTD were underpinned by phenotype-specific patterns of white and grey matter degeneration. Our study also confirmed the descriptive role of multi-parametric quantitative neuroimaging in FTD. Details in chapter 8.

5.3.9.4 Neurophysiologic evaluation (MUNIX) results

We reached two main findings by performing a two-year longitudinal MUNIX study in FTD patients (Total of 39 participants; 22 bvFTD, 9 nvPPA, 6 FTD-MND & 2 svPPA):

Firstly, there was no decline in MUNIX values over the study period in nvPPA and svPPA patients.

Secondly, in bvFTD patients some decline in MUNIX values was seen in 3 patients out of 22 (13.6% bvFTD of patients). Moreover, the rate of decline in MUNIX values for those 3 patients was much less than that seen in ALS patients.

Of note, the pattern of decline of MUNIX in FTD-MND subgroup is detailed in the ALS section (section 10.2). MUNIX study details are discussed in **chapters 10** of this thesis.

5.4 Discussion

We summarized the demographic characteristics of our FTD participants. Only clinical characterisation was described in this chapter. Findings of family aggregation study is presented in the next chapter (chapter 6), genetic profile is detailed in chapter 7 while

imaging is described in chapter 8 and findings of the neurophysiological study (MUNIX) is described in chapter 9 of this thesis.

BvFTD was the commonest diagnostic subgroup accounting for about one third of the cohort. In congruence with previously published work, there were significant differences in diagnostic breakdown and age at symptom onset between FTD subgroups as nfvPPA patients were significantly older at presentation when compared to those to either bvFTD or svPPA (229).

Regarding symptom onset to diagnosis, bvFTD patients had the longest delay period (mean 32 ± 16.9 months) in comparison to other subgroups in our cohort. This duration is shorter than previously published delay time in the seminal FTD epidemiology papers, for instance; Rosso et al reported mean duration of symptoms at ascertainment for two FTD cohorts from the Netherlands between 3.7 years and 4.3 years (96). We ascribe this difference to the disparity in design between the two studies (clinic-based vs population based), as well as the fact that the study by Rosso et al was conducted in 2003 when FTD awareness was much lower.

We did not detect significant differences among subgroups as regards to gender, symptom onset to diagnosis (slightly shorter duration for the FTD-MND subgroup), educational attainment or MOCA metrics.

While full neuropsychological assessment was performed by a neuropsychologist during clinical diagnostic workup, the Montreal Cognitive Assessment (MOCA) testing was performed systematically on all participants at least twice as part of this research. MOCA was introduced as a brief cognitive screening test for AD and MCI in 2005 with a global total cut off score of ≤ 25 (238). It has been validated in 2012 as a cognitive screening test for bvFTD in Portuguese population by Freitas et al (239). In this validation study performed by Freitas et al, MOCA displayed high levels of sensitivity (78%), specificity (98%), Positive predictive value (98%), Negative Predictive Value (82%) and classification accuracy of 82% for bvFTD at a cut-off point of < 17 (239).

Kristy et al (240) examined the overall and standard item performance on the MOCA for patients with the 6 FTD subtypes of bvFTD, svPPA, nvPPA, PSP, CBS, and FTD-MND: the standard cut-off point of ≤ 25 successfully detected cognitive impairment in 87.2% of patients who were ultimately diagnosed with FTD patients (240).

A number of reasons (for instance, different population characteristics, the clinical heterogeneity of FTD cohorts and methodological differences) preclude both generalization of results of our study or comparing those results to previously discussed studies.

Disease severity measurement tools are needed before further studies examining the correlation between MoCA scores and disease severity scores among FTD subtypes can be carried out to aid comparison of MoCA scores across disorders at similar disease severities and to determine whether MoCA can serve as a proxy measure for disease severity.

Our finding of decline in MUNIX values on longitudinal testing in 13.6% (3/22) of bvFTD patients is dissimilar to a previous needle EMG study conducted in FTD patients by Lomen-Hoerth and colleagues (136). In the study by Lomen-Hoerth and colleagues it was reported that 33% of FTD patients showed EMG changes in the ALS range after one year follow up. Whether this disparity was due to overestimation by the other study or relatively more sensitivity of conventional needle EMG in comparison to MUNIX needs to be determined in future research.

5.5 Conclusions

Deep phenotyping of FTD provides a rich data source for biomarker profiling and genotype-phenotype correlations. We performed deep phenotyping of a clinic-based cohort of FTD patients but our findings need to be replicated by studying larger cohorts across research centers using different strategies to further explore

epidemiological, imaging, physiological and biological features of various FTD subtypes.

Full account of the family aggregation study that described the pattern of aggregation of neurodegenerative and neuropsychiatric disorders in participants' kindreds is given in the next chapter (chapter 6).

6 Chapter 6: Results and Discussion of study 3: Family aggregation study of FTD patients

6.1 Introduction

The previous 2 chapters described the experience of our cognitive clinic over the research period and gave an overview of FTD phenotyping investigation plan as well as a brief account on main findings of FTD studies that we carried out.

A through family aggregation study could also seek to determine the true rate of aggregation of neurodegenerative and neuropsychiatric conditions in FTD kindreds.

The rate of aggregation of some of those diseases among first-degree relatives of patients with FTD has been reported in only few previous family aggregation and case studies, however, none of these studies determined the rate of aggregation of all conditions that studied in this research.

6.2 Aims

The primary aim of this study was to carry out a comprehensive family aggregation study, comparing family members of patients with FTD to family members of controls. This would identify neurodegenerative and neuropsychiatric conditions that occurred at a higher degree among family members of people with FTD compared to relatives of controls. Such a method could divvy the risk to other family members and inform future genetic studies.

Specific aims:

1. To determine familial recurrence risk (λ_R) for neurodegenerative diseases (ALS, Alzheimer Dementia, Parkinson disease) and neuropsychiatric diseases (schizophrenia, depression, learning disability and suicide) among relatives of FTD patients,

2. To stratify this risk according to the absence or presence of any genetic mutation in the proband.
3. To determine the rate of familial FTD and the mode of inheritance in those familial cases.
3. To use information collected to direct future genetic studies.

To achieve these aims, a family aggregation study was carried out where incident and prevalent FTD patients in the three-year study period 2013-2015 were recruited from both St James's hospital cognitive clinic and Beaumont hospital neurodegenerative clinic, both in Dublin, Ireland and a detailed family history taking technique was used to identify all neurodegenerative and neuropsychiatric conditions in the families. The same data was collected from age and sex matched controls. Comparisons were made between cases and controls including family history, family structure and size and epidemiological data.

6.3 Results

6.3.1 Recruitment of FTD patients

In the three-year study period from January 2013 to December 2015, 55 people were diagnosed with FTD at our two clinics and were recruited in the study: 18 bv-FTD, 13 nfvPPA, 2 svPPA, 15 FTD-MND, 4 FTD-PSP and 3 FTD-CBS. All patients were approached and were asked to take part in this family history study.

Of 55 FTD patients contacted, 50 agreed to be included in the study and 5 patients declined participating. 45 completed the questionnaire and the questionnaire was not returned by 5 participants. This gave a response rate of 82% (figure 6.1).

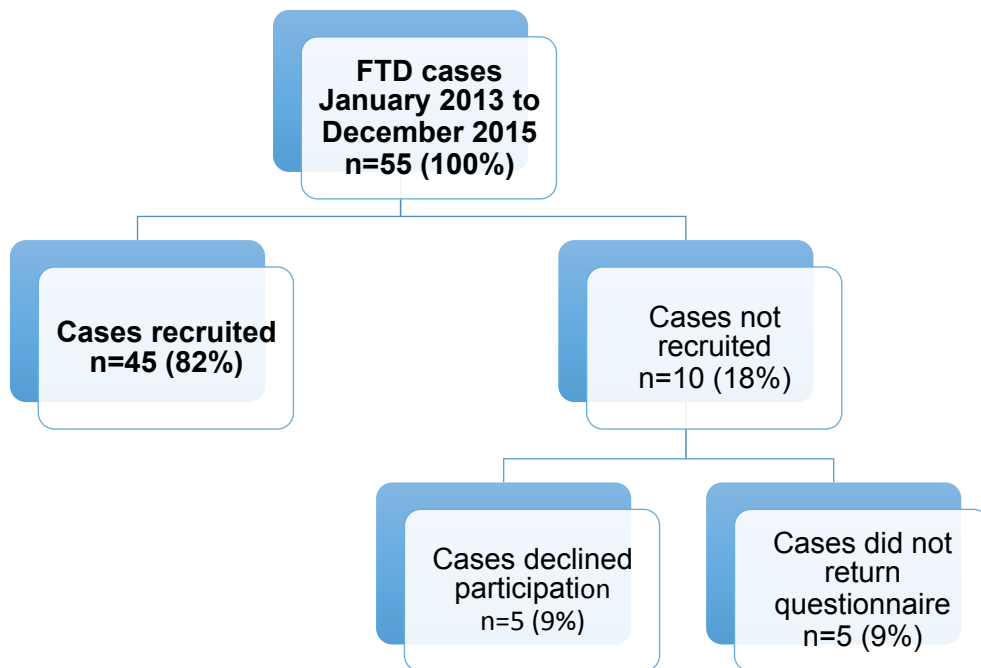


Figure 6.1: Flow chart showing recruitment to the family aggregation study

There were no differences between those who completed the questionnaire and those who declined in terms of gender, age at onset or symptom onset to diagnosis. Table 6.1 compares baseline characteristics of between those who completed a family history questionnaire and those who did not.

Table 6.1: Demographic details of those patients who participated in the family aggregation study versus those who did not participate in the study

	Participated in the study (n=45)	Did not participate in the study (n=10)	
Onset age (years)	60.6 (SD7.1)	60.4 (SD7.2)	P=0.89

Onset to diagnosis (months)	23.2 (SD9.7)	23.0 (SD9.8)	P=0.83
Gender	22 Females (49%)	5 Females (50%)	P=0.86

6.3.2 Recruitment of controls

Controls were matched for age (within 1 year) and gender. 98 healthy population-based controls, without neurological diseases, were contacted through patient general practitioners, and 71 returned questionnaires and participated in semi-structured interviews giving a response rate of 72.4%. All returned control questionnaire were included in analysis.

6.3.3 Quality of data collected

Family histories were rated as having complete data capture, good data capture, incomplete data capture and poor data capture (table 6.2). Data collected from controls yielded higher grades of complete data collection and lower rates of incomplete data collection ($X^2=19.0$, $P<0.001$).

Table 6.2: Comparisons of qualities of family histories collected between cases and controls

	Cases n= 45	Controls n= 71
Complete data capture (all 1st & 2nd degree relatives)	20 (44.4%)	38 (53.5%)

Good data capture (most 1st & 2nd degree relatives)	16 (35.6%)	28 (39.4%)
Incomplete data capture (have 1st degree & 2nd degree relatives)	8 (17.8%)	5 (7.1%)
Poor data capture (data on 1st degree relatives only)	1 (2.2%)	0 (0%)

6.3.4 Demographic comparison between cases and controls

Family history information on 45 patients and their families and 71 controls and families was collected. 49% percent (22 of 45) of the cases were females, compared to 51% (36 of 71) of the controls ($P= 0.86$). Patients and controls were equally matched, with a mean age of 60.6 years (SD 7.1 years) in patients and 60.3 years (SD 8.9 years) in controls at the time of family history data collection ($p=0.53$). Female cases had a mean age of 62.6 years (SD 8.6 years), compared to 61.4 years (SD 9.7 years) in controls ($P=0.51$). Males were similarly matched, with a mean age of 58.8 years (SD 10.4 years) in cases and 59.1 years (SD 11.5) in controls ($P= 0.87$).

Complete data were available for 1059 first- and second-degree relatives of 45 cases and 1813 first- and second-degree relatives of 71 controls.

6.3.5 Number of first-and second-degree relatives

Initial information was available on 1059 first-and second-degree relatives of 45 cases (44 pedigrees) and 1813 relatives of 71 controls (71 pedigrees). 63 relatives of cases and 72 relatives of controls were excluded from analysis (only available information

was gender). Therefore, analysis was carried out on 996 first-and second-degree relatives of cases and 1741 first-and second-degree relatives of controls. Table 6.3 details the breakdown of number of each category of relatives and comparison of the mean ages for different categories of relatives between cases and controls.

Table 6.3: Breakdown of numbers of relatives and comparison of mean ages for different categories of relatives between cases and controls

		Number of relatives of cases	Number of relatives of controls	Mean ages for relatives of cases	Mean ages for relatives of controls	P values for age differences
1 st degree relatives	Offspring	100	162	37.2	35.4	P=0.008
	Siblings	181	249	60.4	59.4	P=0.005
	Parents	82	119	74.3	74.3	P=0.999
2 nd degree relatives	Aunt	111	237	68.6	69.7	P=0.254
	Uncle	109	241	69.4	70.1	P=0.12
	Grandmother	50	105	73.2	73.6	P=0.672
	Grandfather	48	102	71.6	69.8	P=0.438
	Niece/Nephew	315	526	36.5	33.7	P=0.001
	Total	996	1741	60.6 years	60.3 years	P=0.51

6.3.6 Causes of death

Forty two per cent (418) of relatives of cases and 44% (766) relatives of controls were deceased (P=0.007). Causes of death were split into eight categories as follows: cardiac, neurological, respiratory, gastro, cancer, stroke, other and unknown (table 6.4).

Table 6.4: Causes of death by category

		Relatives of cases n= 409	Relatives of controls n= 761
Cardiac	Count	108	204
	% Within group	26.4%	26.8%
Neurology	Count	60	98
	% Within group	14.8%	12.9%
Respiratory	Count	39	85
	% Within group	9.5%	11.2%
Gastro	Count	2	16
	% Within group	0.6%	2.1%
Cancer	Count	90	146
	% Within group	22.1%	19.2%
Other	Count	83	71
	% Within group	20.3%	9.3

Unknown	Count	26	65
	% Within group	6.3%	8.5%
Total	Count	409	761
	% Within group	100%	100%

The commonest cause of death was cardiac, which was equal in both groups; 26.45 compared to 26.85. The death due to cancer was slightly higher among relatives of FTD cases compared to relatives of controls (22.15 compared to 19.2%).

6.4 Results of aggregation study: Rate of familial FTD

Of 45 FTD cases, 18 cases (40%) reported a family history of FTD. This included 5 patients (11%) who reported a family history consistent with an autosomal dominant mode of inheritance fulfilling score 1 of the criteria proposed by Goldman's et al (defined as the presence of at least 3 affected people in 2 generations with 1 person being a first-degree relative of the other 2). (230). This resulted in a familial FTD rate of 40% and an autosomal dominant inheritance in 11% of our cohort.

Two healthy controls (2.8%) reported a family member with FTD (1 second-, and 1 third-degree relative). Hence, 1.4% (1 of 71) of healthy controls had a second-, and 1.4% (1 of 71) had a third-degree relative with FTD, and no healthy control had a first-degree relative with FTD.

It was beyond the scope of this research to calculate the lifetime HR of developing FTD for probands or controls, as our study was not population-based.

6.5 Results of Aggregation study: Neuropsychiatric and Neurodegenerative diseases

6.5.1 Analysis to look for effects of case clustering in families

The number of cases of Alzheimer dementia, PD, MND, Schizophrenia, suicide, depression, learning disability and Personality disorder in each family, for cases (n=45 families) and controls (n=71 families), was compared visually using a histogram to ensure that there was no evidence of clustering which may have driven results.

Binary logistic regression in a generalized linear model was used to compare the frequency cases for each of above diseases separately in relatives of cases and controls, taking into account the individual kindred size. No significant differences found between cases and controls (e.g $P= 0.143$ for AD), suggesting that there was no statistical evidence for a cluster effect.

6.5.2 Analysis results

This study compared the rates of neuropsychiatric and neurodegenerative disorders in 996 first-and second-degree relatives of 45 clinic-based FTD cases and 1741 first-and second-degree relatives of 71 age-and sex-matched controls. Analysis of the rates of those disorders in family members was carried out on the entire cohort. A number of Neuropsychiatric and Neurodegenerative illnesses were over-represented in kindreds from the FTD cohort.

The relative risk and hazard rate of each of conditions studied are reported in table 6.5.

Table 6.5: Comparisons of relatives of cases and controls, demonstrating a significant relative risk of Schizophrenia, Learning disability, suicide and MND in relatives of FTD patients compared to relatives of controls

	Relatives of cases n=996	Relatives of controls n=1741	Risk ratio	X², P-value	Hazard rate	P-value	95% CI
Frontotemporal dementia	9 affected	1 affected	15.7	9.6, P=0.000	15.9	P=0.000	2.6-12.1
Schizophrenia	3 affected	1 affected	4.3	5.4, P=0.0031	4.8	P=0.0003	1.5-12.3
Depression	6 affected	10 affected	1.1	0.1, P=0.0892	1.1	P=0.685	0.7-1.6
Learning disability	3 affected	1 affected	4.3	5.4, P=0.0031	4.8	P=0.0003	1.5-12.3
Suicide/attempted suicide	4 affected	1 affected	6.7	9.8, P=0.0002	6.9	P=0.000	2.5-13.4
Alzheimer disease	36 affected	59 affected	1.1	1.4, P=0.256	1.2	P=0.053	0.9-1.4
Motor neurone diseases	3 affected	1 affected	4.3	5.4, P=0.0031	4.8	P=0.0003	1.5-12.3
Parkinson disease	6 affected	11 affected	1	0.3, P=0.897	0.8	P=0.299	0.5-1.3

The relative risk of schizophrenia in relatives of FTD patients in our study was 4.3. This result was similar to a previous family aggregation study conducted Schoder et al. Schoder et al reported in their study an increase morbid risk for Schizophrenia in relatives of FTD patients compared to relatives of patients with Alzheimer's disease

(155): They reported a relative risk of Schizophrenia of 4.1 among 741 first-degree relatives of hundred FTD patients.

The rate of FTD in relatives of cases was even higher (HR 15.9, P=0.000, CI 2.6-12.1).

The similarly high rate of familial FTD in the cohort was discussed in section 5.5 above.

A family history of depression was not increased in relatives of FTD patients in comparison with relatives of controls (discussion section of this chapter).

A family history of suicide was also more frequent in families from the FTD cohort, with a high risk of death by suicide in their first- or second-degree relatives with HR of 6.9 (95% CI 2.5-13.4, $p < 0.0001$; Table 6.5). The previous paper by Schoder and colleagues reported that up to 70% of all schizophrenic patients attempt suicide on at least one occasion, with 15% succeeding (155). The timing of suicide in relatives of FTD patients in our cohort was independent of the timing of the diagnosis of FTD and in nearly all cases preceded the onset of FTD in another relative.

Compared to controls, the relative risk of Alzheimer dementia in first- and second-degree relatives in the FTD cohort was 1.1 ($p=0.256$), and the HR was 1.2 ($p=0.053$). Therefore, relatives of cases had a higher rate of Alzheimer dementia but this increase was not statistically significant. However, it was noticed that relatives of patients with FTD developed Alzheimer dementia at a younger age than relatives of controls.

The rate of ALS was also increased in relatives of cases in comparison to relatives of controls (HR 4.8, P=0.0003, CI 1.5-12.3).

Parkinson disease was prevalent in relatives of both FTD and control cohorts, with no significant difference between the 2 groups.

6.6 Discussion

6.6.1 General points

Our study, which is a family aggregation study of 116 FTD cases and controls, as designed to establish the rate of FTD, ALS, PD, AD, Schizophrenia, Learning disability, Depression and Suicide in 2737 first- and second-degree relatives.

This cohort was clinic-based that comprised incident and prevalent FTD cases over a three-year study period and was well matched with the control group.

6.6.2 Aggregation of Neuropsychiatric and Neurodegenerative disorders

The study compared the presence or absence of neuropsychiatric and neurodegenerative disorders in 996 first- and second-degree relatives of 45 FTD cases and 1741 first- and second-degree relatives of sex-and age-matched controls.

It showed that neuropsychiatric and neurodegenerative conditions occur at a higher rate in kindreds of FTD patients when compared to families of normal healthy controls.

With respect to the rate of Alzheimer's dementia, our data indicated that relatives of FTD patients have a slightly elevated risk (risk rate of 1.2) when compared to relatives of controls. A nationwide family aggregation population-based Dutch study concluded that 10% of FTD probands had two or more first-degree relatives with dementia, but only five (0.9%) of the control subjects had two or more affected first-degree relatives (10). The differences in results between this study and our study may be attributed to the two different methodologies and study populations.

The high rate of familial FTD reported in this research is congruent with previously published literature (11,12, 231). Byrne et al (243) reported comparably higher rates of neuropsychiatric conditions in kindreds of patients with ALS in comparison to relatives of controls, lending support to the theory of shared biological factors among conditions in the FTD-ALS spectrum.

Specifically, we have demonstrated an increased aggregation of schizophrenia, learning disability and suicidal behavior in families of FTD patients. To date, there are no studies have been undertaken to determine the rate of FTD within the schizophrenia population. Notwithstanding, these higher rates of schizophrenia and psychiatric conditions in FTD kindreds may suggest the possibility that some forms of FTD are neurodevelopmental in origin. While FTD and Schizophrenia are both neurobehavioral syndromes characterized by an alteration in social interaction with varying degrees of frontal dysfunction, the two syndromes differ in that the age of onset is markedly younger in Schizophrenia, the course is more variable, and hallucinations and delusions are prominent features, compared with an older age of onset with progressive decline in FTD, and delusions reported much less commonly. Because of similarities between FTD and Schizophrenia Schoder and colleagues (155) examined each relative diagnosed with Schizophrenia, wherever possible, and confirmed that these relatives did indeed had Schizophrenia rather than a misdiagnosed FTD case. They also reported two cases where Schizophrenia and FTD segregated with PRGN and VCP mutations, both of which are associated with FTD. None in our study tested positive for either of these genes. The overlap independently identified between ALS and FTD (104, 106), FTD and Schizophrenia (155) and ALS and Schizophrenia is compelling evidence for a link between these conditions. The reasons for this link are likely to be varied; There may be a common gene resulting in different phenotypes; there may be a common pathological mechanism that result in different phenotypes depending on interaction with other genes or environment; or these diseases may simply result from an abnormal protein aggregates mediated by a similar mechanism but depositing in regions that specifically cause anterior horn cell dysfunction (ALS), frontal lobe dysfunction (FTD) or altered synaptic connectivity (Schizophrenia).

In addition to schizophrenia, we have found an increased rate of suicide among relatives of FTD patients. The timing of suicide in relatives of FTD patients in our study

was independent of the timing of the diagnosis of FTD and in almost all cases preceded the onset of FTD in a relative. Despite the increased rate of suicide among relatives of patients with FTD, the rate of depression was not increased. This observation may suggest that suicides in this group were related to impulsivity rather than altered mood state. Of note here is that it was not possible to access the detailed clinical notes of those who died by suicide to confirm whether this was the case. We propose the same explanation for the reported increased rate of cigarette smoking and excessive alcohol consumption. Of note, this study did not specifically look at addiction among relatives of FTD patients.

A disproportionate number of FTD patients in this study reported that they had relatives who were “very odd”, or “spent many years in psychiatric hospitals”, or “disappeared in their twenties/thirties”. None of these were classified as particular condition as the diagnosis was unknown, however, and as we have shown in this family aggregation study, there is definite evidence for aggregation of psychiatric conditions and FTD. These personality traits may represent a certain behavioural endophenotype that could be used in future genetic studies.

In this study, a number of cases psychotic disorders among relatives were reported by the proband or his/her family members, but no specific diagnosis could be confirmed. In these cases a diagnosis of ‘unspecified psychotic disorder’ was recorded but was not included in data analysis. Unconfirmed rates of psychotic disorder was also increased among relatives of cases than among relatives of controls.

The numbers of C9orf72 repeat expansion positive patients were too small to draw any firm conclusions. However, it was noticed that the rate of aggregation of FTD, Schizophrenia, Suicide, Depression, MND and Learning disability were all increased in first-and second- degree relatives of FTD patients carrying the repeat expansion when compared with relatives of those not carrying the repeat expansion and with relatives of

controls. There was no difference in the rate of Parkinson diseases and Alzheimer disease between the three groups.

6.6.3 Genetic testing (including C9orf72 repeat expansion status)

DNA was available for analysis for all of the 45 FTD patients. Only two FTD-MND patients had pathogenic mutations in C9orf72 repeat expansion but no patients had a pathogenic variant in other known FTD gene; as no mutations in MAPT, GRN, FUS, CHMP2B, PSEN1, PSEN2 or TBK1 were identified.

6.6.4 Study limitations

This study has some limitations. Firstly, possible presence of shared environmental exposures and genetic factors such as local founder effect and genetic homogeneity within a population may render this study population specific. A second point is that, although the rate of suicide in the FTD cohort was higher than in controls (HR=6.9), it must be emphasized that the total number of suicides was relatively small. Thirdly, information for this study was collected using a questionnaire completed by relatives of FTD patients. Whether or not the person completing the questionnaire reported a family history of a certain illness obviously depends on whether they were aware of the diagnosis. This was based on a semi-structured interview, however no specific diagnostic tool was used. A meta analysis by Hardt and Franke (230) demonstrated high reliability and validity of relatives' reports of major psychiatric illnesses such as Schizophrenia and addition.

6.7 Conclusions

This was a clinic-based cohort family aggregation study looking at family history among patients with FTD compared to controls. FTD was familial in 40% of our cohort and 11%

of patients had evidence of an autosomal dominant inheritance. Higher rates of other neurodegenerative disease including MND, neuropsychiatric disorders including Schizophrenia, Learning disability and suicide are present in first and second-degree relatives (N=996).

Information on aggregation of neurodegenerative and neuropsychiatric disorders in kindreds of FTD patients can be used to determine patterns of comorbidity and may help in customizing interventions to different groups of individuals. Also, further work on the link between the pathophysiology of FTD and neuropsychiatric diseases may lead new discoveries in the field of protein aggregation and neurogenetics.

7 Chapter 7: Results and Discussion of study 4: Genetics of Irish FTD patients

7.1 Introduction

There is a strong genetic component underlying FTD and it tends to cluster in families as FTD is inherited in about 40-50% of patients. An autosomal dominant mode of inheritance reported in 10-27% of all FTD patients (96). Furthermore, bvFTD is familial in 30-50% of cases whereas patients with svPPA or nfvPPA have a much lower frequency (58). A familial rate of 40% and an autosomal mode of inheritance of 11% were detected in this research (chapter 6, section 6.4 of this thesis). Genes recognized to play an important role in autosomal dominant FTD include: 1) MAPT, encoding microtubule-associated protein tau, 2) PGRN, encoding the protein progranulin, and 3) C9ORF72, a recently identified hexanucleotide repeat expansion on chromosome 9 (11.12). These gene mutations explain the majority of autosomal dominant FTD cases as mutations in the remaining known genes; VCP, charged multivesicular body protein 2B (CHMP2B), TAR-DNA binding protein (TARDBP), and FUS genes are found in less than 5%. There are still families with FTD in whom no gene has yet been found.

In this study we performed genetic analysis on DNA samples collected from 51 FTD patients to detect the presence of any mutation or mutations for known FTD genes. Repeat-primed PCR technique was used for the purpose of screening for the presence of a GGGGCC repeat expansion while targeted enrichment next-generation sequencing technique was used to sequence the exons of other genes known to be involved in FTD. Of note here is that identifying novel mutations would require matched population of controls, which is beyond the scope of this work.

7.2 Aims

Specific aims:

- 4- To use blood samples (whole blood) collected from participants to identify patients carrying the C9orf72 repeat expansion
- 5- To examine the phenotype and radiological features associated with the C9orf72 repeat expansion in FTD patients
- 6- To use whole blood samples of participants to identify the presence of any one or more of the known FTD gene mutations. Genes specifically tested for were: MAPT, GRN, FUS, CHMP2B, PSEN1, PSEN2 and TBK1.

7.3 Results

7.3.1 Genetic testing for hexanucleotide repeat expansion in C9orf72 (n=51)

Of 51 banked DNA samples collected from research participants, 4% (2/51) had the characteristic appearance of a GGGGCC hexanucleotide expansion consisting of a decaying series of 24 or more peaks. Both positive samples had 30 or more repeats. Both positive cases were of FTD-MND phenotype.

7.3.2 Genetic testing for other known FTD genes (excluding C9orf72)

Pathogenic Variants: The 168 retained SNPs and 16 retained INDELS were compared to the following literature to identify cohort mutations which have previously been associated with ALS or FTD: ALS Online Database (ALSoD) (178), Alzheimer's Disease and FTD Mutation Database (AD&TFDMDB) (179), Kenna et al. (180), Fogh et al. (181), Van Rheenen et al. (182) and (Williams et al. (183).

16 mutations were found to be present in the literature. These were filtered as follows:

- 7 were synonymous mutations (changed DNA but did not change amino acid) and thus were deemed to not be pathogenic

- Nonsynonymous mutations were compared to the exons of 60,706 individuals present in the Exome Aggregation Consortium (ExAc) database (Lek et al., (184).
- Mutations that are present at too high frequency in ExAc were deemed to be non-pathogenic as they are common in the population.
- The remaining 9 mutations were present at too high a frequency in ExAc to be deemed pathogenic.

7.3.3 Conclusion

In summary, there were no significant pathogenic mutations in our cohort of 51 FTD patients, which are reported in the literature within the genes tested.

8 Chapter 8 Results and Discussion of study 5: Neuroimaging of FTD patients

8.1 Introduction background and study rationale

The striking clinical, genetic and pathological heterogeneity of frontotemporal dementia and its overlap with other neurodegenerative conditions is well recognized [185]. The distinctive clinical phenotypes of this spectrum disorder are defined by the most dominant neuropsychological deficits such as behavioural or language impairment or association with amyotrophic lateral sclerosis (ALS). Despite the considerable deficits in phenotype-defining neurocognitive domains, visuospatial impairment, perception deficits, apraxia and amnesic deficits are seldom observed. There is great clinical and pathological overlap between FTD and ALS, notwithstanding, catastrophic motor dysfunction, autonomic, sensory and visual symptoms are seldom reported (186).

The neuroimaging literature of FTD encompasses publications from both FTD and ALS research groups, which explains the relatively distinct perspective and diverse patient cohorts included in these studies. ALS groups seldom include FTD patients without motor symptoms and focus on ALS phenotypes with varying degree of cognitive and behavioural impairment [187]. The anatomical substrate of cognitive impairment is relatively well characterized in ALS. Extra-motor frontal lobe pathology has been consistently reported both at cortical [188], subcortical [189] and white matter levels [190]. Interestingly, extra-motor pathology can also be readily detected in ALS patients with no overt cognitive or behavioural deficits on extensive neuropsychological testing. [191].

From an imaging methodological perspective, visual atrophy rating scales (192), PET (193), SPECT (194), voxel-based morphometry (VBM) (187, 195, 196), and diffusion tensor imaging (DTI) (196) have all been utilized to characterize FTD-ALS patients. Despite the growing imaging literature of FTD-ALS continuum, relatively few studies

evaluate their cohorts with both grey and white matter imaging techniques. Furthermore, white matter studies of the FTD-ALS spectrum often rely on a single diffusivity parameter, most often fractional anisotropy, which provides limited insights into the pathological nature of white matter degeneration.

FTD-ALS studies and ALS-dementia studies predating the discovery of the hexanucleotide expansions in C9orf72 have suggested distinct imaging signatures, but many of these studies suffered from relatively small patient samples: n=4 (Neary et al., 1990, Tanaka et al., 1993), n=8 (Talbot et al., 1995). The discovery of the C9orf72 GGGGCC hexanucleotide repeat expansions in 2011 gave a major impetus to FTD-ALS research, (54, 67) and it has been swiftly identified that the genotype is associated with shorter survival, younger age of onset, and marked behavioural impairment in ALS (197). The neuroimaging profile of ALS patients carrying the hexanucleotide repeat expansion has been linked considerably to orbitofrontal and temporal lobe pathology, consistent with the clinical phenotype (198). It is clear however that the hexanucleotide repeats in C9orf72 are not accountable for all FTD-ALS patients, and relatively little is known of the imaging profile of FTD-ALS patients who don't carry the hexanucleotide repeats. With the exception of a large PET study (193), C9orf72 negative FTD-ALS patients remain a poorly characterized patient cohort, with considerable neuropsychological deficits and poorly understood genetic vulnerability.

Comorbid frontotemporal dementia in ALS has significant management and survival implications as it is relatively well established that cognitive and behavioural impairment in ALS affect compliance with assistive devices (199), insight into end-of life decisions (200), caregiver burden (201) and survival (202). FTD imaging studies without ALS cohorts often focus on extra-motor brain regions driving the most salient clinical symptoms and seldom assess motor regions, despite growing evidence that ALS patients may present with cognitive and/or behavioural deficits prior to motor

disability and that subtle motor dysfunction can be detected in a significant proportion of FTD patients. (203)

A total of 100 participants underwent comprehensive multimodal neuroimaging, genetic testing and neuropsychological evaluation. Seven patients with behavioural variant FTD (bvFTD), eleven patients with non-fluent-variant primary progressive aphasia (nfvPPA), two patients with semantic-variant primary progressive aphasia (svPPA), ten patients with amyotrophic lateral sclerosis and FTD carrying the *C9orf72* hexanucleotide repeat (ALS-FTD C9+), ten patients with ALS-FTD without hexanucleotide repeats (ALS-FTD C9-), twenty ALS patients without behavioural or cognitive deficits (ALSnci) and forty healthy controls (HC) were included in a prospective quantitative neuroimaging study. Phenotype-specific patterns of grey matter atrophy were evaluated using both whole-brain voxel-wise statistics as well as region-of-interest analyses. White matter alterations were explored using multiple diffusivity indices: radial diffusivity, axial diffusivity and fractional anisotropy.

The objective of this study was to characterise the imaging signatures of FTD phenotypes along the FTD-ALS spectrum using multiple complementary imaging techniques.

Of note, the abbreviations FTD-ALS and ALS-FTD were used interchangeably to describe the same cohort of patients throughout this chapter.

8.2 Aims

1- The main objective of the study was comprehensive characterization of in vivo pathological changes in FTD phenotypes.

2- Additionally, we aimed to specifically evaluate the neuroimaging profile of *C9orf72* positive and negative FTD-ALS patients. Motor cortex and corticospinal tract alterations

were evaluated in FTD cohorts without ALS. Unilateral and bilateral patterns of neurodegeneration were explored along the FTD-ALS continuum. Grey matter pathology was specifically evaluated in key cortical regions, such as Broca's and Wernicke's area, orbitofrontal cortex, pre- and post-central gyrus. Unaffected brain regions were also assessed using both whole-brain and region of interest (ROI) statistics.

The main hypothesis of the study is that FTD-ALS is a spectrum disorder, where the main clinical phenotypes are manifestations of focal pathology, a continuum where various degree of motor cortex pathology can be identified in patients without ALS and extra-motor changes in ALS patients without cognitive deficits.

8.3 Results

8.3.1 Grey matter analyses

8.3.1.1 Whole –brain phenotype specific imaging signatures versus controls

Whole-brain analyses revealed considerable grey matter pathology along the FTD-ALS spectrum. While FTD-ALS patients showed widespread and bilateral patterns of cortical atrophy, language variant FTD cohorts showed more asymmetric and focal grey matter alterations, as shown in Figure 8.2.

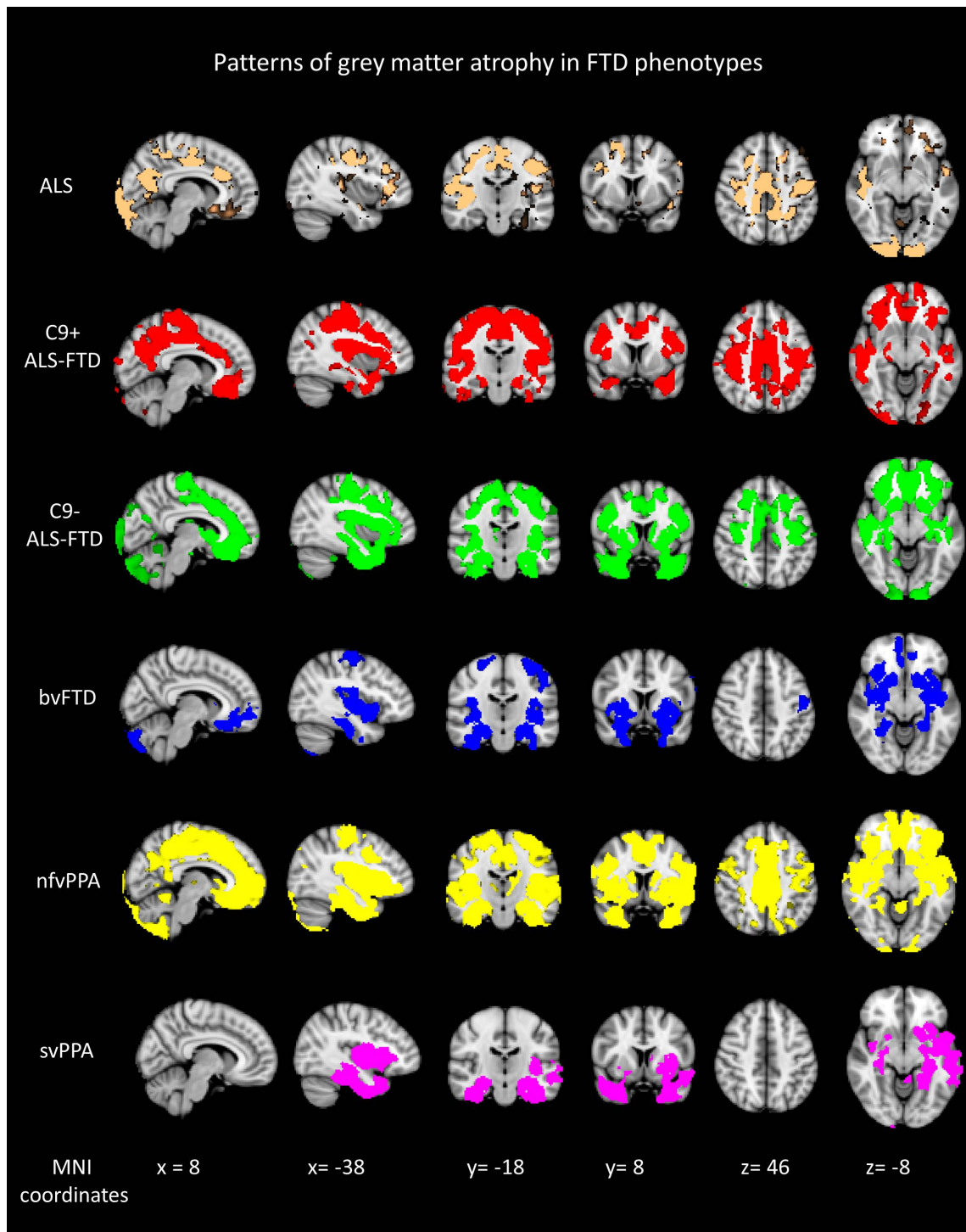


Figure 8.2: Patterns of grey matter atrophy in ALS and FTD phenotypes at $p < 0.01$ TFCE, corrected for age. Representative sagittal, coronal and axial views are shown with the corresponding MNI coordinates indicated at the bottom. ($P < 0.05$ for svPPA, uncorrected p values are shown for ALSnci)

8.3.1.2 ALS versus APT and ATD

In comparison to ALSnci patients, FTD-ALS cohorts demonstrated ample grey matter pathology, which was less significant in C9orf72 positive patients than in C9orf72 negative patients (Figure 8.3).

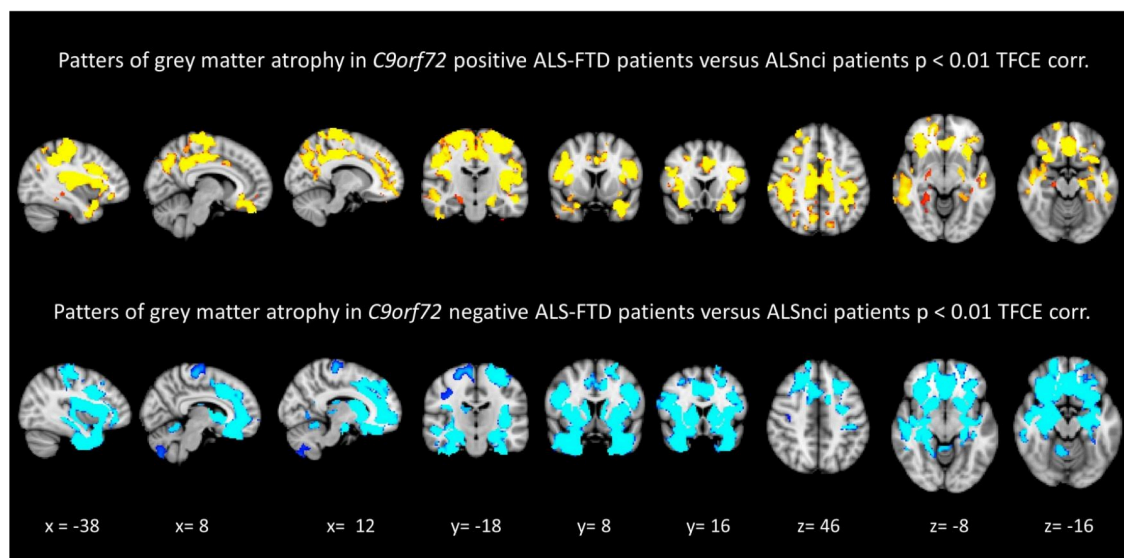


Figure 8.3: Patterns of grey matter atrophy at $p < 0.01$ TFCE, corrected for age, in ALS-FTD cohorts versus ALSnci patients. Representative sagittal, coronal and axial views are shown with the corresponding MNI coordinates indicated at the bottom

8.3.1.3 Region of interest (ROI) based grey matter analyses

Region of interest grey matter analyses in the precentral gyrus, postcentral gyrus, Broca's area, Wernicke's area, orbitofrontal cortex, frontal, temporal, parietal, and occipital lobes showed relatively selective involvement of cortical regions in the different phenotypes (Figure 8.4 and Figure 8.5). As shown by the box plots of T1-signal intensity distributions, Broca's area was most severely affected in nvPPA, Wernicke's area in svPPA, the precentral gyrus in C9-ALS-FTd and nvPPA, and the orbitofrontal cortex in C9-negative ALS-FTD and nvPPA. The most marked temporal cortex alterations were seen in language variant FTD cohorts. As demonstrated by

previous studies, the post central gyrus, parietal and occipital lobe were relatively spared compared to phenotype-defining cortical regions. (Ref selective vulnerability)

The regional grey matter density profile of FTD phenotypes and controls

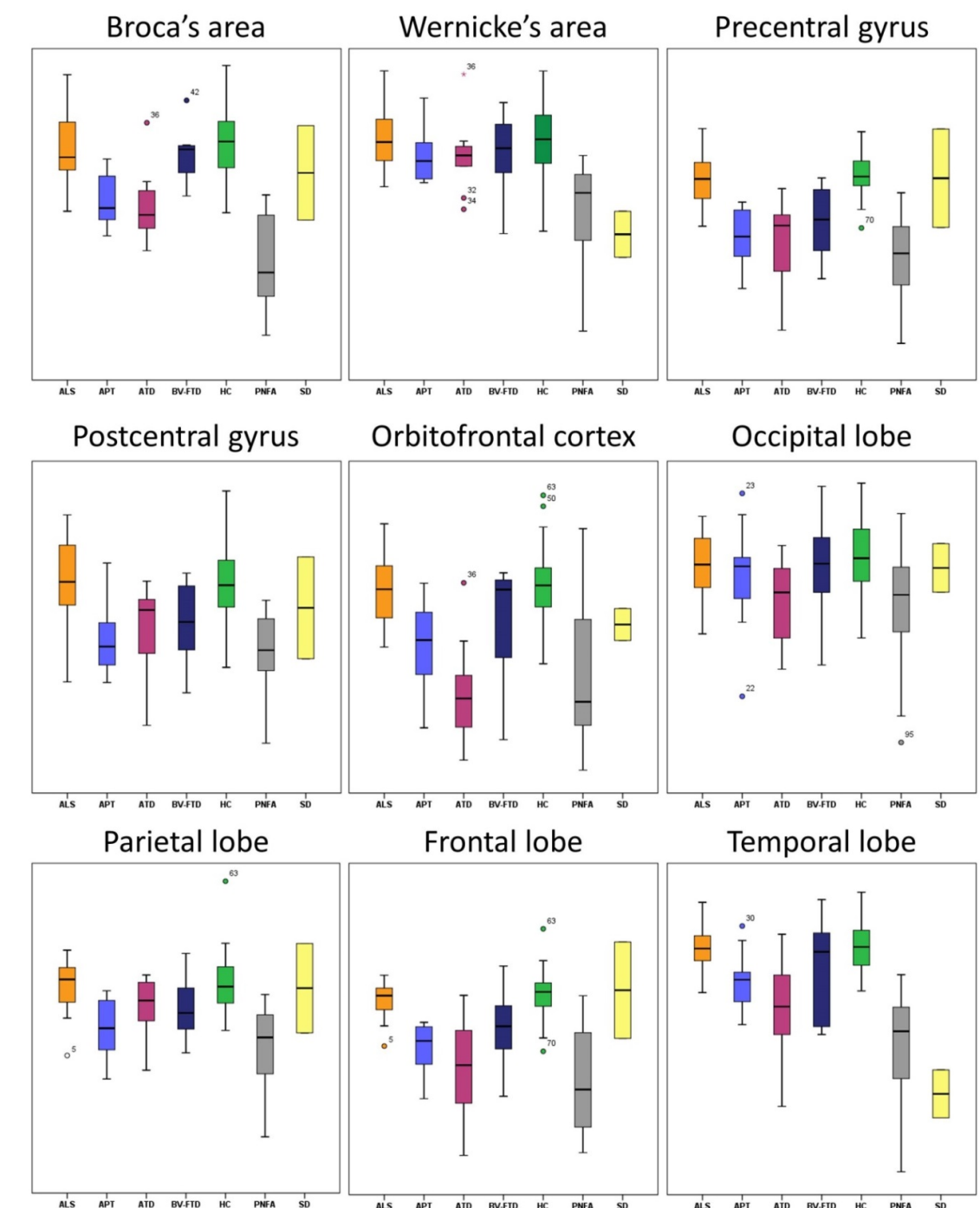


Figure 8.4: the phenotype specific grey matter profiles in key cortical grey matter regions. **ALS**: ALS patients with no cognitive impairment (orange), **APT**: C9orf positive ALS-FTD patients (aqua), **ATD**: C9orf negative FTD-ALS patients (purple), **bvFTD**: Behavioural variant FTD (blue), **HC**: Healthy controls (Green), **nvPPA**: non-fluent variant primary progressive aphasia (Grey), **svPPA**: semantic variant primary progressive aphasia.

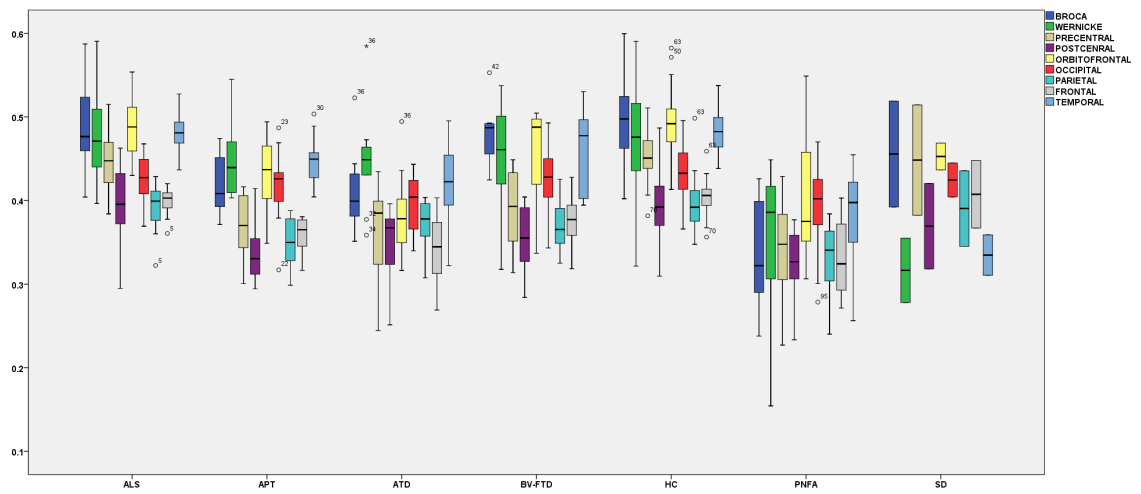


Figure 8.5: The regional grey matter density profile of FTD phenotypes. The Y axis indicates average signal intensity in the following nine cortical regions; Broca's area (Blue), Wernicke's area (Green), Precentral gyrus (Beige), Postcentral gyrus (purple), orbitofrontal cortex (yellow), occipital lobe (red), parietal lobe (aqua), frontal lobe (grey), temporal lobe (light blue). X axis represents the seven phenotypes along the FTD-ALS spectrum; **ALS**: ALS patients with no cognitive impairment, **APT**: C9orf positive FTD-ALS patients, **ATD**: C9orf negative ALS-FTD patients, **bvFTD**: Behavioural variant FTD, **HC**: Healthy controls, **nvPPA**: non-fluent variant primary progressive aphasia, **svPPA**: semantic variant primary progressive aphasia.

Table 8.2: The descriptive grey matter profile of FTD phenotypes in key cortical regions, mean, standard deviation, estimated marginal means and p-values are provided for age-corrected group differences (ANCOVA). **ALSnci**: ALS patients without behavioural or cognitive deficits, **C9+ ALS-FTD**: ALS-FTD carrying the C9orf72 hexanucleotide repeat, **C9- ALS-FTD**: 10 patients with ALS-FTD without hexanucleotide repeats, **bvFTD**: behavioural variant FTD, **HC**: healthy controls, **nfvPPA**: non-fluent-variant primary progressive aphasia, **svPPA**: semantic-variant primary progressive aphasia.

ROI	Descriptive statistics	ALSnci	C9+ALS-FTD	C9-ALS-FTD	bvFTD	HC	nfvPPA	svPPA	p-Value
Broca's area	Mean	0.48502	0.419487	0.410065	0.480019	0.498355	0.340512	0.455633	0.000
	Std. Deviation	0.044648	0.037043	0.049759	0.040767	0.048523	0.062824	0.089396	
	Est.Marg.Mean	0.483	0.41	0.416	0.482	0.497	0.352	0.457	
Wernicke's area	Mean	0.473266	0.448512	0.44925	0.451018	0.470324	0.344345	0.316524	0.000
	Std. Deviation	0.047437	0.044335	0.060599	0.07814	0.067229	0.098061	0.054384	
	Est.Marg.Mean	0.473	0.445	0.452	0.452	0.47	0.349	0.317	
Precentral gyrus	Mean	0.445671	0.369987	0.36542	0.389272	0.453854	0.337282	0.448401	0.000
	Std. Deviation	0.035149	0.037906	0.059498	0.054217	0.029039	0.06361	0.093242	
	Est.Marg.Mean	0.443	0.356	0.374	0.393	0.451	0.355	0.45	
Postcentral gyrus	Mean	0.395406	0.334637	0.348906	0.354374	0.394748	0.328659	0.369339	0.000
	Std. Deviation	0.040507	0.035324	0.042932	0.045441	0.034826	0.040567	0.072218	
	Est.Marg.Mean	0.392	0.318	0.359	0.359	0.392	0.349	0.372	
Orbitofrontal cortex	Mean	0.487823	0.43309	0.384237	0.451867	0.492712	0.404235	0.452626	0.000
	Std. Deviation	0.03183	0.043556	0.052468	0.065902	0.035687	0.076967	0.022622	
	Est.Marg.Mean	0.486	0.426	0.388	0.454	0.491	0.413	0.454	
Occipital lobe	Mean	0.426546	0.416372	0.398858	0.424612	0.436166	0.390055	0.424567	0.058
	Std. Deviation	0.026061	0.04732	0.034995	0.047448	0.03108	0.058328	0.028673	
	Est.Marg.Mean	0.424	0.402	0.408	0.428	0.434	0.407	0.427	
Parietal lobe	Mean	0.393124	0.349649	0.372015	0.370712	0.394204	0.329175	0.390423	0.000
	Std. Deviation	0.025912	0.027508	0.030058	0.035471	0.028126	0.045928	0.063824	
	Est.Marg.Mean	0.391	0.338	0.379	0.374	0.392	0.343	0.392	
Frontal lobe	Mean	0.39962	0.358658	0.343427	0.375568	0.402836	0.331786	0.407513	0.000
	Std. Deviation	0.014467	0.023208	0.040889	0.035615	0.019256	0.047082	0.057092	
	Est.Marg.Mean	0.399	0.353	0.347	0.377	0.402	0.338	0.408	
Temporal lobe	Mean	0.479931	0.448234	0.416634	0.45717	0.48229	0.37968	0.334748	0.000
	Std. Deviation	0.022913	0.031311	0.053934	0.056474	0.023908	0.063341	0.034081	
	Est.Marg.Mean	0.478	0.438	0.423	0.46	0.48	0.391	0.336	

8.3.2 White matter analyses

8.3.2.1 Fractional anisotropy analyses: Whole-brain single phenotypes versus controls

Using fractional anisotropy widespread bilateral white matter alterations were identified in all FTD phenotypes with the exception of svPPA patients who exhibit more focal left temporal lobe white matter pathology (Figure 8.6).

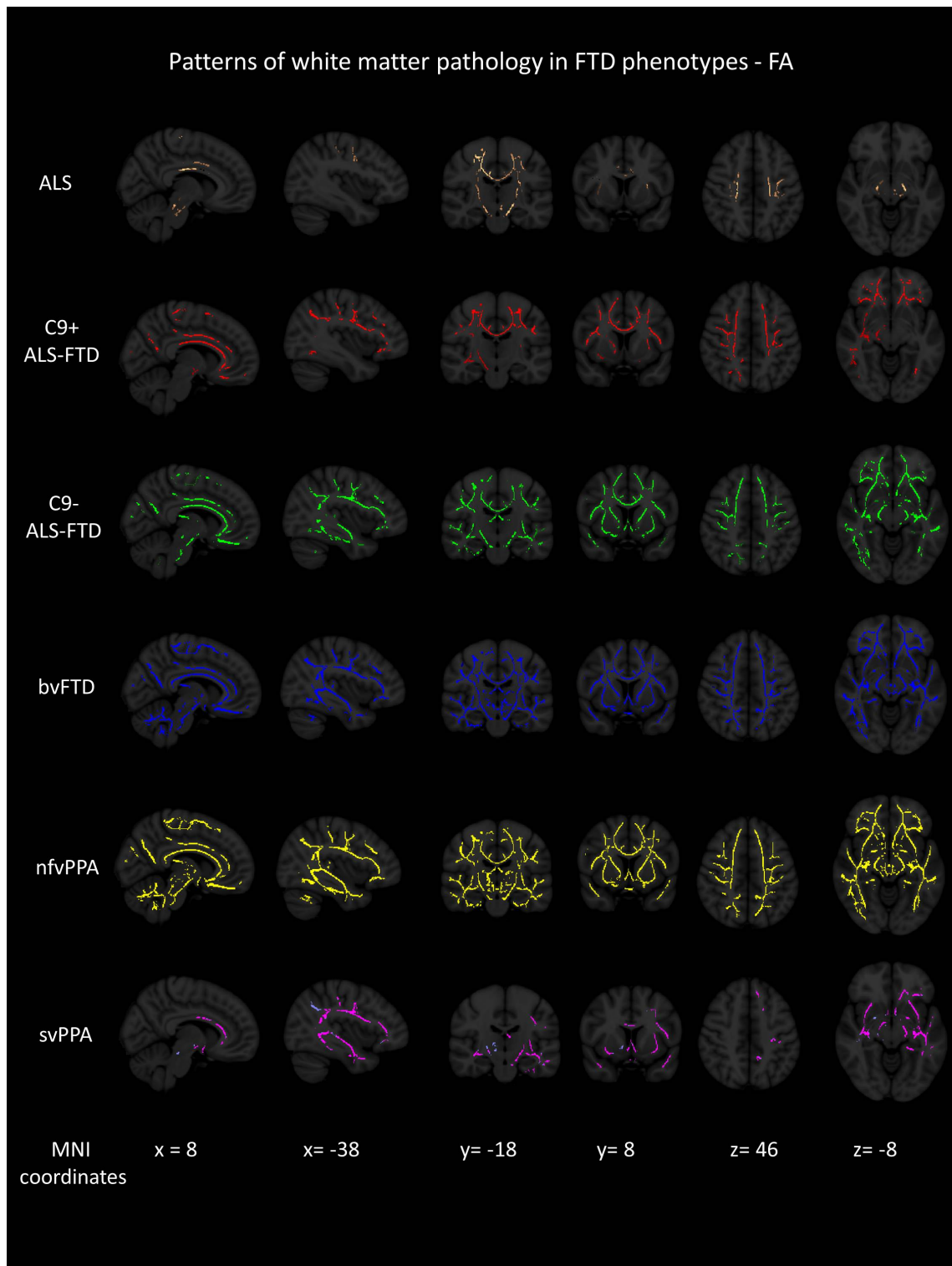


Figure 8.6: Patterns of white matter degeneration among FTD-ALS spectrum highlighted by fractional anisotropy alterations (FA) at $p < 0.01$ TFCE, corrected for age. Representative sagittal, coronal and axial views are shown with the corresponding MNI coordinates indicated at the bottom. (Uncorrected p -values shown for ALSnci).

8.3.2.2 ALS-FTD cohorts versus ALSnci

Similarly to our grey matter analyses, ALS-FTD patients showed ample extra-motor white matter pathology in comparison to ALSnci patients, which was more pronounced in ALS-FTD patients without the C9orf72 hexanucleotide repeats. Similarly to previous reports, FTD-ALS patients carrying the hexanucleotide repeats demonstrated orbitofrontal, cingulate and opercular white matter pathology (Figure 8.7).

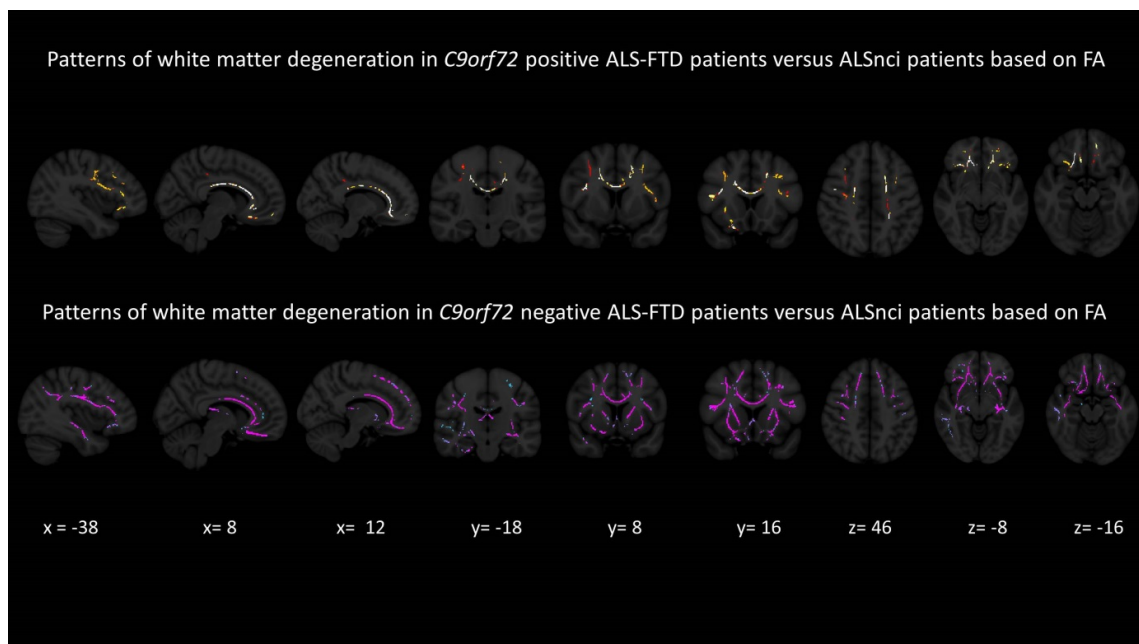


Figure 8.7: Patterns of white matter degeneration at $p < 0.01$ TFCE corrected for age, in ALS-FTD cohorts versus ALSnci patients. Representative sagittal, coronal and axial views are shown with the corresponding MNI coordinates indicated at the bottom.

8.3.3 Radial diffusivity analyses

8.3.3.1 Whole-brain single phenotypes versus controls

The patterns of white matter pathology identified by radial diffusivity were similar to those highlighted by fractional anisotropy. Widespread changes were captured in FTD-

ALS cohorts and relatively focal orbitofrontal changes in bvFTD and left temporal changes in svPPA patients (Figure 8.8).

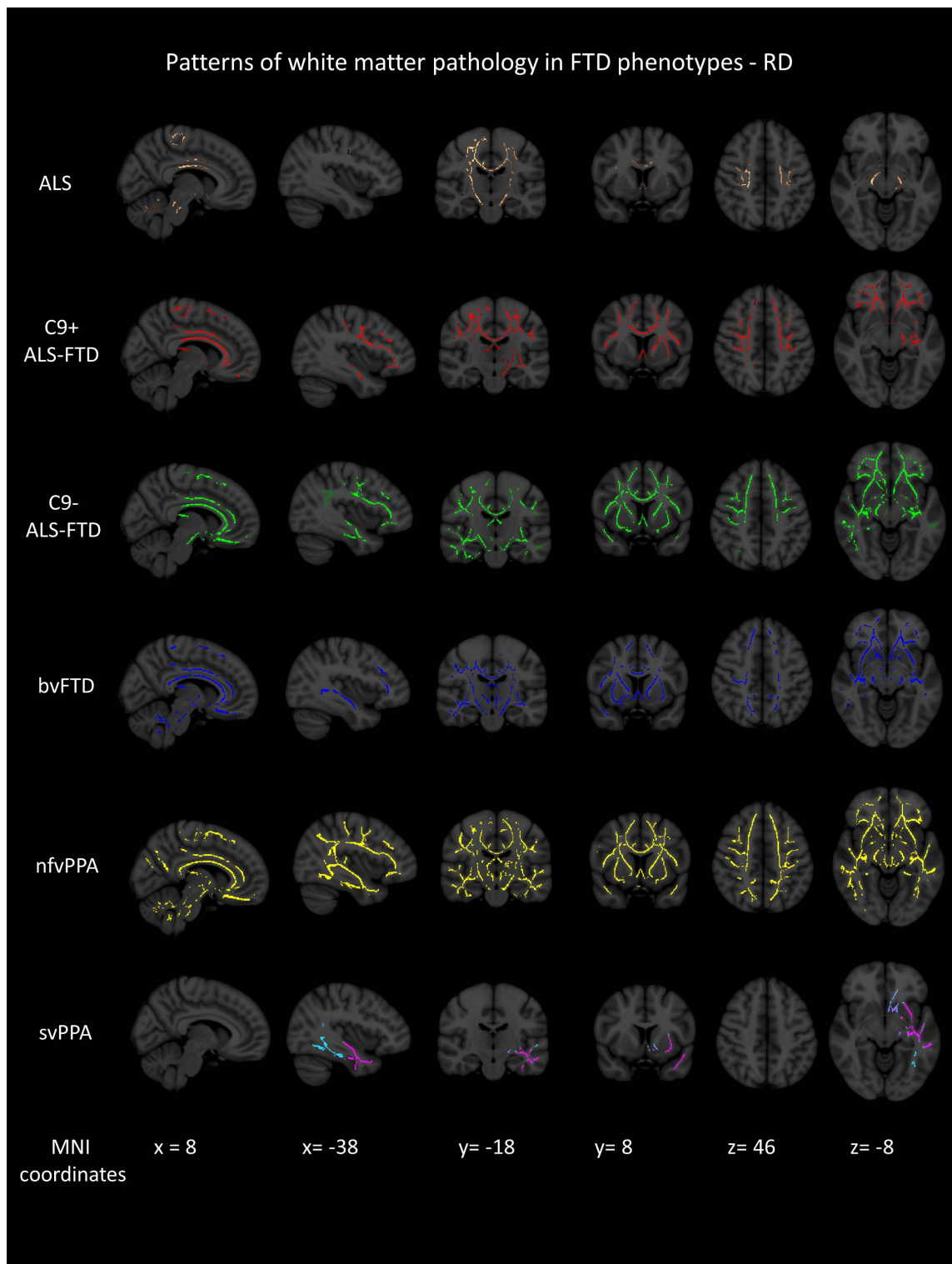


Figure 8.8: Patterns of white matter degeneration in ALS-FTD spectrum highlighted by radical diffusivity (RD) alterations at $p < 0.01$ TFCE, corrected for age. Representative sagittal, coronal and axial views are

shown with the corresponding MNI coordinates indicated at the bottom. (Uncorrected p -values shown for ALSnci)

8.3.3.2 ALS-FTD cohorts versus ALSnci

C9orf72 positive ALS-FTD patients showed orbitofrontal, cingulate and opercular radial diffusivity changes in comparison to ALS patients without cognitive or behavioural impairment (Figure 8.9).

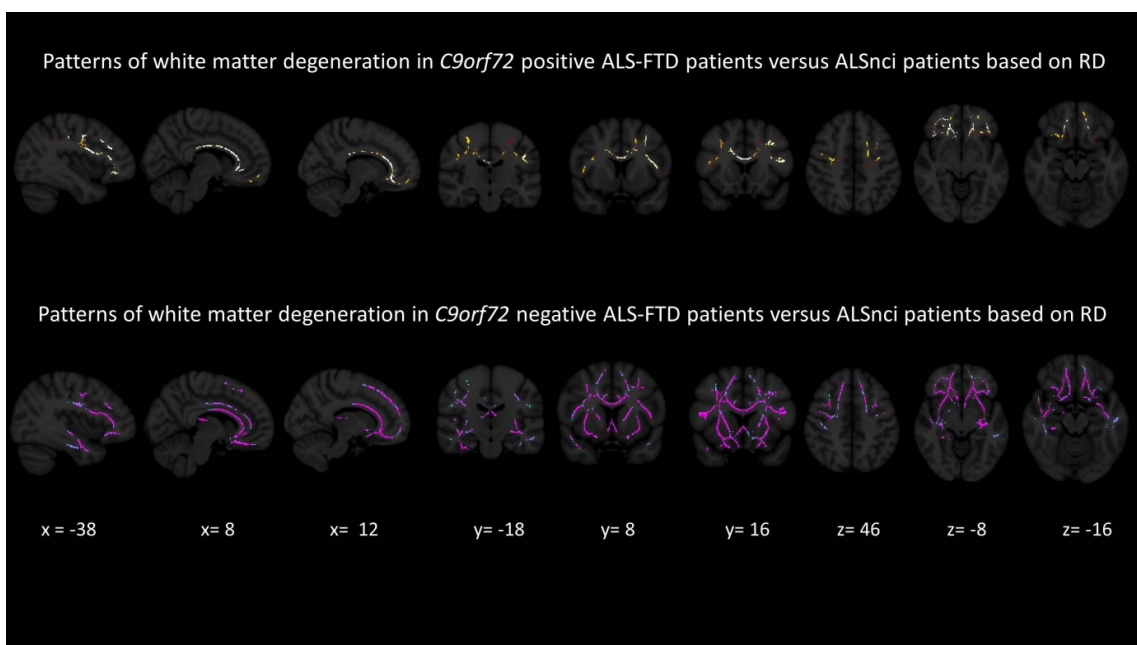


Figure 8.9: Patterns of white matter degeneration at $p < 0.01$ TFCE corrected in ALS-FTD cohorts versus ALSnci patients. Representative sagittal, coronal and axial views are shown with the corresponding MNI coordinates indicated at the bottom.

8.3.4 Axial diffusivity analyses

8.3.4.1 Whole-brain single phenotypes versus controls

Axial diffusivity changes were considerably less widespread than RD and FA alterations. Focal left inferior frontal lobe white matter changes were captured by AD subjacent to Borca's area in nvPPA and in the left temporal lobe in svPPA. Extensive bifrontal white matter changes were identified in the two ALS-FTD cohorts (Figure 8.10).

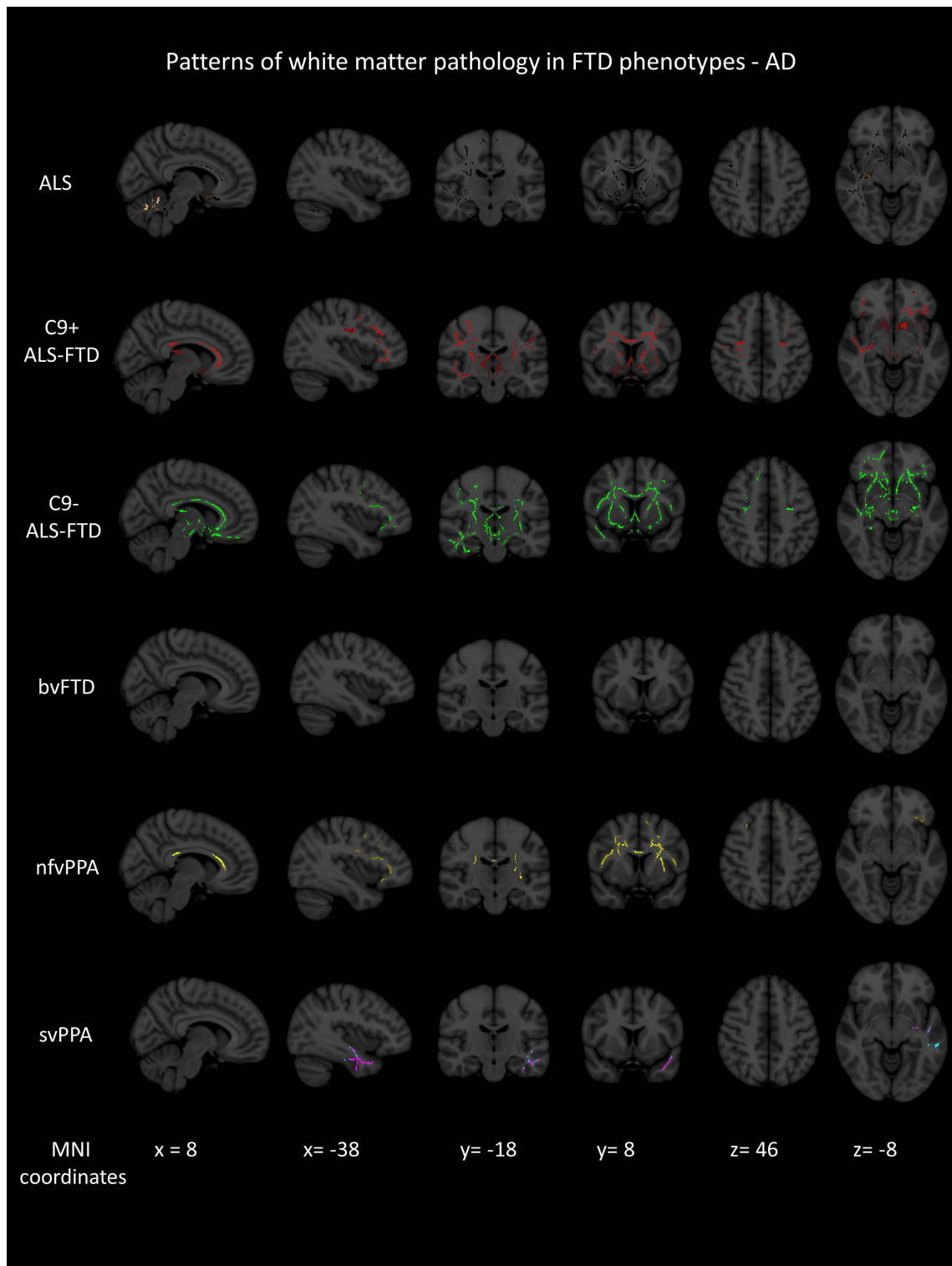


Figure 8.10: Patterns of white matter degeneration along ALS-FTD spectrum highlighted by axial diffusivity alterations at $p < 0.05$ TFCE, corrected for age. Representative sagittal, coronal and axial views are shown with the corresponding MNI coordinates indicated at the bottom. (Uncorrected p -values shown for ALSnci)

8.3.4.2 ALS-FTD cohorts versus ALSnci

Similarly to the other white matter indices, orbitofrontal white matter pathology was identified in C9orf positive ALS-FTD patients, but overall, pathological change was less extensive than in C9orf negative ALS-FTD patients (Figure 8.11).

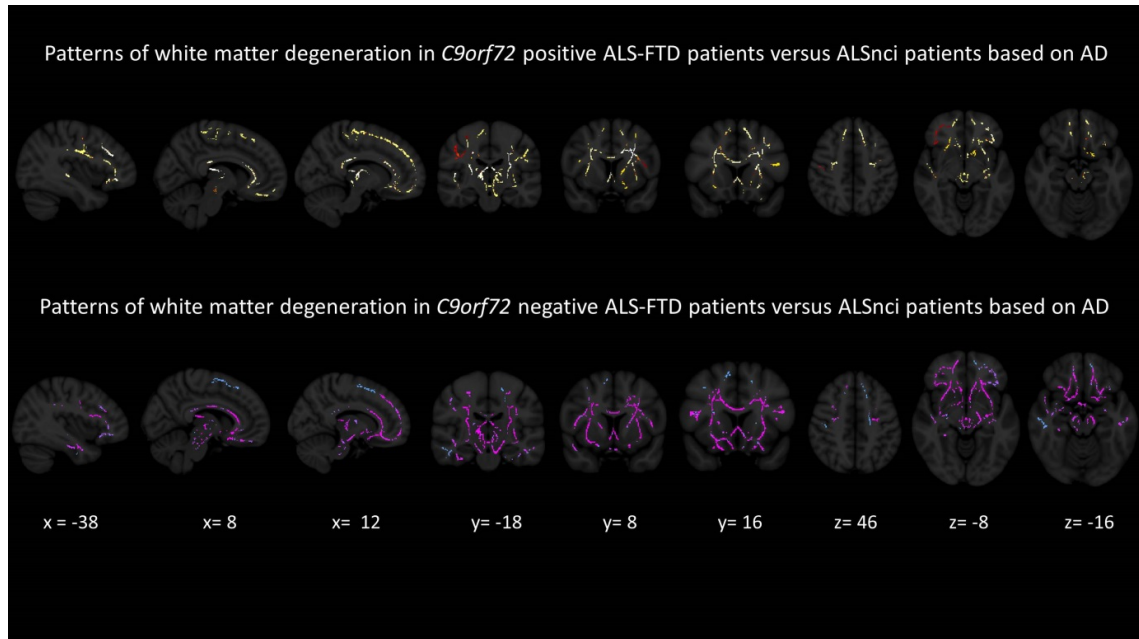


Figure 8.11: Patterns of white matter degeneration at $p < 0.05$ TFCE corrected in ALS-FTD cohorts versus ALSnci patients. Representative sagittal, coronal and axial views are shown with the corresponding MNI coordinates indicated at the bottom.

8.4 Discussion

The novelty of the study was the in vivo characterization of C9orf72 negative ALS-FTD patients who exhibited widespread extra-motor changes, which were more extensive than those observed in FTD-ALS patients carrying the hexanucleotide repeat in C9orf72. This is a particularly vulnerable patient cohort with both considerable motor and cognitive impairment with no established genetic susceptibility. With marked interest in recent years in patients carrying the hexanucleotide repeat relatively little

information is available on ALS-FTD patients who have a similar neurocognitive profile without the mutation. As demonstrated by this study, these patients exhibit more widespread frontotemporal pathology than those with the hexanucleotide repeat, highlighting that hexanucleotide repeats only account for a proportion of the ALS-FTD phenotype and that urgent genetic studies are needed to clarify susceptibility profile of this cohort. Similar to previous imaging studies, C9orf72 patients included in this study showed more unilateral changes than those without the hexanucleotide repeats. Interestingly, the white matter pathology highlighted by RD analyses localized to the left temporal lobe and subjacent to Broca's area.

As shown in Figure 8.3, statistically significant precentral gyrus atrophy was identified in ALS-FTD patients in comparison to ALS patients without cognitive deficits. The marked motor cortex pathology was captured in both C9orf positive and negative ALS-FTD cohorts. The findings are in keeping with the shorter survival of these patients and points beyond the survival effect of behavioural impairment. The findings suggest that ALS-FTD is associated with more marked motor cortex involvement.

In keeping with the emerging literature of upper motor neuron dysfunction of FTD patients, we have also identified precentral gyrus i.e. motor cortex pathology in bvFTD patients without a diagnosis of ALS. This result, combined with our other important finding from the neurophysiology arm of this research of lower MUNIX values in some patients with FTD without MND (chapter 9 of this thesis), can lay the foundation for future studies aiming to explore further the degree of upper and lower motor neuron dysfunction in FTD. Furthermore, we have identified corticospinal tract alterations in bvFTD and nfvPPA patients in the internal capsule, corona radiata and cerebral peduncles based on FA and RD statistics demonstrating upper motor neuron degeneration in FTD cohorts without clinical evidence of ALS or PLS. These findings may have significant management implications. We suggest that FTD patients should be regularly assessed for upper and lower motor neuron signs and undergo

neurophysiological testing if possible, as overlap with ALS is not uncommon. The classical categorisation of ALS patients as bulbar or spinal onset could be expanded to spinal-, bulbar-, respiratory-, and behavioural or cognitive onset. The demonstration of extensive frontotemporal changes in ALS-FTD cohorts reconfirms the urgency for neuropsychology assessment to be firmly integrated in multidisciplinary ALS care. Routine cognitive screening of newly diagnosed ALS patients is increasingly undertaken in view of the availability of ALS specific cognitive screening tools (213). Furthermore, the presence of recently validated ALS-specific behavioural tools, for instance, Beaumont Behavioural Inventory (BBI), (214) made comprehensive screening available and timely referral to specialist neuropsychological evaluation possible based on screening results.

Our study demonstrated various degree of cerebellar atrophy among the ALS-FTD spectrum. While cerebellar pathology is recognized in ALS (211, 215), the cerebellum is increasingly linked to a range of integrative cognitive functions (216) and has not been studied in depth in FTD to date.

The direct correlation of neuropsychological measures and imaging parameters remain contentious (217). While promising studies have been previously undertaken (218), there is a consensus that correlation of MRI measures to cognitive performance may be simplistic and overlooks the complexity of cortico-basal networks implicated in neurocognitive processes (217).

From a broader neuroimaging perspective, the study showcases the benefit of including multiple phenotypes along a spectrum disorder and mimic-neurodegenerative conditions. Many ALS studies only include controls and ALS patients and motor cortex changes are attributed to ALS (219). As shown in this study however, motor cortex atrophy, cerebellar degeneration can also be readily identified in FTD cohorts. Therefore the inclusion of 'mimic-controls' or 'disease-controls' helps to identify imaging signatures which are unique to a specific condition and is superior to merely including

healthy controls. Inclusion of mimic conditions has special relevance to classification studies of blinded data sets. Classification studies are increasingly proposed as precursors to viable diagnostic applications. Classifying blinded imaging data in a binary fashion as 'ALS' or 'healthy control' has been successfully performed in a number of recent studies (220). The methodological challenge is to correctly sub-categorize a blinded dataset among a number of neurodegenerative diagnoses. Accordingly, the detailed and comparative characterization of phenotypes along the ALS-FTD spectrum has implications for the refinement of classification studies and ultimately for the development of accurate diagnostic biomarkers.

Both our whole-brain and ROI analyses highlighted relative sparing of parietal and occipital brain regions, which are in sharp contrast to commoner neurodegenerative conditions such as Alzheimer's disease. The selective involvement and sparing of specific brain regions is a key feature of neurodegenerative conditions (186) and may have implications for the development of imaging based diagnostic markers.

As discussed in the introduction, many imaging studies continue to solely use fractional anisotropy (FA) to appraise white matter degeneration. Other diffusivity measures however, such as AD and RD added considerable insights to the pathological characterization of white matter alterations (43, 204). Axial diffusivity (AD) is generally considered as a sensitive axonal marker (221, 222), and radial diffusivity as a myelin related proxy (223, 224). Fractional anisotropy (FA) is calculated based on the all three eigenvalues and is a sensitive composite markers of white matter integrity. The other advantage of including AD and RD analyses is that they not only reflect on different pathological aspects of white matter degeneration, but are mathematically independent measures.

Limitations: The presented study is not without limitations. Relatively small number of FTD patients were included, especially patients with semantic dementia. While a comprehensive, multimodal cross-sectional analysis was provided; longitudinal

imaging would provide further information on patterns of pathological spread and rate of progression in various phenotypes. The addition of other mimic neurodegenerative subgroups, such as posterior cortical atrophy, Parkinson's disease or Huntington's disease patients would enable the demonstration of the selective anatomical vulnerability of these conditions further. Presymptomatic studies of pathological mutation carriers would add valuable information on this relatively arcane phase of the disease, which may precede clinical symptoms by years (225). Furthermore, it is clear that the pathology of subcortical grey matter structures contributes to the diverse neuropsychological deficits observed in these patient cohorts (218), and the comprehensive evaluation of these structures may provide further insights. Nonetheless, the study dissected the anatomical heterogeneity of FTD and demonstrated the unique patterns of neurodegeneration underpinning the clinical phenotypes. In a condition where diagnosis is notoriously challenging and definite diagnosis can often only be provided by post mortem examination, multimodal neuroimaging offers unrivalled anatomical insights in vivo.

Another potential confounding factor is the inclusion of three left handed patients in the nvPPA cohort. While only a small minority of left handed people have their Broca's area in the right hemisphere (226), the inclusion of these patient may have decreased the anatomical homogeneity of the nvPPA cohort in this study. One of the confounders of our analyses was disease duration. While the primary objective was to identify focal cortical and white matter changes in each phenotype by assessing multiple imaging metrics, in some cohorts such as nvPPA we could only capture widespread bilateral changes, which is likely to be explained by relatively prolonged disease duration and scanning the patients long after their diagnosis. It is conceivable that analyzing imaging data from nvPPA patients scanned soon after their diagnosis a more focal pathological pattern may be captured. To account for this limitation, ROI-based cortical were carried

out using anatomical masks for Borca's and Wernicke's area which have convincingly demonstrated phenotype-defining grey matter density alterations.

Finally, no genetic screening was performed on the healthy controls of his study. While a detailed family history and medical history was taken from each control and only volunteers with no family history of neurodegenerative conditions were included in the study, it is possible that asymptomatic mutation carriers were included among the controls.

8.5 Conclusions

We have shown that clinical heterogeneity of the FTD-ALS spectrum is driven by relatively distinct pathological patterns of neurodegeneration. We have also demonstrated that FTD-ALS patients who tested negative for hexanucleotide repeats in C9orf72 have considerable extra-motor frontotemporal pathology, which was even more prominent than that observed in C9orf72 positive patients. While larger studies are needed to comprehensively untangle the heterogeneity of the FTD-ALS spectrum, it is clear that multifaceted imaging analyses remain an indispensable tool in characterizing the in vivo processes underpinning these conditions. Our findings demonstrated that the clinical manifestations of FTD are underpinned by phenotype-specific patterns of white and grey matter degeneration. Our study also confirmed the descriptive role of multi-parametric quantitative neuroimaging in FTD.

9 Chapter 9 Results and Discussion of study 6: MUNIX in FTD patients: a comparative study of FTD subtypes, ALS and Poliomyelitis.

9.1 Introduction

In degenerative diseases such as amyotrophic lateral sclerosis (ALS), the number of motor units (MUs) is reduced, and estimating the number of MUs has been shown to be of value in prognosis, follow-up, and also evaluation of drug treatments (140). Currently there are two methods in use to evaluate muscle motor units - Motor Unit Number Estimate (MUNE) and Motor Unit number Index (MUNIX).

Motor Unit Number index (MUNIX) is a measure estimated by a noninvasive method that requires minimal electrical stimulation. It gives an index of functioning motor units. The technique involves utilizing the surface-recorded compound muscle action potential (CMAP) and electromyographic (EMG) interference pattern to compute the motor unit number index (MUNIX).

MUNE is now well established as a biomarker in ALS. However, the methodology is time consuming (20 to 30 minutes per muscle), and requires use of intramuscular needles or hundreds of stimuli.

By contrast, MUNIX is a noninvasive method that can be applied to both proximal and distal muscles. MUNIX techniques utilize a compound muscle action potential (CMAP) obtained after one supramaximal stimulation of the nerve, and surface electromyographic (EMG) interference pattern (SIP) recorded during voluntary muscle contraction. MUNIX uses a mathematical model based on the CMAP and the surface interference pattern following their import into analysis software created by Nandedkar et al (142). The result is presented as a plot and a numeric value reflecting the number

and size of motor units recruited at various force levels. The result of the examination is directly related to the number of functioning motor neurons in a given muscle.

While the number of muscles that can be evaluated using MUNE is limited, MUNIX has a greater degree of flexibility and can be used to evaluate a larger number of muscles when compared to MUNE (169). Both MUNE and MUNIX have been evaluated as biomarkers of motor unit decline in ALS. However there have been only few MUNIX studies of healthy volunteers, and the degree of variance across healthy normal subjects has not been fully established, nor has the effect of gender or ageing been fully evaluated (145). MUNIX has not been studied before in Irish patients with ALS and there is no published work in the literature investigating MUNIX in FTD or poliomyelitis.

The technical aspects of MUNIX measurement, mathematical model and computation were described in chapter 1 of this thesis.

Details of MUNIX study participants' selection criteria, procedure, investigatory plan and the practical steps of muscle testing were given in the methodology section of this thesis (chapter 3, section 3.6). A brief summary of findings is given in this section while detailed descriptions and findings of specific individual studies are written in the next sections (section 9.5 through section 9.8).

9.2 Aims

The aims of the MUNIX studies were:

1- To develop a lower motor neuron repository by collecting MUNIX values from:

- (a) Healthy volunteers so as to establish normative data profile and to determine effects of age and gender before applying this technique in patients with neurological diseases, where appropriate. This normative data profile will also be used for the purpose of improving the quality of reports issued by the neurophysiology department of our hospital (Beaumont Hospital Dublin).

(b) A cohort of patients with poliomyelitis. Poliomyelitis was chosen as an example of a non-progressive lower motor neuron disorder, i.e., to develop a lower motor neuron MUNIX repository.

2- To perform longitudinal MUNIX measurements on three disease groups: FTD, ALS and Poliomyelitis with the aim of determining the rate of decline in MUNIX value with time in patients with those three diseases.

This would help in better understanding of disease processes to identify patients with above conditions earlier so that any potentially disease modifying therapies can be offered as early as possible.

3- While ALS and poliomyelitis are examples of conditions that primarily affect the lower motor neuron; the objective of the FTD arm of the MUNIX study was to test neurophysiologically the hypothesis that FTD, a primarily upper motor neuron disease, also causes lower motor neuron (LMN) dysfunction and this LMN dysfunction varies in the different FTD subtypes of bvFTD, nvPPA, svPPA and FTD-MND.

4- A specific objective of the ALS study was to compare the rate of MUNIX decline over time in ALS patients with that of the well established marker of diseases progression, ALSFRS-R with the possibility of establishing MUNIX as an other equality reliable marker for the same purpose (monitoring disease progression).

9.3 Results of MUNIX studies: brief descriptions

A summary of main findings of our four MUNIX studies (normative data, Poliomyelitis, FTD and ALS and) is provided here. Detailed description of each of these studies is written separately under appropriate sections in this chapter (section 9.5 through section 9.8).

9.3.1 Summary of results of MUNIX data: normative data

MUNIX normative data repository was built by collecting MUNIX, MUSIX and CMAP from 40 healthy control subjects. This study yielded the following main findings:

- The lower and upper values as well as the mean \pm SD of MUNIX, CMAP and MUSIX for each of the six muscles in the two age groups are detailed in table 9.2.
- The mean MUNIX values differed for each muscle (i.e. there was significant Muscle effect).
- MUNIX was lower in the elderly (significant Age effect) but there was no significant Gender effect.
- Using Spearman's rank correlation, there was a highly significant ($p < 0.00001$) positive correlation ($r = 0.79$) between MUNIX and CMAP. This finding persisted for overall data as well as for each one of the 6 muscles individually. This pattern was not affected by gender or age (Figures 9.1a and 9.1b).
- A highly significant ($p < 0.00001$), but medium ($r = -0.46$) negative correlation was noted between MUNIX and MUSIX (Figure 9.2).

9.3.2 Summary of results of MUNIX data: Poliomyelitis patients

Longitudinal MUNIX data was collected from 41 poliomyelitis survivors. Mixed results were obtained:

- There was no change in MUNIX/MUSIX/CMAP values over study period in the majority of participants. MUNIX values lower than normal were recorded in weak muscles (polio affected muscles).
- Disproportionally high MUSIX values were recorded from hypertrophied muscles while low MUNIX and CMAP values were recorded in weak atrophied muscles.

- A temporal increase in MUSIX values (without concomitant change in CMAP and/or MUNIX values) were recorded in 8 patients (20% of participants) around the time when patients experienced muscle pain. This was followed by return of MUSIX values to about 90-80% of the baseline (values before pain onset) after pain subsided. Some possible mechanisms explaining these findings are proposed in section 9.6.4 of this thesis.

9.3.3 Summary of results of MUNIX data: FTD patients

MUNIX, MUSIX and CMAP were collected longitudinally from 39 FTD patients (22 bvFTD, 9 nvPPA, 2 svPPA and 6 FTD-MND patients) as per protocol outlined in the methodology section. Results varied in different FTD subgroups:

- All nvPPA and svPPA patients: MUNIX, MUSIX and CMAP measurements were within normal range and showed no decline in MUNIX values over the study period.
- FTD-MND subjects: MUNIX values were below normal in the FTD-MND Patients and decreased over time, as expected.
- BvFTD participants: None displayed values in the ALS range, however, MUNIX values were below normal range (but above ALS range) in only 3 subjects (in 14% of bvFTD participants). Among the 22 bvFTD subjects 1 patient's data were below normal values in three muscles but showed no further decline on longitudinal follow up. This was observed in two upper limb muscles (APB and BB) and one lower limb muscle (AH). MUNIX values were also below normal in 2 further bvFTD patients. MUIX values in the latter 2 subjects showed further decline after 9 months (test 4 onwards), a decline at a rate that was lower than that observed in ALS patients. This decline was seen in all 6 muscles tested.
NB: there was no concomitant clinical features to suggest lower motor neuron involvement clinically in this subgroup of bvFTD patients with lower MUNIX values, i.e. there was no muscle weakness, fasciculations or wasting.

9.3.4 Summary of results of MUNIX data: ALS patients

MUNIX was recorded in 43 ALS patients. Of the 43 patients, (M: F ratio: 30:13), twenty-five patients were of spinal disease onset (58.1%), 12 of bulbar onset (27.9%) and 6 FTD-MND (14%). In the spinal onset group, 13 (30.2%) patients had upper limb and 12 (27.9%) patients had lower limb onset.

When comparing rates of decline for the three parameters of MUNIX, ALSFRS-R and CMAP with disease progression, MUNIX started to decline earlier than ALSFRS-R and it continued to decline at a significantly faster rate. CMAP declined at a slower rate when compared to MUNIX. MUNIX, CMAP and ALSFRS-R all declined in a parallel fashion but at different rates and at different start times: for all participants global MUNIX declined at an average rate of -3.62% (± 0.04) per month. Furthermore, the monthly rate of MUNIX decline was different in different muscles as it ranged between -2.6% (± 0.06) and -4.43 (± 0.07). The highest rate of MUNIX decline was recorded in the two small upper limb muscles of APB (-4.43% ± 0.07) per month followed by ADM (-4.24% ± 0.12) per month. The lowest decline rate was seen in BB (-2.61% ± 0.06) per month followed by AH muscle (-3.62% ± 0.06) per month. The mean monthly relative decline of ALSFRS-R from its baseline (which was defined as 100%) was -2.28% (± 0.05) per month or 0.82 ALSFRS-R points/month.

These findings suggest that MUNIX is a sensitive biomarker of diseases progression in ALS with a potential of therapeutic applications. Full description of findings is written in section 9.8 below.

9.4 Conclusions and main outcomes of MUNIX studies:

In summary, the main outcomes of our MUNIX studies included:

1. MUNIX normative data repository (LMN pool) was compiled from local healthy volunteers and from a cohort of patients with Poliomyelitis. We

used this pool in studying our disease groups and it can be used to improve our neurophysiology department reports.

2. The rate of decline of MUNIX overtime was determined for the three diseases that affect the lower motor neurone: FTD, ALS and Poliomyelitis. I.e. the role of MUNIX in monitoring disease states.
3. MUNIX was shown to be a promising marker of disease progression in ALS. When compared to ALSFRS-R, MUNIX started to decline earlier in ALS and continued to decline faster. This suggests that MUNIX can serve as an early and sensitive marker of both motor dysfunction and disease progression with a possibility of therapeutic applications in the future.
4. Potential for MUNIX as a new tool as an adjunct to careful clinical evaluation.
5. Potential for MUNIX to be used as a tool for earlier diagnosis of motor neuron disease and related conditions. Earlier diagnosis is essential as it may allow earlier access to therapeutic interventions.
6. The MUNIX findings from the longitudinal FTD study were interesting in many ways. Firstly, there was no decline over the study period in patients with nvPPA or svPPA. Secondly, MUNIX declined longitudinally in 20% of patients with bvFTD. Notwithstanding this finding was in congruence with reports by Burrell et al of lower motor neuron dysfunction in FTD using the neurophysiological index measure (203), it was in variance with previous claims by Catherine Lomen-Hoerth et al of needle EMG features to suggest MND in 33% of patients with bvFTD after one year follow up. This point is discussed in section 9.7 of this thesis.

Details of findings of MUNIX studies performed as part of this research, combined with full discussions of the significance of those findings are delineated in the next sections of this chapter.

9.5 MUNIX normative data study

9.5.1 Introduction

Motor Unit Number index (MUNIX) is a measure estimated by a noninvasive neurophysiological method that requires minimal electrical stimulation. It gives an index of functioning motor units. The technique involves utilizing the surface-recorded compound muscle action potential (CMAP) and electromyographic (EMG) interference pattern to compute the motor unit number index (MUNIX).

Both MUNE and MUNIX have been evaluated as biomarkers of motor unit decline in ALS. However there have been few MUNIX studies of healthy volunteers, and the degree of variance across healthy normal subjects has not been fully established, nor has the effect of gender or ageing been fully evaluated (145).

9.5.2 Objectives

- 1- The aim of this study was to establish MUNIX normative data profile by prospectively collecting normative data for MUNIX values in an Irish population to determine the degree of variance, effect of gender, and whether MUNIX declines in normal ageing.
- 2- A specific objective of this study was to utilize results of MUNIX normative data to improve quality of reports of our neurophysiology department and to study disease conditions.

9.5.3 Results

A total of 40 volunteers participated. All subjects were free of any neurological condition or conditions that might have affected MUNIX measurements as they were all free of any history of central or peripheral nervous system disease and their clinical neurological examination was normal. Subjects were positioned on a supine comfortable position during testing and all measurements were performed by the same examiner (T.O). Participants tolerated the procedure and reported experiencing no or minimal discomfort. The time required obtaining MUNIX measurements of all 6 muscles varied between 20 and 35 minutes. Mean age of participants was 47.2 years (range 23–80 years). 29 subjects were <60 years (11 males, 18 females) and 11 subjects were ≥60 years of age (8 males, 3 females) (Table 9.1).

Table 9.1: Demographic characteristics of MUNIX normative data study participants.

Study participants	Males =19 Females =21 Total =40
Age (all participants)	Mean: 47.2 years Range: 23-80 years
<60 years of age	Males =11 Females =18 Total = 29
≥60 years of age	Males = 08 Females = 03 Total =11

The three measures of MUNIX, CMAP and MUSIX values were recorded for the following muscles on the right side: APB, ADM, BB, TA, EDB and AH.

MUNIX decreased with patient's age as the mean MUNIX values (\pm SD) for all muscles defined by the two age groups were 175 (\pm 84.2) for those < 60 and 130 (\pm 57) for those \geq 60 years of age. For the healthy subjects the mean MUNIX was 163 with a standard deviation (SD) of 80.2 and the range was 52–501. Similarly the mean, and SD of CMAP amplitude were 9.3 mV and 3.7 mV respectively.

The lower and upper values as well as the mean \pm SD of MUNIX, CMAP and MUSIX for each of the six muscles in the two age groups are detailed in table 9.2.

Table 9.2: Showing lower and upper normal values of MUNIX, CMAP and MUSIX for six different muscles, for the whole cohort and stratified by 2 age groups.

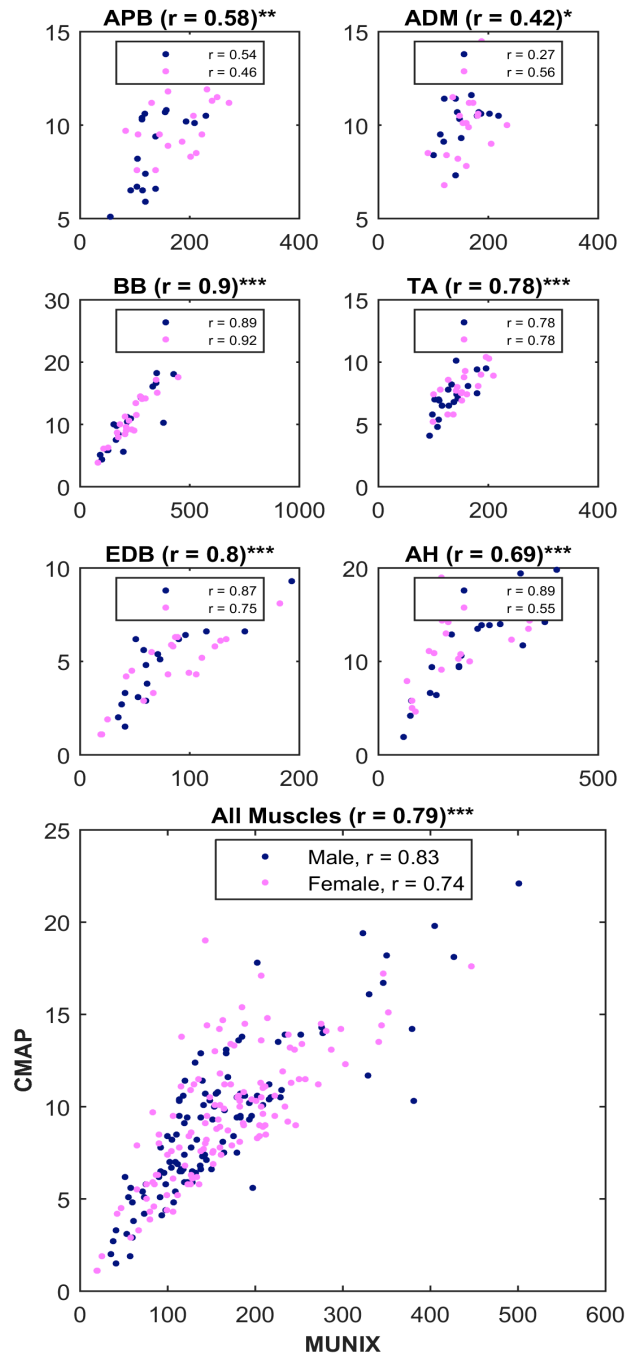
Muscle	MUNIX			CMAP			MUNIX			CMAP			MUNIX									
	APB	ADM	BB	TA	EDB	AH	APB	ADM	BB	TA	EDB	AH	APB	ADM	BB	TA	EDB	AH				
All	Mean (±sd)	164.5 (±79.3)	9.4 (±3.7)	61.0 (±17.4)	164.6 (±58.3)	161.7 (±34.6)	236.2 (±100.9)	145.6 (±34.7)	83.9 (±38.9)	192.0 (±89.9)	9.8 (±2.2)	10.7 (±1.9)	11.0 (±4.4)	7.7 (±1.7)	5.2 (±1.9)	11.8 (±4.1)	65.0 (±19.9)	67.3 (±15.0)	47.8 (±6.5)	53.8 (±8.6)	63.9 (±17.0)	68.7 (±21.7)
	Min	25.0	1.5	28.2	55.0	90.0	80.0	93.0	25.0	65.0	5.1	6.8	3.9	4.1	1.5	4.2	40.0	35.8	28.2	41.6	38.1	35.6
	mean c _i	154.8	8.9	59.0	147.1	151.6	207.2	135.4	73.0	166.4	9.1	10.1	9.7	7.2	4.6	10.5	59.6	62.7	45.8	51.3	58.8	62.6
	mean c _h	175.0	9.9	63.5	182.5	172.3	269.1	156.3	97.7	223.0	10.4	11.2	12.4	8.2	5.8	13.1	72.0	72.0	49.7	56.6	69.4	76.2
	Max	501.0	22.1	132.5	287.0	240.0	501.0	211.0	193.0	405.0	13.9	14.5	22.1	11.1	9.3	19.8	118.7	95.3	65.3	74.2	101.7	132.5
<60 years	Mean (±sd)	176.3 (±83.6)	10.0 (±3.7)	61.5 (±18.5)	179.3 (±59.7)	164.7 (±31.8)	264.5 (±96.0)	154.6 (±35.0)	87.5 (±41.3)	204.2 (±94.0)	10.4 (±1.8)	11.1 (±1.9)	12.2 (±4.0)	8.2 (±1.6)	5.4 (±1.9)	12.8 (±3.8)	64.4 (±21.5)	67.1 (±14.3)	47.5 (±6.0)	53.9 (±9.5)	65.4 (±17.7)	71.0 (±24.2)
	Min	25.0	1.9	35.6	83.0	113.0	80.0	93.0	25.0	65.0	6.5	6.8	3.9	4.1	1.9	5.0	40.0	35.8	36.7	41.6	40.7	35.6
	mean c _i	164.8	9.5	59.0	157.9	154.2	232.9	142.3	73.8	173.4	9.7	10.4	10.8	7.6	4.7	11.5	57.4	61.8	45.6	50.9	59.3	63.0
	mean c _h	189.7	10.6	64.4	200.5	176.9	302.2	167.1	104.0	241.4	11.0	11.7	13.7	8.7	6.1	14.2	73.1	72.1	50.0	57.7	72.1	80.4
	Max	501.0	22.1	132.5	287.0	240.0	501.0	211.0	193.0	405.0	13.9	14.5	22.1	11.1	9.3	19.8	118.7	95.3	65.3	74.2	101.7	132.5
≥60 years	Mean (±sd)	132.7 (±55.4)	7.6 (±3.0)	59.7 (±14.0)	125.6 (±31.5)	153.6 (±41.7)	161.5 (±73.9)	122.0 (±20.3)	73.7 (±30.7)	156.5 (±68.6)	8.2 (±2.4)	9.6 (±1.5)	7.7 (±3.6)	6.5 (±1.3)	4.4 (±1.6)	9.0 (±3.9)	66.7 (±15.5)	67.9 (±17.4)	48.4 (±8.0)	53.6 (±6.2)	59.9 (±14.6)	62.0 (±9.9)
	Min	41.0	1.5	28.2	55.0	90.0	91.0	98.0	41.0	73.0	5.1	7.3	4.4	4.8	1.5	4.2	48.3	48.3	28.2	44.3	38.1	49.6
	mean c _i	120.2	6.9	56.5	104.8	129.9	130.8	111.6	60.9	117.4	7.0	8.8	6.2	5.9	3.4	6.7	58.8	59.1	42.0	50.1	51.4	57.1
	mean c _h	147.6	8.4	63.3	140.5	176.0	222.1	134.9	102.3	198.6	9.8	10.5	10.6	7.4	5.3	11.4	76.3	78.5	51.8	57.1	68.9	69.1
	Max	346.0	16.7	94.7	167.0	218.0	346.0	158.0	150.0	252.0	13.1	12.4	16.7	9.3	6.6	13.9	92.6	94.7	57.6	62.1	84.3	81.1

The mean MUNIX values differed for each muscle (significant Muscle effect). MUNIX was lower in the elderly (significant Age effect). The mean MUNIX values differed for each muscle, and MUNIX recordings also change as a function of age. This effect was primarily observed in BB. There was also significant Muscle x Age effect). There was no significant Gender effect overall.

Descriptive statistics and ANOVA results were individually calculated for each measure of MUNIX, MUSIX and CMAP.

Figures 1a and 1b show the correlation between MUNIX and CMAP for each muscle and for groups of muscles, using Spearman's rank correlation. There was a highly significant ($p < 0.00001$) positive correlation ($r = 0.79$) between MUNIX and CMAP. This finding persisted also for each one of the 6 muscles individually. This correlation was generally stronger in larger muscles (strongest in BB) and was weaker in small muscles (weakest in ADM). This pattern was not affected by gender or age (Figure 9.1a and figure 9.1b).

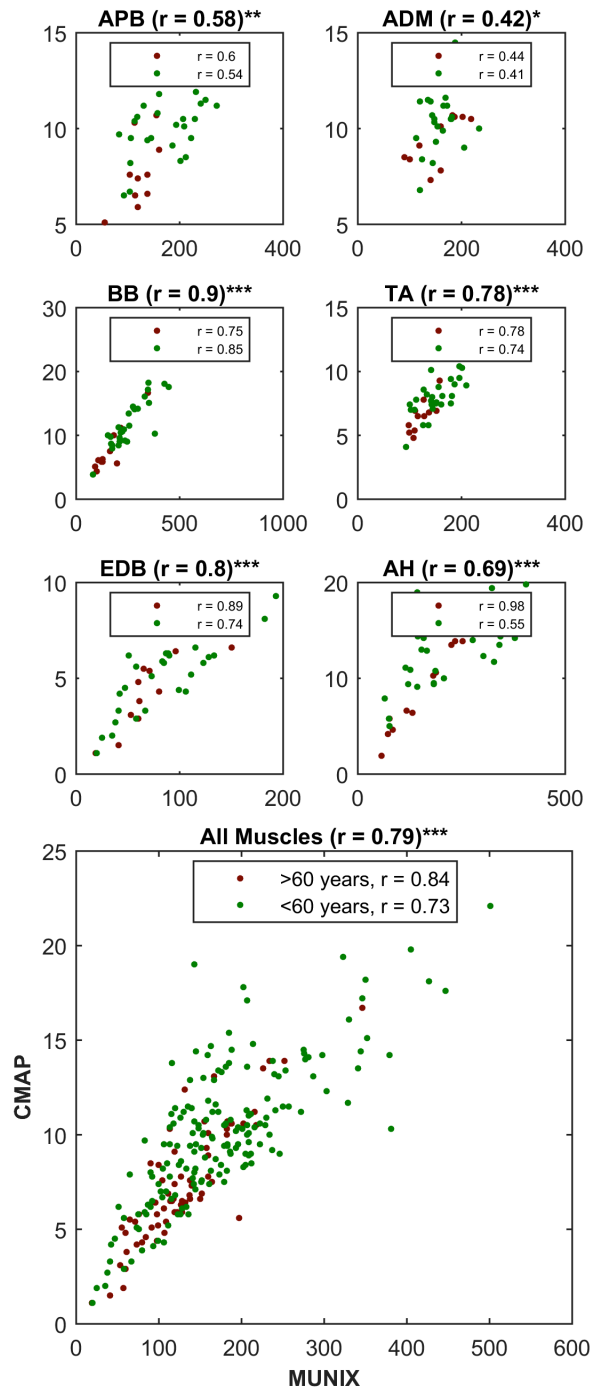
MUNIX - CMAP, Spearman Correlation



* $p < 0.05$, ** $p < 0.001$, *** $p < 0.00001$

Figure 9.1a: The relationship between CMAP and MUNIX in pooled and individual muscles, separated by gender. MUNIX value is strongly determined by CMAP amplitude, a relation that holds for overall values as well as for each of the individual muscles and is not affected by gender

MUNIX - CMAP, Spearman Correlation

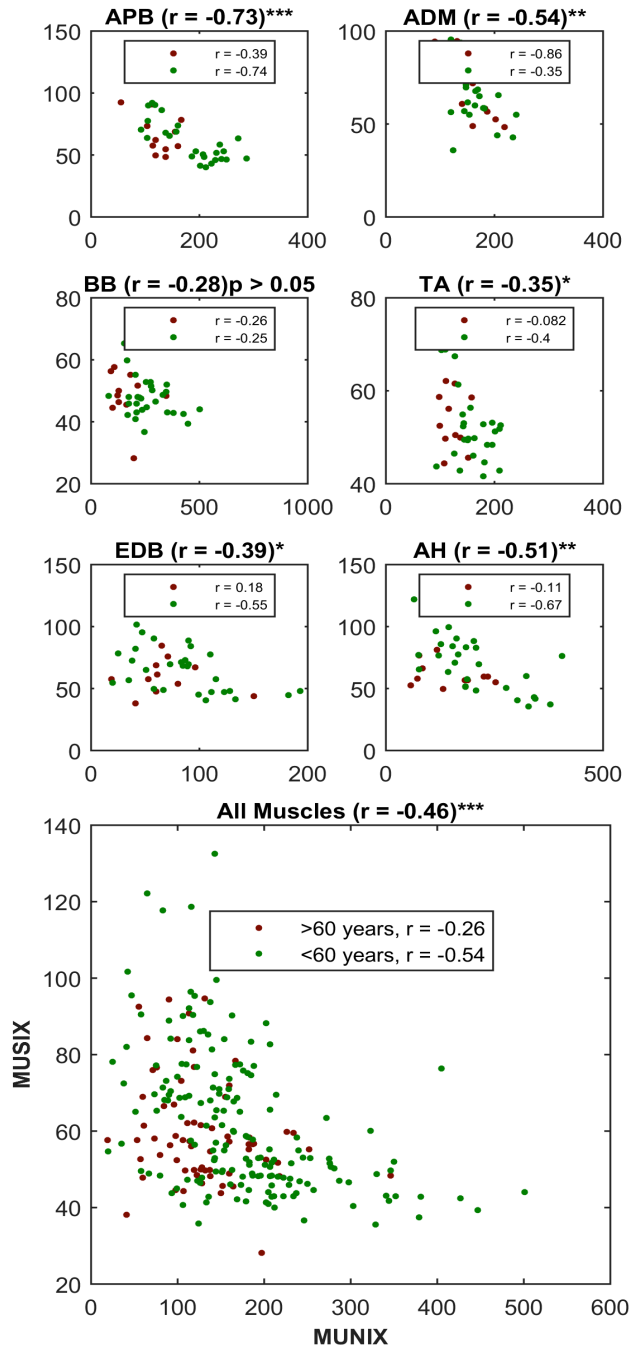


* $p < 0.05$, ** $p < 0.001$, *** $p < 0.00001$

Figure 9.1b: The relationship between CMAP and MUNIX in pooled and individual muscles, separated by age. MUNIX value is strongly determined by CMAP amplitude, a relation that holds for overall values as well as for each of the individual muscles and is not affected by age.

A highly significant ($p < 0.00001$), but medium ($r = -0.46$) negative correlation was noted between MUNIX and MUSIX. This correlation persisted for overall data as well as for each individual muscle (figure 2). Of note is that in those ≥ 60 this correlation degraded, especially in TA, AH and EDB (Figure 9.2).

MUNIX - MUSIX, Spearman Correlation



* $p < 0.05$, ** $p < 0.001$, *** $p < 0.00001$

Figure 9.2: The relationship between MUSIX and MUNIX in pooled and individual muscles, separated by age. A significant but moderate negative correlation is shown between MUNIX and MUSIX, persisting for overall data as well as for each of the 6 muscles individually

9.5.4 Discussion

The main advantage of MUNIX when compared to MUNE is that MUNIX is fast, non-invasive and much more convenient to patients. This advantage enables testing about 5 to 6 muscles at the same time length that is needed to test one muscle using any MUNE technique. The aim of our study was to prospectively collect normative data of MUNIX values in a number of muscles. Our data indicated that MUNIX measurements are reproducible (reproducibility is defined as mean of the absolute individual differences in test–retest study), as the intra-rater reproducibility analysis, which was performed in four subjects, revealed a mean Coefficient of Variation (COV) of 0.13 or below 9% when using standard deviation and mean. These data are congruent with previously published figures from studies using well-established MUNE techniques (159-161). Simmons et al has studied MUNE reproducibility in normal controls (162). Although they reported good intra-operator correlation, coefficient of variation was not reported in their study and the number of subjects was small and recruited within a narrow range. One MUNIX study of a comparable scale exists in the literature; Neuwirth et al (171) collected MUNIX normative data by testing 5 muscles of 66 subjects from 6 centers. Normative data for our population is comparable to results of this multi-centre study in many ways despite the fact that our study showed slightly smaller than expected MUNIX values in the EDB and we ascribe this to the variability of the CMAP in this muscle.

Our data indicated strong correlation between MUNIX and the CMAP amplitude. We found relatively stronger correlation between MUNIX and CMAP in larger muscles when compared to smaller ones indicating greater relative contribution of CMAP variability to MUNIX variability in the former muscles. Participants were tested unilaterally (right side muscles) and all 6 muscles tested in all participants were included in final analysis. However, our finding that MUNIX changes with age is

congruent with previously documented age related decrease in muscle mass preferentially affecting postural muscle and reflecting age-related loss of motor neurons (170). The reduction in MUNIX could also signal the presence of mild, asymptomatic radiculopathy in the older age group.

There are a number of reasons for choosing the age of 60 as a cut-off point: Previous studies proved that the most basic finding in aged motor unit morphology is a loss in total unit number by approximately 1% of the total number per year, beginning in the third decade and dramatically increases in percentage at 60, suggesting that the age of 60 is a marker for radical age-related decrease in the number of motor units (163-165). Furthermore, both Tomlinson et al (166) and Gawel et al (167) reported that no evidence exists of loss of motor neurons up to the age of 60 years, but beyond that age, and although individual counts vary considerably, motor neuron counts of only 50% of the counts in early adult life or middle age.

Of note, we did not intend to compare MUNIX values with published MUNE data however; a general agreement was seen for ADM, BB and TA (150). A future multicenter study comparing MUNE to MUNIX will be required to further test this hypothesis.

From a neurophysiology perspective, these data are particularly valuable for several reasons. First, the data presented above are consistent with prior reports that established MUNIX normative data; we have now extended these findings to an Irish population.

Although our study, which is the largest single centre age-specific normative dataset for MUNIX is limited by number of subjects above the age of 60, the observation that MUNIX can be shown to decline with age in a similar pattern to CMAP is of interest and requires further exploration. A second limitation is that the intra-rater reliability test was performed in 4 subjects only but this would not have affected results as the first MUNIX measurement from the right side was used for defining the lower normal limit as per

study design. The finding also underpins the importance of using age-specific normative values in the elderly population.

9.5.5 Conclusions

We have shown that MUNIX is easy to record in both proximal and distal muscles in patients of all ages and can be done with minimal discomfort to patients. Generation of a normative dataset is of value to further studies in patients with diseases of motor nerve and possibly other neurological conditions, and our important observation of age-related decline in MUNIX in those over the age of 60 reflected this.

Our study established MUNIX data profile of a cohort of normal controls and determined the degree of variance as well as the effects of gender and age.

Our data confirmed that MUNIX technique has potential as an alternative to MUNE, given the practical advantages of the noninvasive former test resulting in minimal discomfort to patients, as there is no need to use repetitive nerve stimulation or needles.

These advantages make MUNIX a potentially ideal tool that is particularly powerful in the follow-up of patients with lower motor neuron loss, where patients serve as their own controls. Our data showed that MUNIX values are comparable to previously published ones and we have contributed to previously published normative data pool.

Following normative data collection, we performed longitudinal MUNIX studies on cohorts of patients with illnesses that primarily affect the lower motor neuron (LMN): ALS (a progressive disease affecting the LMN) and Poliomyelitis (a non-progressive disease of the LMN). Poliomyelitis study also served the function of compiling a repository of pure LMN MUNIX data). Longitudinal MUNIX testing was also performed on FTD patients where the possibility of neurophysiologic evidence of lower motor

neuron dysfunction in this condition was examined. Detailed descriptions of findings from these longitudinal studies are given in the next sections.

9.6 MUNIX Poliomyelitis study

9.6.1 Introduction

Poliomyelitis was studied as an example of a non-progressive disorder affecting the lower motor neuron. The clinical features of polio range from mild asymptomatic illness (abortive poliomyelitis), aseptic meningitis (nonparalytic poliomyelitis), to paralytic poliomyelitis. Poliomyelitis survivors often live with residual physical deficits associated with their condition. Many present with late effects of polio including reduced mobility, deformities, pain and deconditioning. Approximately 50 percent of Polio survivors develop a range of symptoms many years after the acute paralytic phase of the illness, known as Postpolio Syndrome (154). Postpolio syndrome symptoms include deterioration of weakness in previously affected limb(s), new onset weakness, fatigue and psychiatric manifestations that can lead to functional disability and can worsen quality of life. There is an estimated 7,500 polio survivors in Ireland following epidemics of Poliomyelitis in the country in the 1940s and 1950s (153).

This longitudinal MUNIX study in polio survivors served the functions of both establishing a repository of pure LMN data and performing a comparative study with FTD and ALS.

9.6.2 Aims (specific aims)

- The aim of this study was to establish MUNIX repository of poliomyelitis patients (polio was studied as an example of a non-progressive lower motor neuron disorder) by performing longitudinal MUNIX measurements on polio survivors and by determining the rate of MUNIX decline, if any, over time.

- Another aim was to compare patient characteristics and disease parameters between the subgroup in which MUNIX declines over time and the rest of the study cohort.

9.6.3 Results

9.6.3.1 Demographics of participants

A total of 41 poliomyelitis patients participated in this longitudinal MUNIX study. Patients were divided into two subgroups (spinal or spinobulbar), depending on their polio onset types. Patients underwent MUNIX testing at 3-monthly intervals for a total duration of 21 months (8 visits). All patients tolerated the procedure well, there were no technical issues and all participants reached the end of the study (visit 8).

The male to female ratio was 19:21. There were 34 participants in the spinal subgroup and 7 participants in the spinobulbar subgroup.

Mean age (\pm SD) of patients was 62.1(\pm 6.8) years and there was no significant difference in age between the two subgroups. Mean disease duration at the time of first measurement was 59.8(\pm 6.6) years for the overall cohort (59.5 \pm 6.9 years and 60.9 \pm 5.0 years for the bulbar and spinobulbar subgroups respectively).

Table 9.3 below details demographic characteristics and disease parameters for overall cohort as well as for each of the two subgroups.

Table 9.3: Participants' demographic characteristics. APP=acute paralytic polio, PPS=post polio syndrome.

	Poliomyelitis onset type (subgroups)		
	Spinal	Spinobulbar	Total
Number	34	7	41
M: F ratio	15:19	4:3	19:22
Age at MUNIX test 1 (y)	61.9±7.2	63.0±4.4	62.1±6.8
Polio duration (y)	59.5±6.9	60.9±5.0	59.8±6.6
APP onset age (y)	2.2±1.9	2.1±1.3	2.1±1.8
APP duration (y)	4.3±3.4	1.8±1.3	3.7±3.1
PPS onset age (y)	51.9±8.8	44.6±6.3	50.3±8.7
PPS duration (y)	8.2±4.9	9.2±4.3	10.5±10.5
APP to PPS (y)	50.0±8.2	42.4±7.7	48.4±8.6

9.6.3.2 General data trends and longitudinal changes

The following mixed results were concluded:

There were no changes in the values of MUNIX, MUSIX or CMAP during the study period in the majority (83%) of participants.

Slight progression (decline in MUNIX values) was recorded in only 7 participants (17%). There was no differences detected when this cohort of polio patients in which MUNIX values declined longitudinally was analysed as a subgroup and was compared to the overall cohort and to the rest of patients (as regards to difference in: age at test1, age at polio onset, gender, polio onset type, polio duration, post-polio syndrome (PPS) symptoms, PPS age at onset, PPS duration and PPS recovery type).

Figure 9.3 shows the MUNIX values corresponding to the six tested muscles for all subjects across the 8 recording sessions. Supplementary figures show the MUNIX, MUSIX and CMAP values in a similar format for all patients, as well as individually for patient subgroups (spinal/spinobulbar) are available from the author.

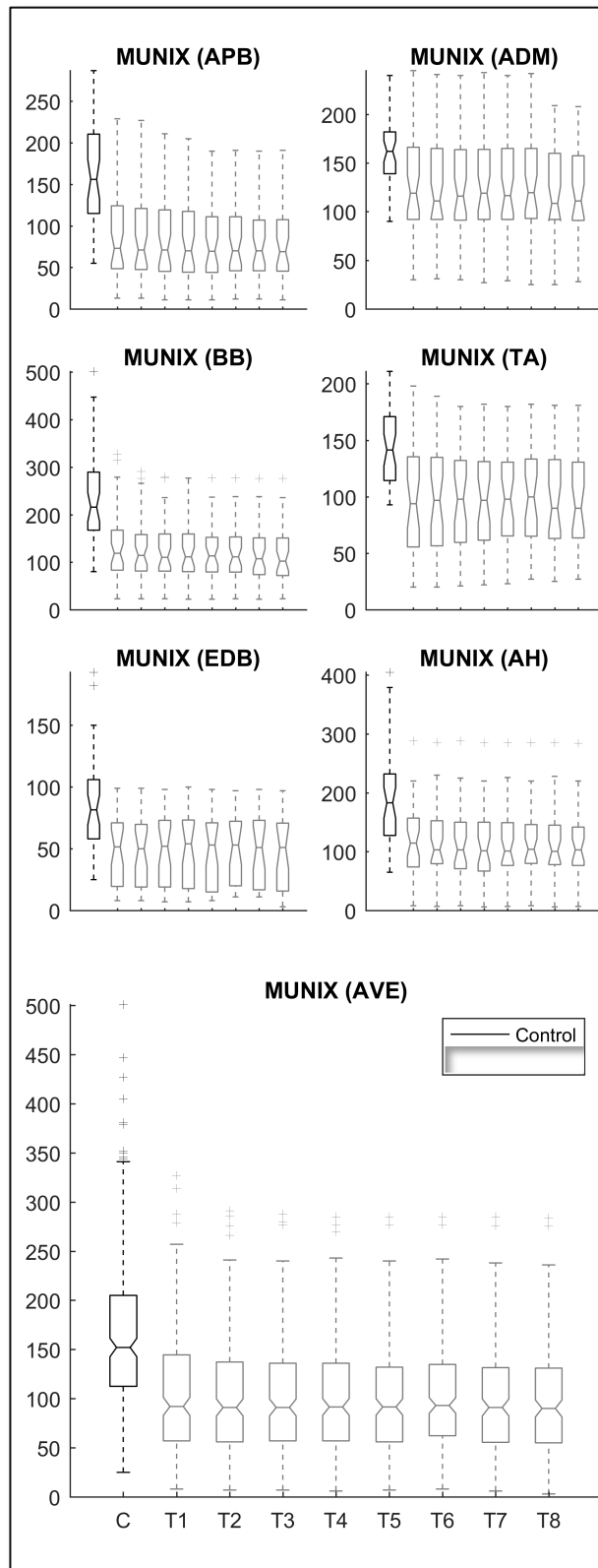


Figure 9.3: MUNIX values corresponding to the six tested muscles for all subjects, across the 8 recording sessions

Lower than normal MUNIX and CMAP values (healthy control values) were recorded in weak muscles (polio affected muscles) as expected, while disproportionately high MUSIX values were obtained from hypertrophied muscles.

9.6.3.3 Change of MUNIX values over study period:

Figure 9.4 shows the MUNIX pattern of change as a function of the time during study period, as well as the corresponding regression line and equation. Similar results for MUNIX, MUSIX and CMAP of all patients and their subgroups are available as supplementary material.

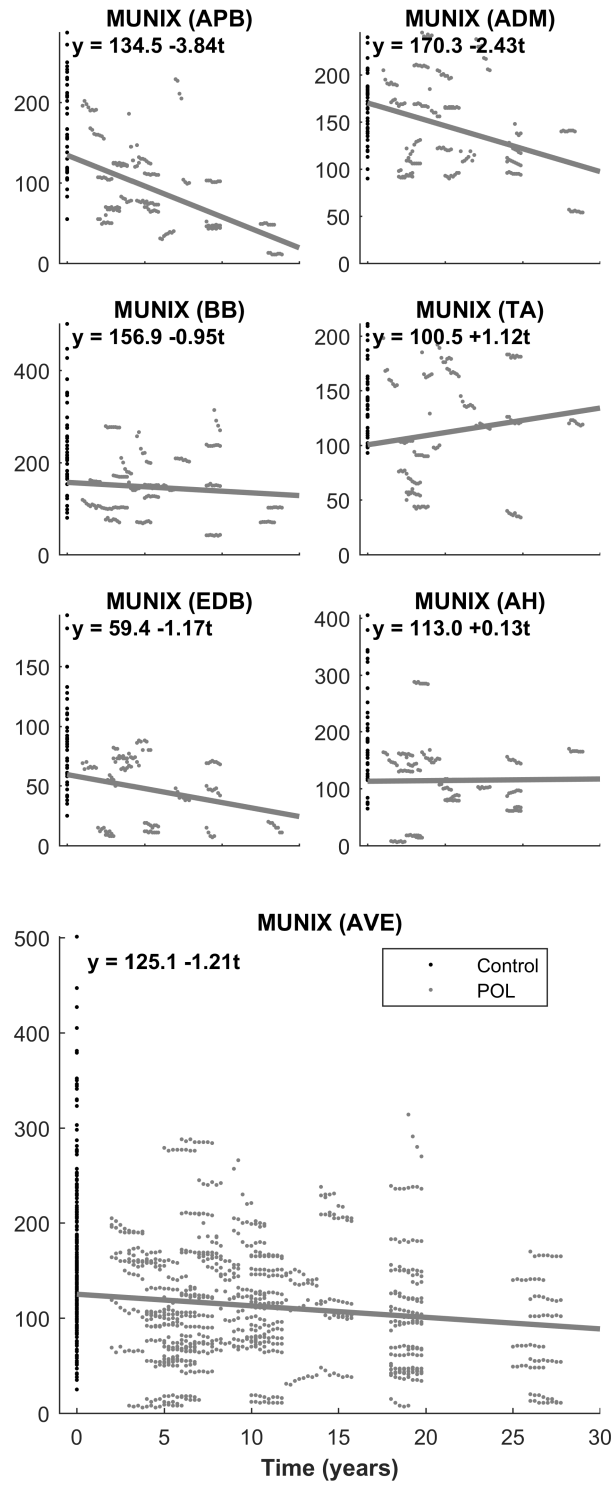


Figure 9.4: showing MUNIX pattern of change as a function of the time during study period, as well as the corresponding regression line and equation.

9.6.3.4 Relationship between MUSIX values and muscle pain in a subgroup of Polio participants

A temporal increase in MUSIX values was noticed in some participants around the time when patients experienced muscle pain (n=8, 19.5%). There was no concomitant change in CMAP and/or MUNIX. This was followed by return of MUSIX values to about 80-90% of the baseline (values before pain onset) after pain subsided. This group was analysed as a subgroup and was compared to the overall cohort and to the rest of participants (rest of participants as a separate group) as regards to: age at test1, age at polio onset, gender, polio onset type, polio duration, post-polio syndrome (PPS) symptoms, PPS age at onset, PPS duration and PPS recovery type. There were no statistically significant differences detected between this subgroup and rest of participants in all above disease metrics. The pattern of change in MUSIX values in the two groups of pain/no pain, across the six muscles tested throughout the study period (8 visits in total) is plotted graphically in figure 9.5.

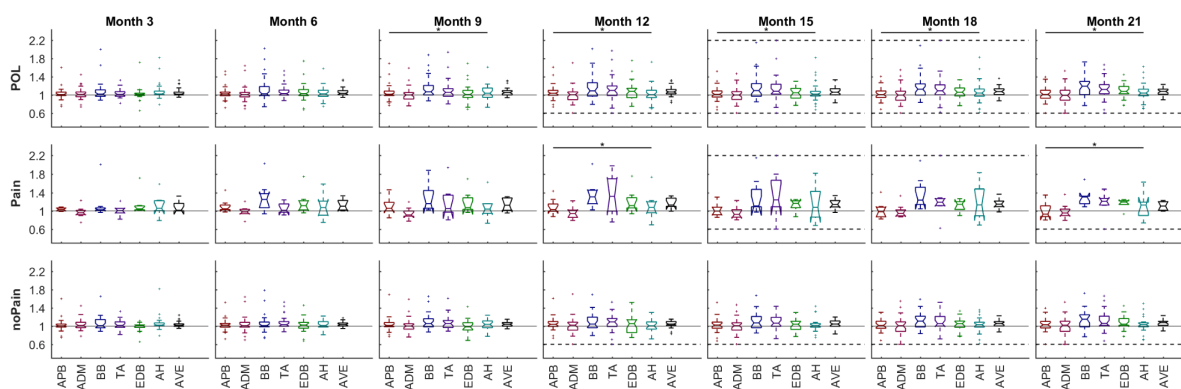


Figure 9.5: Diagrammatic presentation comparing the of the pattern of change in MUSIX values in the two polio groups of pain/no pain in all visits.

The ranges of MUSIX values were wider in the group of patients who experienced pain when compared to the rest of the cohort (figure 9.7).

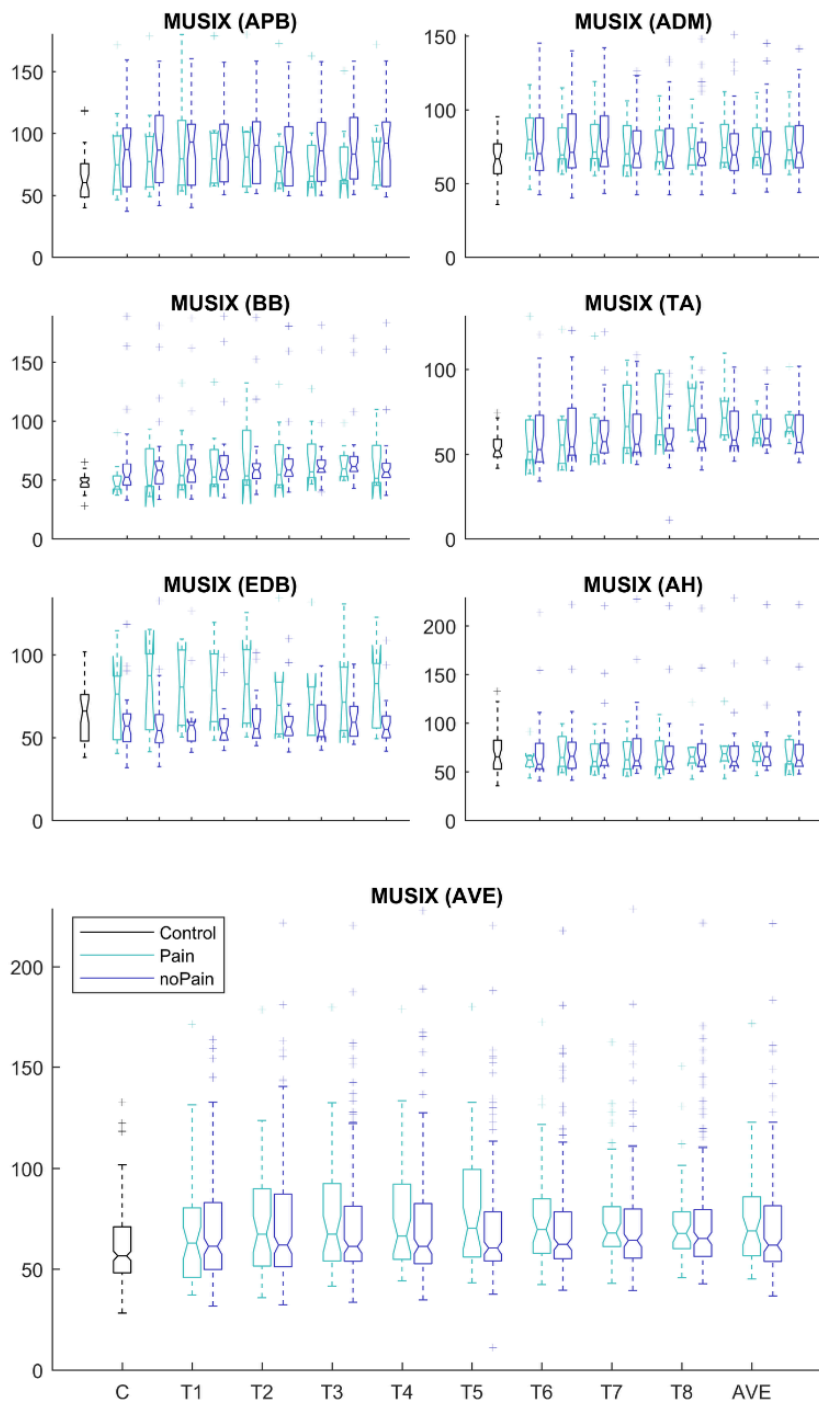


Figure 9.7: showing a relatively wider ranges of MUSIX values in the group of polio patients who experienced muscle pain compared to the group with no pain.

9.6.3.5 Effect of Muscle, Age, Gender, and ALS Phenotype on MUNIX/MUSIX/CMAP:

The results from analysis of variance are provided in Table 9.4 for MUNIX, MUSIX and CMAP, showed significant effects for age, phenotype, muscle, but no effect for time.

Table 9.4: The results from analysis of variance for MUNIX, MUSIX and CMAP, showing significant effects for age, phenotype, muscle, but no effect for time.

	MUNIX	MUSIX	CMAP
Muscle	P<0.00001	P<0.00001	P<0.00001
Age	P<0.001	P<0.05	P<0.00001
Time	P>0.05	P>0.05	P>0.05
Phenotype	P<0.05	P<0.00001	P<0.01

9.6.3.6 Normalised Decline and Rate of Decline:

To reduce inter-individual variability, the values for each measure in tests 2-8 were normalised by the values in test 1. To further account for inter-individual variability and more objectively quantify the rate of decline, the percent change in any measure in

each 3-month interval was used to find the normalised rate of decline in percent change per month.

The ANOVA showed no significant effect of gender, Polio type, or time for the decline rates of either measures (MUNIX, MUSIX, CMAP). There was a significant muscle effect for MUNIX (Table 9.5). There was no effect of partial or complete recovery.

Table 9.5: ANOVA showing no significant effect of gender, Polio type, or time for the normalized decline rates of either measures (MUNIX, MUSIX, CMAP) but significant muscle effect for MUNIX.

	MUNIX	MUSIX	CMAP
Muscle	P<0.00001	P<0.05	P<0.001
Phenotype	P>0.1	P.0.1	P>0.01
Time	P>01	P>0.1	P>0.1
Muscle*Phenotype	P>0.1	P>0.1	P.0.1
Phenotype*Time	P>0.1	P>0.1	P>0.1

Figure 9.5 shows the normalised rate of decline (% per month) for MUNIX in the 6 tested muscles, and on average. Stars (*) denote significant difference between subgroups, based on Kruskal-Wallis test, after multiple-comparison correction using adaptive false discovery rate (Benjamini and Hochberg 1995; Benjamini et al. 2006). Supplementary Figures showing the same rate of decline for MUNIX, MUSIX and CMAP in all patients and Polio subgroups are available.

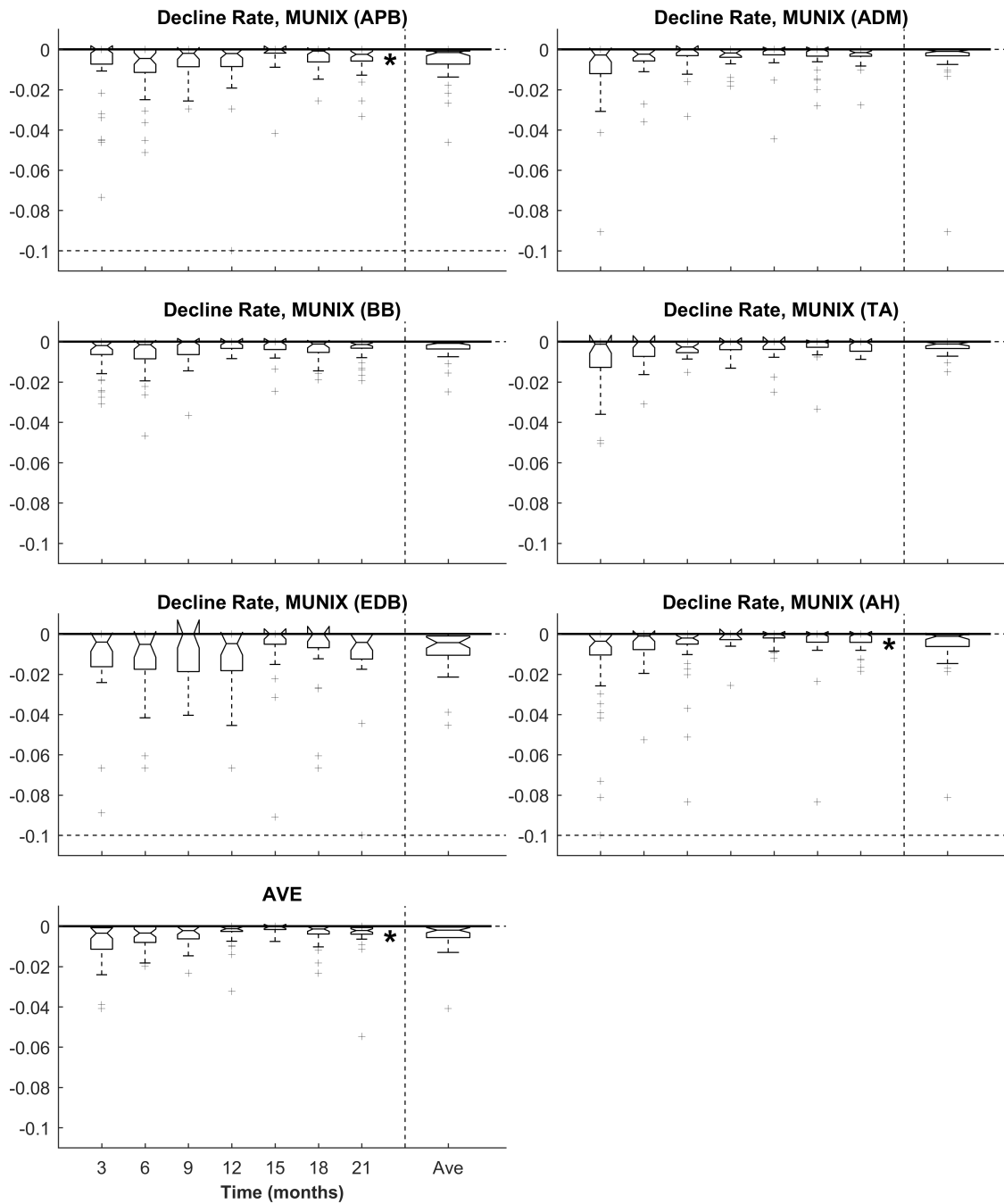


Figure 9.5: The normalised rate of decline (% per month) for MUNIX in the 6 tested muscles, and on average. Stars (*) denote significant difference between subgroups.

9.6.4 Discussion

Late neurophysiologic changes of Poliomyelitis has been described in the literature using conventional EMG (232), but up to this date, MUNIX has been applied in only one study where only one muscle was studied in Poliomyelitis (144). In this study the researchers applied MUNIX technique to demonstrate the reduced number of MUs and increased MU size in Tibialis anterior muscle in 33 prior polio patients (144).

Our study is the first ever study that applied MUNIX in multiple muscles that included upper and lower limb, proximal and distal muscles in Poliomyelitis.

We noticed temporal increase in MUSIX values in 8 participants (19.5%) around the time when patients experienced muscle pain. There was no concomitant change in CMAP and/or MUNIX values. MUSIX values returned to about 80-90% of its baseline value (values before pain onset) after pain subsided. This subgroup was not different when compared to the overall cohort and to the rest of participants (rest of participants as a separate group), as regards to the metrics of: age at test1, age at polio onset, gender, polio onset type, polio duration, post-polio syndrome(PPS) symptoms, PPS age at onset, PPS duration or PPS recovery type. This observation is interesting and needs further research. We postulate some theories to explain this temporal increase in MUSIX values during muscle pain periods: some form of an inflammatory process might have occurred at the time of muscle pain and this proposed inflammation might have caused temporal local oedema and swelling that resulted in increasing the size of motor units and hence increased MUSIX values. A second possibility is that this observation probably was the result of technical difficulty/inaccuracy during pain periods. A third possibility is that pain might have triggered a pathway leading to a surge in reinnervation and motor unit instability.

This longitudinal poliomyelitis study yielded MUNIX values that helped in compiling pure LMN repository. The pattern of MUNIX progression over time in Poliomyelitis also provided meaningful insights to studying lower motor neuron dysfunction in FTD using the same neurophysiologic technique.

The main limitation of this study is its relatively short duration. A longer research would be better in determining longitudinal changes in such chronic disease like poliomyelitis.

9.6.5 Conclusion

In summary, this is the first study of MUNIX in Poliomyelitis of this scale. Our observation of the effect of pain on MUSIX needs to be studied further in future research perhaps using a combination of neurophysiological techniques.

9.7 MUNIX longitudinal FTD study

9.7.1 Introduction

Frontotemporal dementia, a primarily upper motor neuron disease, may also cause lower motor neuron (LMN) dysfunction (203). There is robust evidence for the overlap between FTD and ALS (section 1.4.6 of this thesis). Such evidence has been confirmed by clinical and epidemiological (103, 104), neuropsychological (108, 109), pathological (6, 21) and genetic (54,67) studies. However, and to the best of the author's knowledge, only two neurophysiological studies exist in the literature that investigated this association between FTD and MND: The first study was conducted by Catherine Lomen-Hoerth et al estimated the rate of MND in FTD patients using needle EMG (section 9.7.4 of this thesis), (107). The second study was conducted by Burrell et al and investigated the lower motor neuron dysfunction in FTD using the Neurophysiological Index technique (section 9.7.4 of this thesis) (203).

Therefore, there was an urging need to quantify neurophysiologically the degree of anterior horn involvement and lower motor neuron dysfunction in FTD. We applied the novel technique of MUNIX longitudinally as a means of neurophysiological biomarker profiling and quantifying LMN dysfunction in FTD and its subtypes.

9.7.2 Aims

- To perform longitudinal study of MUNIX in the FTD cohort to quantify lower motor neuron dysfunction in FTD by analysing the pattern of progression over the study period. The hypothesis here was that FTD, a primarily upper motor neuron disease, also causes lower motor neuron (LMN) dysfunction and this LMN dysfunction varies in the different FTD subtypes of bvFTD, nfvPPA, svPPA and FTD-MND.
- To compare findings among FTD subtypes of: bvFTD, svPPA, nfvPPA and FTD-MND (group comparison). Groups were compared with reference to: FTD subtype, age at symptom onset, symptom onset to MUNIX test1 and gender.
- To apply MUNIX as a means of neurophysiological biomarker profiling in FTD.

9.7.3 Results

9.7.3.1 Demographic characteristics of participants

Thirty-nine FTD patients (22 bvFTD, 9 nfvPPA, 2 svPPA and 6 FTD-MND) participated in this longitudinal MUNIX study. The male to female ratio was 19:20. Participants underwent MUNIX testing at 3-monthly intervals for a total duration of 21 months (8 visits), as per protocol described in the methodology chapter of this thesis.

The mean age (\pm SD) of patients at test1 was 65.3 (\pm 7.0) years while mean symptom duration was 27.6 (\pm 11.3). The demographic characteristics of participants is described in table 9.6.

Table 9.6: Demographic characteristics of FTD MUNIX participants

FTD subtype	Number	M: F	Age at test1, yrs (\pm SD)	Symptom duration, months (\pm SD)
bvFTD	22	12:10	64.5 (\pm 7.1)	31.2 (\pm 10.2)
nfvPPA	9	3:6	69.0 (\pm 5.7)	18.9 (\pm 10.0)
svPPA	2	1:1	56.5 (\pm 2.1)	23.0 (\pm 7.8)
FTD-MND	6	4:2	72.3(\pm 8.5)	15.7(\pm 15.0)
Total	39	19:20	65.3 (\pm 7.0)	27.6 (\pm 11.3)

9.7.3.2 Results of subgroup analyses and longitudinal changes in MUNIX, MUSIX and CMAP

All nfvPPA and svPPA patients' MUNIX, MUSIX and CMAP measurements were within normal range and showed no decline in MUNIX values over the study period.

Figure 9.6 shows the MUNIX values corresponding to the six tested muscles for all subjects across the 8 recording sessions. Supplementary figures showing MUNIX, MUSIX and CMAP values in a similar format for all patients, as well as individually for patient subgroups (<60/ \geq 60 years of age, male/female, bvFTD/nfvPPA/svPPA,FTD-MND) are available with the author.

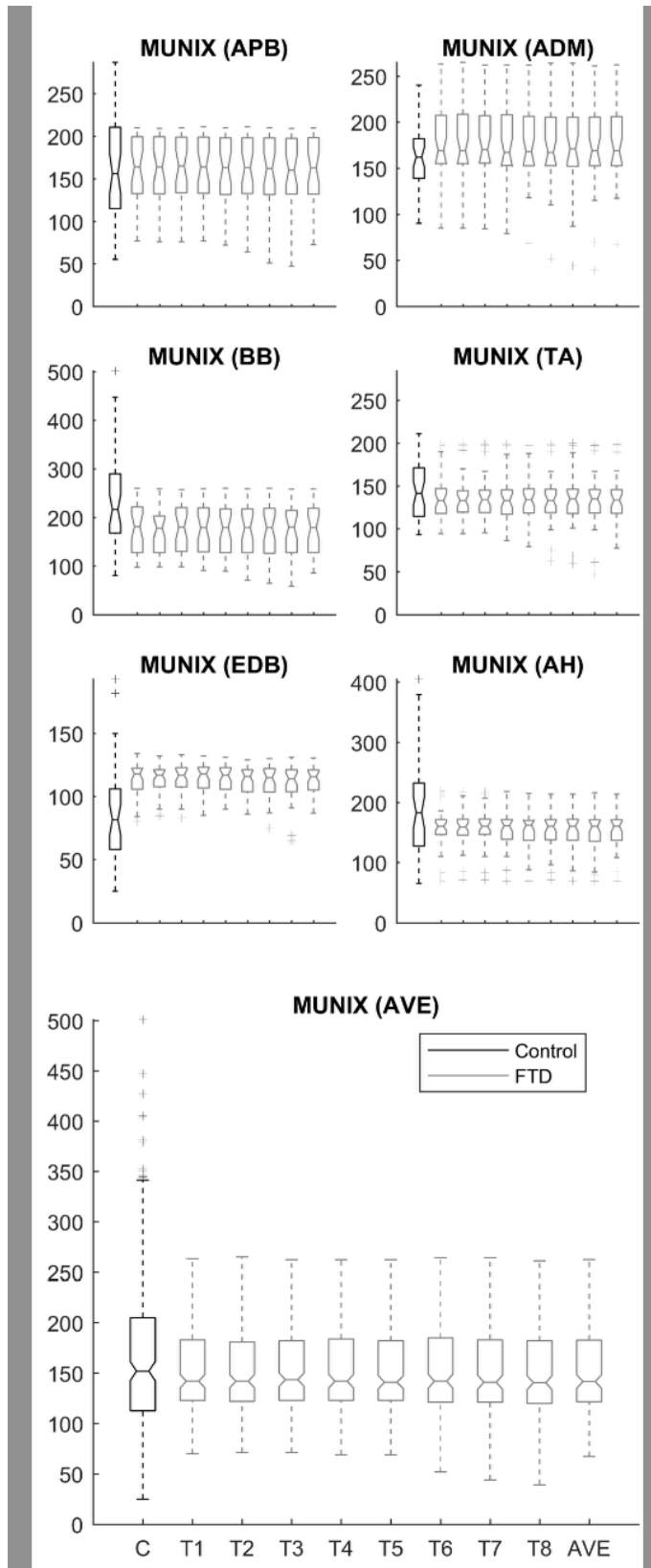


Figure 9.6: MUNIX values for FTD participants corresponding to the six tested muscles for all subjects across the 8 recording sessions

MUNIX values for FTD-MND subgroup were below normal control values, as expected.

9.7.3.3 Pattern of longitudinal MUNIX decline in the bvFTD subgroup:

None of the 22 bvFTD patients displayed MUNIX values in the ALS range, however, interestingly MUNIX values were below normal range (but above ALS range) in a subgroup of 3 subjects (14%).

Among the 22 bvFTD subjects, one patient's MUNIX values were below normal range in three muscles at test1 but showed no further decline on longitudinal follow up. These low MUNIX values for this participant were observed in two upper limb muscles (APB and BB) and one lower limb muscle (AH).

MUNIX values were also below normal in 2 further bvFTD patients at test1. Low MUNIX values in the latter two subjects remained stable before showing further decline after 9 months of follow up (test 4 onwards), a decline at a rate that was lower than that observed in ALS patients. This decline was seen in all 6 muscles tested.

NB: There were no detectable clinical signs of lower motor neuron involvement in those 3 patients, i.e., there was no muscle weakness, wasting or fasciculations.

9.7.4 Discussion

The main findings of our longitudinal MUNIX study in FTD were: firstly, there was no decline in MUNIX values after 2 years follow up of nvPPA and svPPA patients. Secondly, in bvFTD patients some decline in MUNIX values was seen in 3 patients out of 22 (14% bvFTD of patients). Moreover, the rate of decline in MUNIX values in those three patients was much less than that seen in ALS patients. This contrasts the results from a previous needle EMG study conducted in FTD patients by Lomen-Hoerth et al (136). In the study by Lomen-Hoerth et al it was reported that 33% of FTD patients showed EMG changes in the ALS range after one year follow up.

While ideally a head-to-head longitudinal study comparing needle EMG to MUNIX can solve this issue, the author feels that the rate of occurrence of MND in FTD patients was possibly overestimated in the other study. This is because the authors of the study conducted by Lomen-Hoerth et al did not provide information about the presence or absence of clinical signs that might have suggested FTD-MND at test1 (for instance, reflex status, MRC muscle grades, wasting, fasciculations, etc). Moreover, they reported that all patients who displayed diagnostic EMG features of MND after 1 year follow up developed either muscle wasting or fasciculation by then.

Of note here is that all our FTD participants in this study had no clinical signs to suggest lower motor neuron involvement as they all had MRC grade 5 and the rest of their motor system examination was normal, in particular there was no muscle wasting or fasciculations. Deep tendon reflexes of all participants in this study were either grade 2+ or 3+, were symmetric and there was no reflex differences detected between arms and legs.

Furthermore, our findings of lower MUNIX values in 14% of bvFTD patients indicated the presence of lower motor neuron dysfunction in FTD. This result is in congruence with previous reports from a neurophysiology study that utilized the Neurophysiological Index technique (203). Our findings also lend further support to the idea of overlap between FTD and MND and to the fact that the two conditions represent two ends of the same spectrum of neurodegenerative disorders.

Despite being not fully understood, a “dying forward hypothesis”, similar to what has been postulated in MND pathogenesis (233, 234), has been proposed by some researchers to explain lower motor neuron dysfunction in FTD (203). It has been suggested in this hypothesis that an upper motor neuron degeneration process initiated by frontal cortical pathology in FTD results in secondary downstream lower motor neuron degeneration.

Additionally, the important finding that reached in this research of lower MUNIX values suggesting lower motor neuron dysfunction in some bvFTD patients, taken in tandem with the other important MRI result of precentral gyrus (i.e. motor cortex) pathology in bvFTD patients without a diagnosis of ALS (chapter 8 of this thesis) can be developed further in future research to determine fully the extent and functional significance of upper and lower motor neuron dysfunction in patients with FTD.

9.7.5 Conclusions

Lower MUNIX values are present in FTD-MND and bv-FTD patients suggesting lower motor neuron dysfunction in some instances.

Larger clinicopathological studies are needed to replicate these findings and to investigate whether these findings are related to certain underlying proteinopathy.

The onset of MUNIX decline in FTD patients with no clinical signs of lower motor neuron involvement and whether abnormalities of MUNIX and other neurophysiological biomarkers predict the development of subsequent ALS in patients with FTD is currently unknown and needs to be investigated in longitudinal studies.

We extended our MUNIX research of the FTD-ALS spectrum by performing longitudinal study on ALS subtypes, including ALS-FTD and findings are presented in the next section (section 9.8)

9.8 MUNIX Longitudinal ALS study

9.8.1 Introduction: ALS/MND

In this study we carried out longitudinal simultaneous MUNIX measurements in the same set of six muscles and ALSFRS-R recordings in order to evaluate MUNIX as a marker of disease progression in an Irish cohort of ALS patients by describing and to comparing the rates of decline in these two measures in various ALS subtypes including ALS-FTD.

9.8.2 Aims

The primary aim of this study was to complete MUNIX biomarker profiling of ALS-FTD continuum by:

- 1- Determining the rate of longitudinal decline in MUNIX value over time in patients with MND using proximal and distal limb muscles and to compare this rate of decline with that of the well-established standardized functional outcome measurement (ALSFRS-R), in order to evaluate the value of MUNIX as a marker of disease progression.
- 2- In addition we sought to describe the rate of MUNIX decline in the subgroups of spinal-onset (upper/lower limb), bulbar-onset and FTD-MND.

9.8.3 Results

9.8.3.1 Demographics of participants and general data trends

A total of 43 ALS patients participated in this longitudinal MUNIX study. Patients were of spinal (upper limb or lower limb) onset, bulbar onset or ALS-FTD phenotypes. Patients underwent MUNIX testing at 3-monthly intervals for a total duration of 21 months (8 visits). 31 participants reached month 12 (visit 5).

Mean age (\pm SD) of patients was 63.6 (\pm 9.7) years. Mean duration (\pm 9.7SD) of symptoms at the time of first measurement (defined as the onset of weakness, muscle wasting, fasciculations, dysarthria, dysphagia, dyspnea, falls, or disturbance of fine finger movements) was 9.3 (\pm 2.9) months. Mean ALSFRS-R at first measurement (\pm SD) was 40.3 (\pm 4.5).

Of the 43 patients, (M: F ratio: 30:13), twenty-five patients were of spinal disease onset (58.1%), 12 of bulbar onset (27.9%) and 6 FTD-MND (14%). In the spinal onset group, 13 (30.2%) patients had upper limb and 12 (27.9%) patients had lower limb onset.

Table 9.7 below details demographic characteristics, phenotypes, and ALS FRS-R values at the time of the first test (T1 or V1) along with time intervals between symptom-onset to first test for all participants.

Table 9.7: Details of Demographic characteristics, ALS phenotypes (Elscorial diagnostic groups), ALSFRS-R values at the time of test 1 and symptom onset to test 1 duration for MUNIX longitudinal ALS study participants.

Group	N	(Male: Female)	(\leq60: >60)	Age (y)	El Escorial (Poss: Prob: Def.)	ALSFRS*	Onset-test (Months)
Total	43	(30: 13)	(15: 28)	63.6 \pm 9.7	(9:17: 17)	40.3 \pm 4.5	9.3 \pm 2.9
Spinal onset	25	(21: 4)	(10: 15)	63.2 \pm 9.7	(6: 9: 10)	40.6 \pm 4.3	9.3 \pm 2.8
UL	13	(11: 2)	(7:6)	60.2 \pm 9.9	(4:3: 6)	42.2 \pm 4.1	9.5 \pm 3.2
LL	12	(10: 2)	(3: 9)	66.4 \pm 8.8	(2: 6: 4)	38.8 \pm 3.9	9.0 \pm 2.5
Bulbar onset	12	(5: 7)	(5: 7)	60.3 \pm 8.3	(1: 7: 4)	40.8 \pm 5.5	8.9 \pm 3.0
ALS-FTD	6	(4: 2)	(0: 6)	72.3 \pm 8.5	(2: 1:3)	38.0 \pm 2.9	10.2 \pm 3.4

* At the time of the first assessment
 UL: Upper Limb, LL: Lower Limb

MUNIX was well tolerated in all participants. There were no major technical issues and measurements of the complete set of all six muscles required 20–40 minutes recording

time. 31 and 27 participants reached follow-up visit at month 12 (visit 5) and month 15 (visit 6) respectively.

9.8.3.2 Longitudinal changes in values of MUNIX, MUSIX, CMAP and ALSFRS-R analysis results

When comparing rates of decline for the three parameters of MUNIX, ALSFRS-R and CMAP with disease progression, MUNIX started to decline earlier than ALSFRS-R and it continued to decline at a significantly faster rate. CMAP declined at a slower rate when compared to MUNIX

MUNIX, CMAP and ALSFRS-R all declined in a parallel fashion but at different rates and at different start times: for all participants global MUNIX declined at an average rate of $-3.62\% (\pm 0.04)$ per month. Furthermore, the monthly rate of MUNIX decline was different in different muscles as it ranged between $-2.6\% (\pm 0.06)$ and $-4.43 (\pm 0.07)$. The highest rate of MUNIX decline was recorded in the two small upper limb muscles of APB ($-4.43\% \pm 0.07$) per month followed by ADM ($-4.24\% \pm 0.12$) per month. The lowest decline rate was seen in BB ($-2.61\% \pm 0.06$) per month followed by AH muscle ($-3.62\% \pm 0.06$) per month (Figure 9.7).

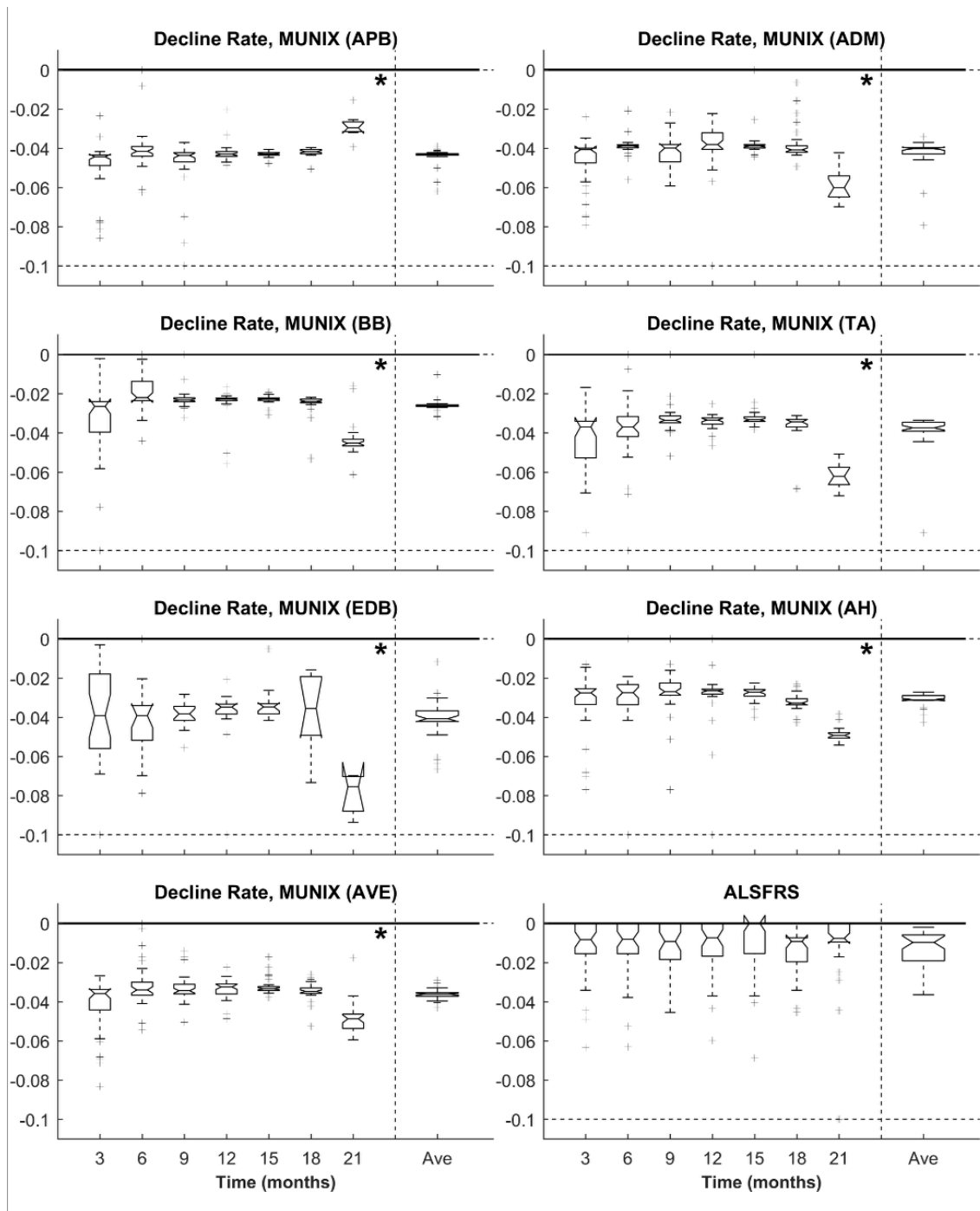


Figure 9.7: Longitudinal and average decline rates for MUNIX in each of the 6 muscles during study period plotted with ALSFRS-R longitudinal and average decline diagram.

The mean monthly relative decline of ALSFRS-R from its baseline (which was defined as 100%) was -2.28% (± 0.05) per month or 0.82 ALSFRS-R points/month.

The overall rate of decline in CMAP amplitude was -2.92% (± 0.05) per month, an intermediate rate between that of MUNIX and that of ALSFRS-R. Of note, and as expected (chapter 9), the longitudinal pattern of decline in CMAP amplitude followed the same pattern of decline in MUNIX albeit slower; on average and for each of the six muscles individually.

MUSIX increased from its baseline value at an average rate of $+3.00\%$ (± 0.06) per month.

Values and patterns of decline of MUNIX and CMAP amplitudes along with MUSIX increase pattern corresponding to the six tested muscles for all subjects across the 8 recording sessions over time are plotted graphically in figures 9.8a, 9.8b and 9.8c. Values and supplementary figures showing the MUNIX, MUSIX and CMAP values in a similar format for all patients, as well as individually for patient subgroups (60/ \geq 60 years of age, male/female, upper -limb-spinal/lower-limb-spinal/bulbar/ALS-FTD) are available with the author.

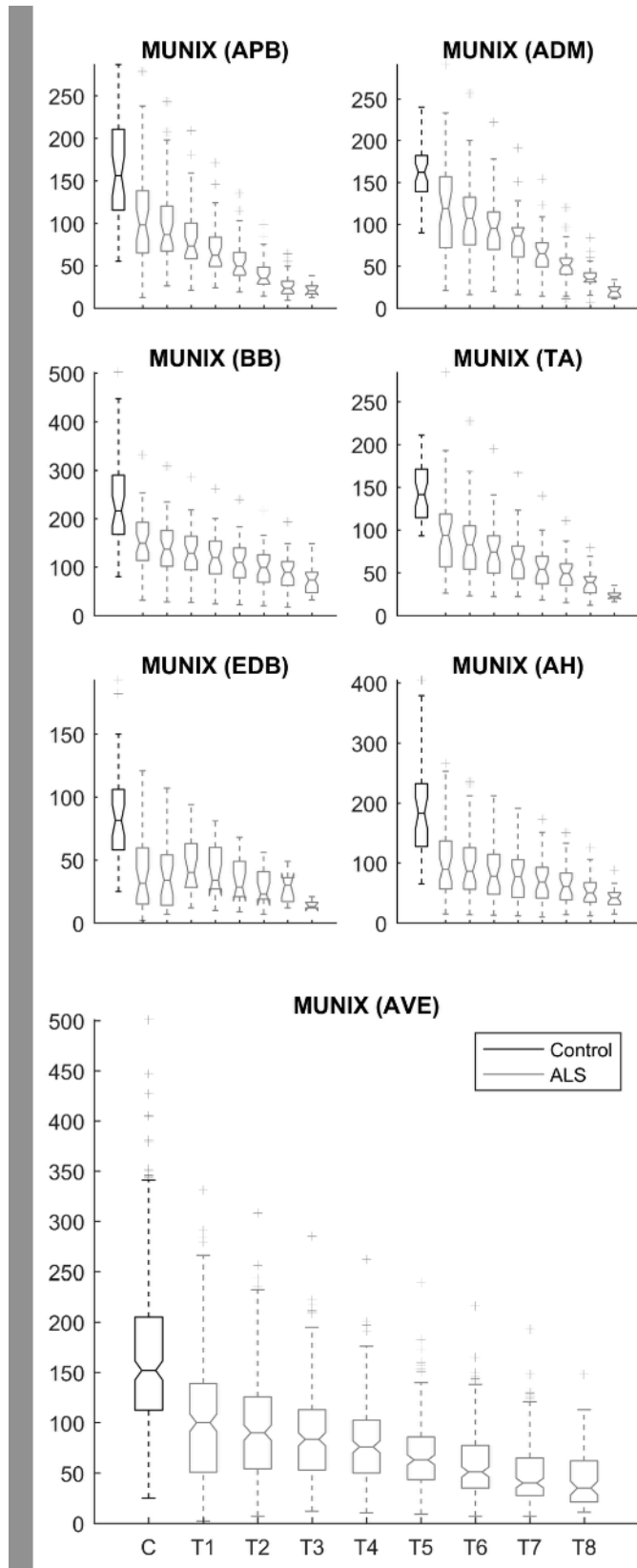


Figure 9.8a: The pattern of longitudinal decline in MUNIX values in ALS: for each of six muscles individually and on average.

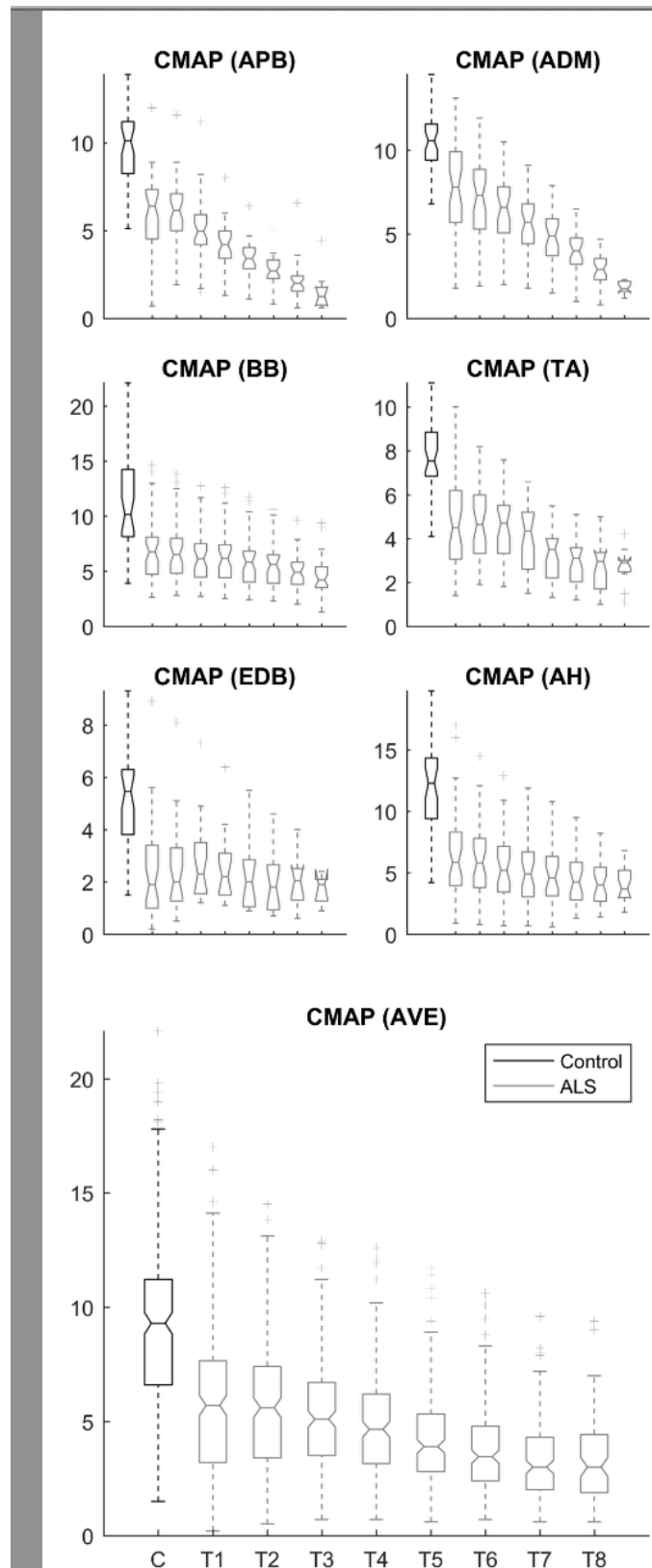


Figure 9.8b: The pattern of longitudinal decline in CMAP values in ALS: for each of six muscles individually and on average.

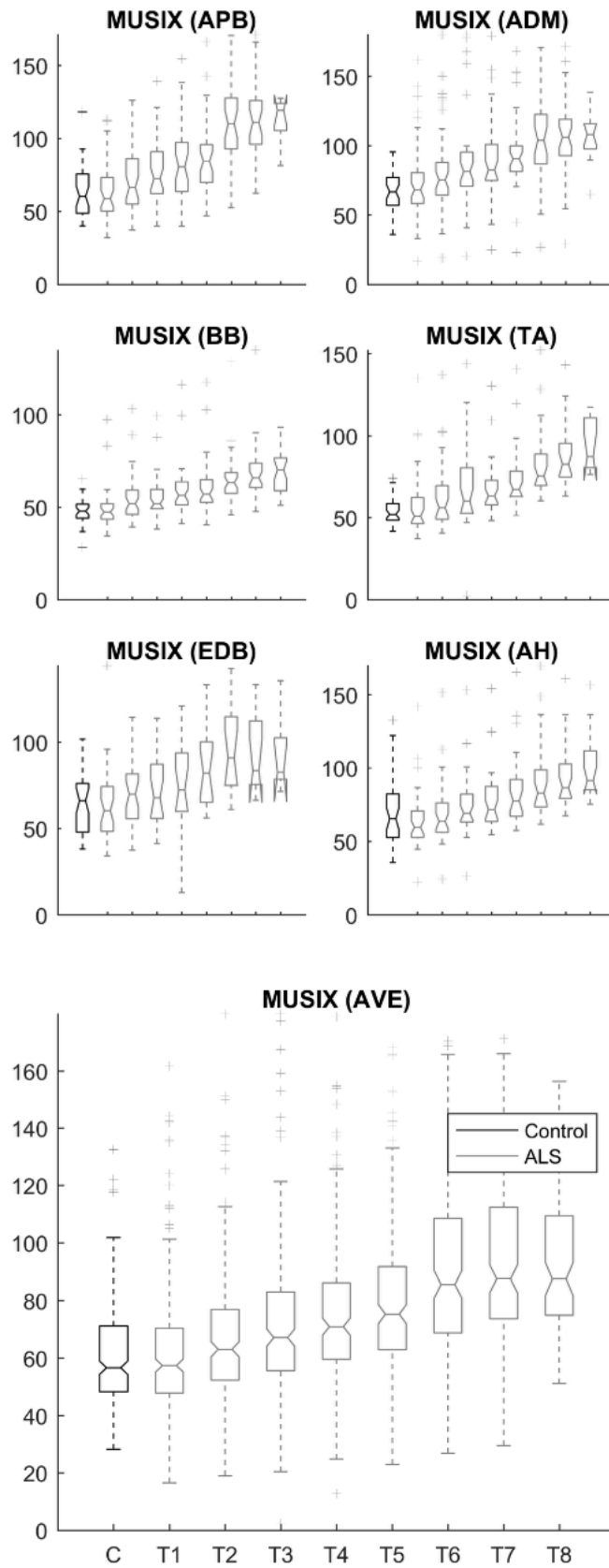


Figure 9.8c: The pattern of increase over time in MUSIX values in ALS: for each of six muscles individually and on average.

Figure 9.9 shows the MUNIX decline as a function of the time since symptom onset, as well as the corresponding regression line and equation. Similar results for MUNIX, MUSIX and CMAP of all patients and their subgroups are available as supplementary material.

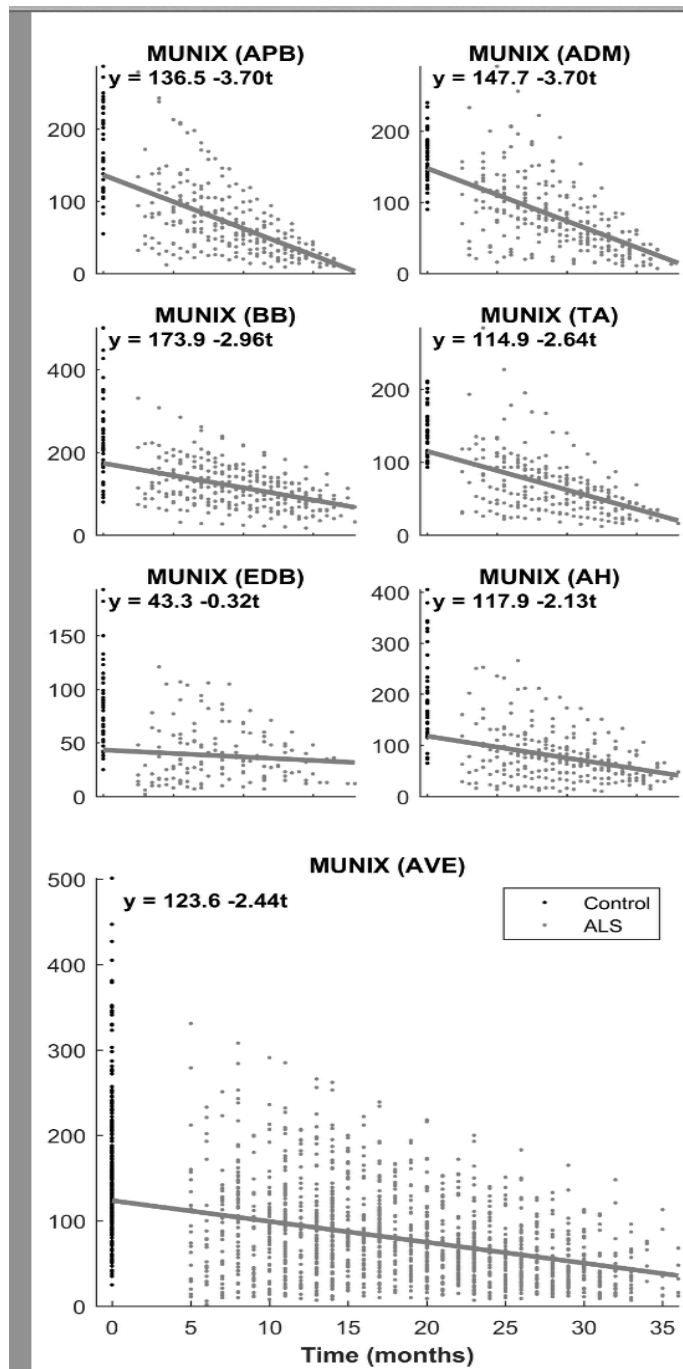


Figure 9.9: Showing MUNIX decline as a function of the time since symptom onset, as well as the corresponding regression line and equation.

Table 9.8 summarizes the rates of change per months for ALSFRS-R, MUNIX, MUSIX and CMAP for overall data (on average) as well as per each of the tested muscles individually.

Table 9.8: Details of absolute and percentage of changes per month in the parameters of MUNIX, MUSIX, CMAP and ALSFRS-R on average as well as for each of the 6 muscles individually.

Table: Mean \pm standard error of mean of the absolute change per month (Δ /month) and percent change per month (%/month) for MUNIX, MUSIX and CMAP.

	APB	ADM	BB	TA	EDB	AH	AVE
Δ MUNIX/month	-3.70 \pm 0.35	-3.70 \pm 0.35	-2.96 \pm 0.35	-2.64 \pm 0.32	-0.32 \pm 0.29	-2.13 \pm 0.39	-2.44 \pm 0.17
Δ MUSIX/month	+2.39 \pm 0.21	+1.48 \pm 0.25	+0.85 \pm 0.10	+1.19 \pm 0.20	+1.05 \pm 0.26	+1.56 \pm 0.18	+1.35 \pm 0.09
Δ CMAP/month	-0.19 \pm 0.02	-0.21 \pm 0.02	-0.10 \pm 0.02	-0.11 \pm 0.01	-0.01 \pm 0.02	-0.06 \pm 0.02	-0.10 \pm 0.01
% ALSFRS/month							-2.28 \pm +0.05
%MUNIX/month	-4.43 \pm 0.07	-4.24 \pm 0.12	-2.61 \pm 0.06	-3.86 \pm 0.16	-4.14 \pm 0.21	-3.16 \pm 0.06	-3.62 \pm 0.04
%MUSIX/month	+5.07 \pm 0.47	+3.10 \pm 0.16	+2.30 \pm 0.11	+2.98 \pm 0.18	+2.86 \pm 0.20	+2.42 \pm 0.11	+3.00 \pm 0.14
%CMAP/month	-4.00 \pm 0.09	-3.79 \pm 0.10	-1.93 \pm 0.06	-2.63 \pm 0.13	-3.70 \pm 0.29	-2.37 \pm 0.10	-2.92 \pm 0.05

9.8.3.3 Normalised Decline and Rate of Decline:

To reduce inter-individual variability, the values for each measure in tests 2-8 were normalised by the values in test 1. To further account for inter-individual variability and more objectively quantify the rate of decline, the percent change in any measure in each 3-month interval was used to find the normalised rate of decline in percent change per month (figure 9.10). Initial ANOVA showed no significant effect for age or gender on the decline rates of any of the measures (MUNIX, MUSIX, CMAP, ALSFRS-R) after normalisation. Therefore, these effects were omitted from subsequent ANOVA. Figure 9.9 below shows the normalised MUNIX values and rates of decline for each of the 6 tested muscles, on average and in comparison to ALS FRS-R. Supplementary figures show the same normalised decline for MUNIX, MUSIX and CMAP in all ALS patients and ALS subgroups are available from the author on request.

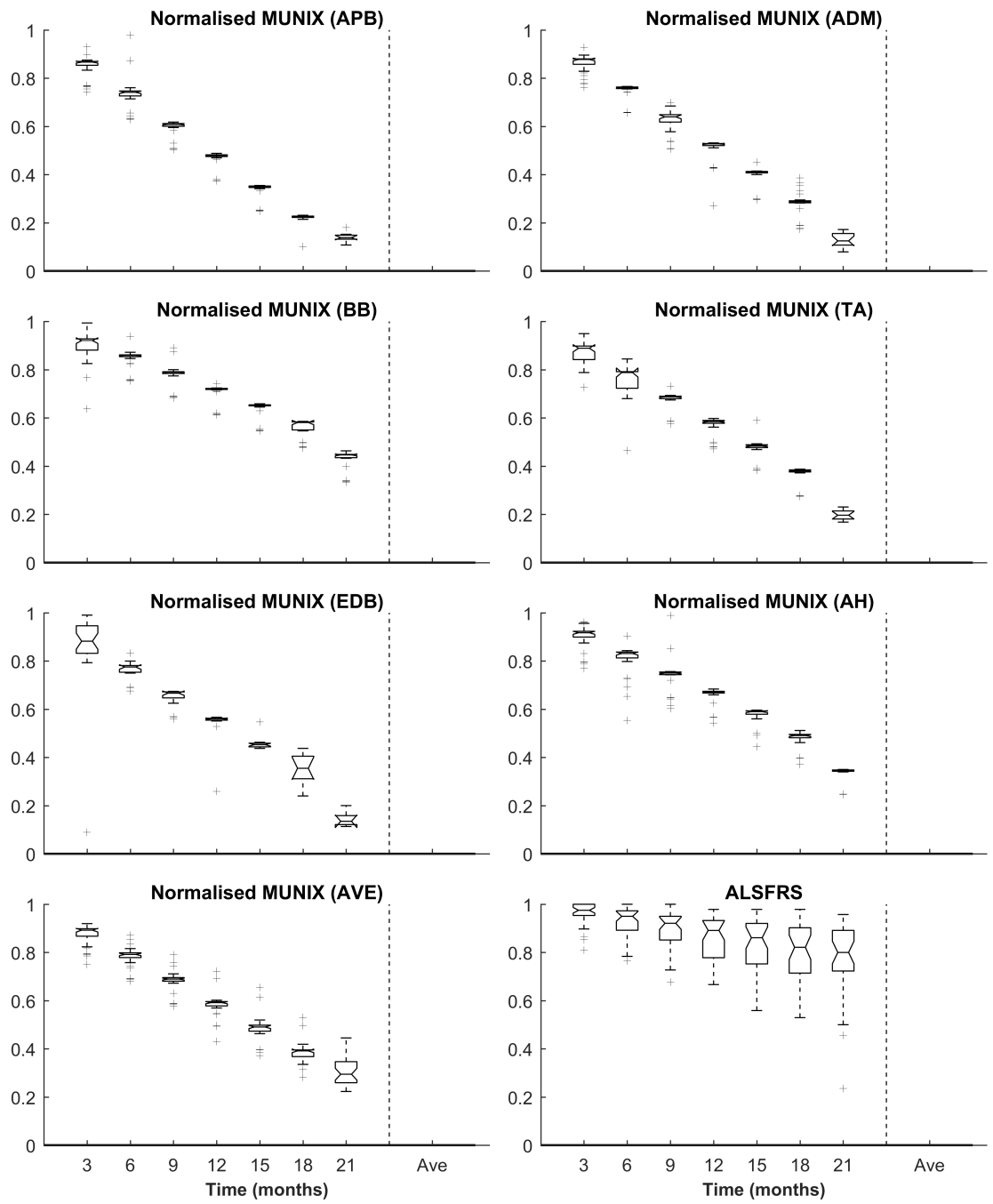


Figure 9.10: The normalised MUNIX values and rates of decline over time in each of the 6 tested muscles, on average and in comparison to ALS FRS-R for ALS patients.

9.8.3.4 Effect of Time, Muscle, Age, Gender, and ALS Phenotype on MUNIX/MUSIX/CMAP

The results from analysis of variance for MUNIX, MUSIX and CMAP are provided in table 9.9 showing a significant effect for time, muscle, age and phenotype, but not for gender, on MUNIX, MUSIX and CMAP. For ALS-FRS there was significant effects for time, age and phenotype, but there was no muscle or gender effect.

Table 9.9: ANOVA result showing significant effects of muscle, age and ALS phenotype on MUNIX/MUSIX/CMAP. There was no significant gender effect on any of the three measures.

Effect	MUNIX	MUSIX	CMAP
Muscle	*** P < 0.00001	*** P < 0.00001	*** P < 0.00001
Gender	P > 0.05	P > 0.05	P > 0.05
Age	*** P < 0.00001	*P < 0.05	*** P < 0.00001
Time (Muscle)	*** P < 0.00001	*** P < 0.00001	*** P < 0.00001
Phenotype	*** P < 0.00001	*** P < 0.00001	*** P < 0.00001

9.8.3.5 Subgroup analyses

Analysis of data acquired from subgroups with bulbar, upper limb, lower limb spinal disease onset and FTD-MND revealed different decline rates of ALSFRS- R (ranged between 2% and 2.8% per month), while MUNIX scores showed similar decline rates among all subgroups (CMAP amplitudes declined at similar, but slower rates compared to MUNIX). Figure 9.11 shows a combined presentation of MUNIX decline, on average and for each of the 6 muscles tested along with ALSFRS-R decrease over study period, for each of the ALS subgroups. Figure 9.12 shows normalised rate of decline (percentage per month) for MUNIX in the 6 tested muscles, on average and in comparison to ALS-FRS is plotted. Stars (*) show significant difference between

subgroups, based on Kruskal-Wallis test, after multiple-comparison correction using adaptive false discovery rate (Benjamini and Hochberg 1995; Benjamini et al. 2006) Similar presentations for MUSIX and CMAP are available as supplementary data.

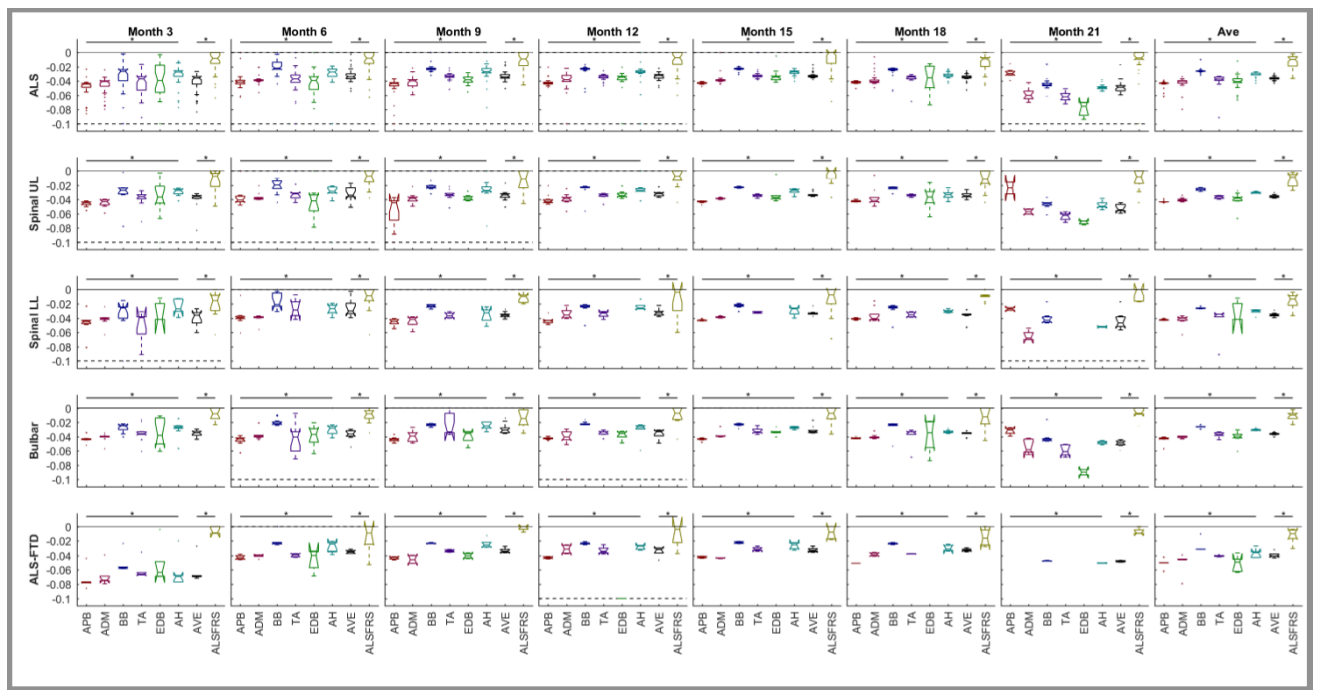


Figure 9.11: A combined diagram of MUNIX decline, on average and for each of the 6 muscles tested, along with ALSFRS-R decrease over study period, stratified by ALS subgroup.

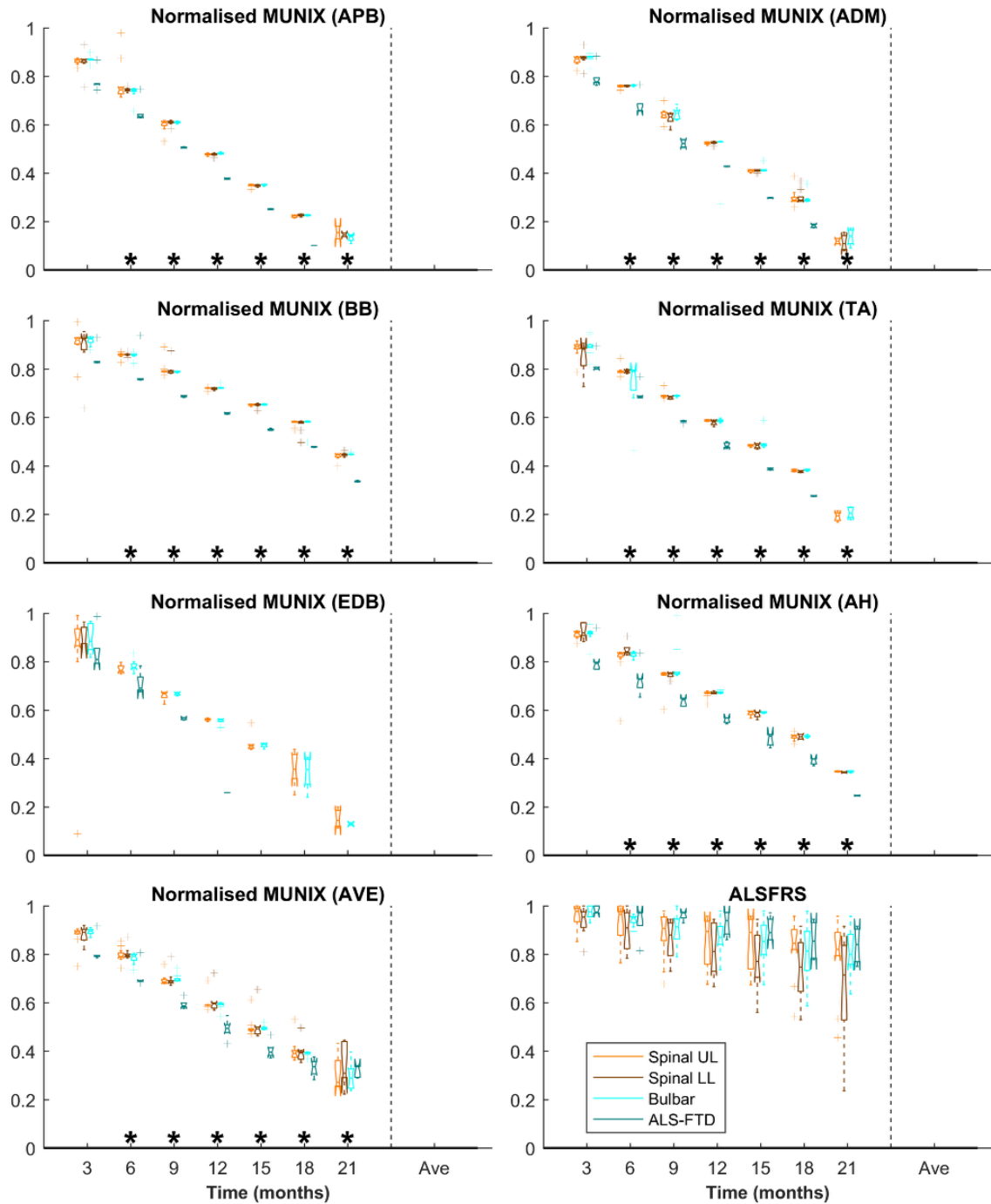


Figure 9.12: Normalised rate of decline (% per month) for MUNIX in the 6 tested muscles, on average and in comparison to ALS-FRS is plotted in figure 10.2.5 below. Stars (*) show significant difference between subgroups, based on Kruskal-Wallis test, after multiple-comparison correction using adaptive false discovery rate (Benjamini and Hochberg 1995; Benjamini et al. 2006)

9.8.3.6 Floor effect of measurements

Possible floor effect on measurements was avoided by careful selection of participants as patients with no severely wasted muscles were recruited at the start of the study. MUNIX of all six muscles did not decline to zero in any participant until the end of follow-up visits.

9.8.4 Discussion

This is the largest single-centre longitudinal study that applied the MUNIX method in ALS patients for the purpose of tracking lower motor neurone loss over time in this condition and comparing this rate of MUNIX decline with that of ALSFRS-R.

Our study reached the important conclusion that the decline of MUNIX in the less affected side was significantly earlier and faster than that of the well-established ALS functional decline measurement tool of ALS FRS-R. This was congruent with previous findings from both a similar MUNIX study conducted by Neuwirth et al (169) and MUNE findings in asymptomatic muscles of SOD1-mutation carriers (168).

The average rate of MUNIX decline per month was found to be identical ($3.62\% \pm 0.04$) per month in all ALS subgroups; regardless the region of disease onset (Bulbar onset, Spinal onset or FTD-MND), while the rate of decline of ALS FRS-R was different in bulbar onset versus spinal onset (range 2 to 2.8 % per month). This may suggest that MUNIX may have the potential to be utilized as a body (neurophysiological scan) with possible further applications in the future, for example to help in diagnosing ALS or in detecting lower motor neuron loss in bulbar onset patients. We would like to make the point that this study was not powered enough to detail subgroup differences due to the small number of participants.

One important advantage of MUNIX over ALSFRS-R that makes MUNIX theoretically more suitable outcome measure in drug trials is that ALSFRS-R represents a functional score that is affected by a number of external factors (such as non-invasive ventilation, percutaneous gastroscopy feeding tube, medical interventions for sialorrhoea etc) while MUNIX reflects lower motor neuronal loss and therefore is less likely to be influenced by external factors and more likely to be measured objectively (169).

A particular advantage of MUNIX is that rapidity in recording time and convenience to patients allow measuring multiple muscles in more than one region in a reasonable time. This is very useful when some muscles are completely wasted and hence reducing the risk of a floor effect that might arise when testing extremely wasted muscles.

Monthly MUNIX decline rate was different in different limb muscles (Figures 9.7, 9.8a). The highest rate of MUNIX decline was recorded in the two small upper limb muscles of Abductor Pollicis Brevis ($-4.43\% \pm 0.07$) per month followed by Abductor Digiti Minimi ($-4.24\% \pm 0.12$) per month while the lowest decline rate was seen in Biceps Brachii ($-2.61\% \pm 0.06$) per month followed by Adductor Hallucis muscles ($-3.62\% \pm 0.06$) per month. This may be explained in light of the previously reported patterns of disease spread e.g electrophysiological split-hand index and spread of disease from the site of onset to the contralateral side as well as to the ipsilateral neighbouring regions (228).

The variability in MUNIX measurements was high than the variability in ALSFRS-R measurements. This can be explained by the much higher range of MUNIX values. A similar observation was made in previous MUNE research (227).

A comparable previous longitudinal study conducted by Neuwirth et al (169) reported that after 15 months, mean MUNIX in several muscles tended to be higher than at month 12. The authors of the study attributed this to a dropout bias of patients with faster disease progression rate. We did not observe this pattern probably due to the very low dropout rate in our study.

9.8.5 Conclusions

MUNIX is a promising marker of disease progression in ALS and it is reproducible. When compared to ALSFRS-R, MUNIX starts to decline earlier in ALS and continues to decline faster. This suggests that MUNIX can serve as an early and sensitive marker of both motor dysfunction and disease progression with potential of therapeutic applications in the future as the benefit from the use of MUNIX as a marker of longitudinal decline is that increased precision could result in improved effect sizes when compared with clinical measures of change.

Our study determined and compared the rates of decline of MUNIX and ALSFRS-R longitudinally in ALS subgroups including ALS-FTD.

10 Chapter 10: Summary of findings, Discussion and Conclusion

10.1 Summary of findings

10.1.1 Cognitive clinic experience and clinical phenotyping of FTD patients

In this study I performed a descriptive analysis of the referral sources, demographics and diagnostic aetiologies of 193 consecutive patients seen at the cognitive clinic during the three-year study period as the cognitive clinic was one of the main sources for patient recruitment for this research. Patients were referred by a number of specialists but general practitioners and neurologists were found to be the main sources of referral. The clinical refined final diagnosis in about 40% of cases and anxiety related symptoms were identified in about 10% and those patients were reassured and further investigations/interventions were avoided. While those results delineated the significance of the clinic, they also highlighted the urgent need for a national referral system and an integrated care pathway for patients with cognitive symptoms and mimics as such undertaking is likely to result in better outcome and proper use of resources. This claim is further supported by the fact that only 23 out of 55 (42%) of FTD patients participated in the clinical phenotyping study were recruited from this clinic. full details are described in chapter 5 (section 5.3.1).

10.1.2 Family aggregation study of FTD

The main finding from this large family aggregation study in which analysis was performed in over 2700 first- and second-degree relatives of 45 FTD patients and 71 controls was the increased relative risk of neurodegenerative (MND) and neuropsychiatric disorders (Schizophrenia, Suicide and Learning disability) in relatives of patients in comparison to kindreds of controls. The commonality of risk was

determined in this study by determining the recurrence rate of the proband's disease, then by determining that other occurring conditions are biologically linked. The author proposes that suicides in this group were related to impulsivity rather than altered mood state as evidenced by the fact that despite the increased rate of suicide among relatives of patients with FTD, the rate of depression was not increased.

10.1.3 Motor Unit Number Index (MUNIX) Estimation in FTD

MUNIX studies constituted the neurophysiologic arm of FTD biomarker profiling. Over 160 patients and normal controls were recruited and studied. A pure lower motor neuron (LMN) MUNIX repository was compiled from 40 normative healthy subjects and 41 poliomyelitis patients at the first instance. Poliomyelitis represented a non-progressive disorder of the LMN. In the second stage a longitudinal comparative study, which was performed on 39 FTD, 41 Polio and 43 ALS patients compared the rate of MUNIX decline to other disease parameters and showed that MUNIX can serve as a reliable biomarker of disease progression. This was important as and may have therapeutic applications in the future.

Another novel result reached in this research was the finding of lower MUNIX values in 14% of bvFTD patients without clinical evidence of lower motor neuron involvement. This finding was particularly interesting as it was congruent with previous reports of LMN dysfunction in FTD using the neurophysiological index technique (203), but more importantly, it also provides another evidence to support applying MUNIX as reliable neurophysiological measure of studying the LMN.

10.2 Using information generated to direct future studies

The main objective outlined in the beginning this PhD thesis was to establish a biomarker repository of FTD and related conditions causing motor neuron degeneration, using a multidimensional approach, to identify disease subgroups and indices.

I have already demonstrated the success of utilizing data from the cognitive clinic to explore referral pathways, diagnostic accuracy, clinical characteristics of patients and to suggest recommendations for improving patients' care. This can serve as a basis for developing an integrated care pathway for patients with neurodegenerative disorders and for establishing a national FTD register similar to the currently existing ALS register.

The family aggregation study demonstrated that patients with FTD had a higher rate of Schizophrenia, suicide and learning disability among family members than among relatives of controls. This association study reported a connection between FTD and other neuropsychiatric diseases. Further investigation of the putative pathways and specific phenotypes in families containing members with FTD and abovementioned psychiatric conditions is required for the purpose of future endophenotype studies.

In the imaging study, phenotype-specific patterns of grey matter atrophy were evaluated and white matter alterations were explored among FTD-ALS spectrum using complementary MRI techniques. This study was novel in reporting that C9orf72 negative ALS-FTD patients exhibited widespread extra-motor changes, which were more extensive than those observed the C9orf72 positive patients. These findings can be developed further in future research by performing longitudinal study on a larger cohort and tracking the changes longitudinally to get more insights into specific imaging signatures of FTD subgroups and to predict the time of onset of cortical and subcortical atrophy in at risk presymptomatic cases.

MUNIX studies revealed a number of important findings and it was novel in many ways as was described in the results section of this thesis. Nonetheless, further research is urgently required to explore issues like the role of MUNIX in disease progression and predicting the onset time for MUNIX decline in asymptomatic individuals.

I also unveiled in this research a strong evidence for both upper and lower motor neuron dysfunction in bvFTD without a diagnosis of ALS. This was evident from the

MRI finding of motor cortex (precentral gyrus) pathology and the lower MUNIX values in some patients respectively. Correspondingly, these findings highlighted the importance of multidimensional approach to FTD biomarker profiling and made important contributions to better understanding of disease pathophysiology and biology. These findings need to be replicated in larger cohorts using similar multidimensional assessments of upper motor (for instance using TME and imaging other extra-motor brain area) as well as lower motor neuron (for example combining MUNIX with muscle MRI/US) functions.

10.3 Conclusion

This thesis has made substantial contribution to the current knowledge on FTD in the fields of epidemiology, family aggregation studies, genetics, imaging and neurophysiology. It has also proven that deep phenotyping provides a rich data source for biomarker profiling. Seminal findings concluded in the course of MUNIX research will be pursued in future studies.

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Appendices

MUNIX: ALS patient's covering sheet

- Name: -----
- Date: -----
- Dob: -----
- Handedness: -----
- Address: -----
- Chosen site and reason: -----

- MRN: -----
- Code: -----
- Visit no: -----
- Phone: -----

- MRC muscle power grade (0-5):
- APB: ----- - TA: -----
- ADM: ----- - EDB: -----
- BB: ----- - AH: -----

- Disease duration (Months): -----

- Pattern(s) of presentation: (1- Bulbar. 2- UE. 3-LE.)

- At onset: -----
- At present: -----

- Revised El Escorial research diagnostic category (1-Possible 2-Probable 3-Definite. 4- PBP):

- ALSFRS-R: -----

- Other points: -----

MUNIX: Poliomyelitis patient's covering sheet

- Name: -----
- Date: -----
- Dob: -----
- Handedness: -----
- Address: -----
- Chosen site and reason: -----

- MRN: -----
- Code: -----
- Visit no: -----
- Phone: -----

- MRC muscle power grade (0-5):
- APB: ----- - TA: -----
- ADM: ----- - EDB: -----
- BB: ----- - AH: -----

- Disease duration (Years): -----
- Acute paralytic Polio phase: - Age at onset: -----
- Symptoms (Limb/ Bulbar): -----

- Recovery type (1- Complete. 2- Partial.): -----
- Age at (or date of) recovery: -----

- Post polio syndrome: - Age at onset: -----
- Symptoms: -----

- Limb(s) affected: -----
- Bulbar symptoms (if any): -----
- Polio type (site of paralysis): 1- Spinal. 2- Bulbar. 3- Spinobulbar. -----

- Previous investigations (Electrophysiology/other): -----

- Other points: -----

Family History Questionnaire – Patient

Thank you very much for taking the time to fill out this questionnaire. We appreciate the effort that you and your family are taking to help with ongoing research into Frontotemporal Dementia. If you have any questions or would prefer to carry out this questionnaire by telephone please contact.



Here are a number of points to make filling in this form easier:

- **Questions are asked about your children over the age of 18, your parents, your brothers and sisters, your aunts and uncles and your grandparents.**
- **We do not need the names of anyone who is alive. For people who are alive their sex (either male or female), their age, and whether they are well or not is enough information.**
- **For relatives who have passed away we endeavor to source death certificates. This helps in verification of the history. In order to get a death certificate it is very helpful to have the name of the person, their age at death, the date or year they died and their place of death.**
- **All information is confidential.**

We thank you again for your participation in research.

Best wishes & thanks,

Taha Omer

1

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

“Proband” is the patient (i.e. the individual with FTD)

02/01/2013

Section 1: Demographic information:

Name : _____ * Maiden name (if applicable): _____

(*please note that the word proband refers to the person whose name is entered here)

Address: _____

Birth date: _____ Phone: _____

Occupation: _____

Date of completion of questionnaire: _____

Names of family members helping to complete questionnaire: _____

Section 2: Information on diagnosis:

What is the proband's diagnosis: _____

On what date did symptoms first appear: _____

What were the first symptoms: _____

On what date was the diagnosis made: _____

Please give the name of the neurologist who made the diagnosis: _____

Are there other family members with a similar illness: _____

Section 3: Information about your family:

Last Name (Maiden) First	Sex M/F	Year and county of birth	Please specify <u>any</u> health problems (including problems such as dementia, Parkinson's, psychiatric disease etc)	If deceased, please specify <ul style="list-style-type: none"> • date/year of death • place of death • cause of death
Proband:				
Spouse:				
Children <u>over 18 years of age</u> :				
1.				
2.				
3.				
4.				

Section 4: Information about your parents:

Last Name (Maiden) First	Sex M/F	Year and county of birth	Please specify <u>any</u> health problems (including problems such as dementia, Parkinson's, psychiatric disease etc)	If deceased, please specify <ul style="list-style-type: none"> • date/year of death • place of death • cause of death
MOTHER:				
FATHER:				

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with FTD)

Section 5: Information about your brothers and sisters (siblings)

Sibling one	Sex M/F	Year and county of birth	Please specify <u>any</u> health problems (including problems such as dementia, Parkinson's, psychiatric disease etc)	If deceased, please specify <ul style="list-style-type: none"> • date/year of death • place of death • cause of death
Brother/Sister:				
Spouse:				
Children over 18 years of age:				
1.				
2.				
3.				
4.				

Sibling two	Sex M/F	Year and county of birth	Please specify <u>any</u> health problems (including problems such as dementia, Parkinson's disease etc)	If deceased, please specify <ul style="list-style-type: none"> • date/year of death • place of death • cause of death
Brother/Sister:				
Spouse:				
Children over 18 years of age:				
1.				
2.				
3.				
4.				

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with FTD)

Section 5: Information about your brothers and sisters (siblings)

Sibling three	Sex M/F	Year and county of birth	Please specify any health problems (including problems such as dementia, Parkinson's disease etc)	If deceased, please specify • date/year of death • place of death • cause of death
Brother/Sister:				
Spouse:				
Children <u>over 18 years of age</u> :				
1.				
2.				
3.				
4.				

Sibling four	Sex M/F	Year and county of birth	Please specify any health problems (including problems such as dementia, Parkinson's disease etc)	If deceased, please specify • date/year of death • place of death • cause of death
Brother/Sister:				
Spouse:				
Children <u>over 18 years of age</u> :				
1.				
2.				
3.				
4.				

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with FTD)

Section 5: Information about your brothers and sisters (siblings)

Sibling five	Sex M/F	Year and county of birth	Please specify <u>any</u> health problems (including problems such as dementia, Parkinson's disease etc)	If deceased, please specify <ul style="list-style-type: none"> • date/year of death • place of death • cause of death
Brother/Sister:				
Spouse:				
Children over 18 years of age:				
1.				
2.				
3.				
4.				

If you have any more brothers or sister please provide information.

If you have any half-brothers or half-sisters please provide information.

Section 6: Information about your maternal grandparents (your mother's parents)

Last Name (Maiden) First	Sex M/F	Year and county of birth	Please specify <u>any</u> health problems (including problems such as dementia, Parkinson's disease etc)	If deceased, please specify <ul style="list-style-type: none"> • date/year of death • place of death • cause of death
Grandmother:				
Grandfather:				

Section 7: Information about your maternal aunts and uncles (siblings of your mother)

Aunt/Uncle One	Sex M/F	Year and county of birth	Please specify <u>any</u> health problems (including problems such as dementia, Parkinson's disease etc)	If deceased, please specify <ul style="list-style-type: none"> • date/year of death • place of death • cause of death
Aunt/Uncle:				
Spouse:				
Children over 18 years of age:				
1.				
2.				
3.				
4.				

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with FTD)

Section 7: Information on your maternal aunts and uncles (siblings of your mother)

Aunt/Uncle Two	Sex M/F	Year and county of birth	Please specify <u>any</u> health problems (including problems such as dementia, Parkinson's disease etc)	If deceased, please specify <ul style="list-style-type: none"> • date/year of death • place of death • cause of death
Aunt/Uncle:				
Spouse:				
Children <u>over 18 years of age</u> :				
1.				
2.				
3.				
4.				

Aunt/Uncle Three	Sex M/F	Year and county of birth	Please specify <u>any</u> health problems (including problems such as dementia, Parkinson's disease etc)	If deceased, please specify <ul style="list-style-type: none"> • date/year of death • place of death • cause of death
Aunt/Uncle:				
Spouse:				
Children <u>over 18 years of age</u> :				
1.				
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DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with FTD)

Section 7: Information on your maternal aunts and uncles (siblings of your mother)

Aunt/Uncle Four	Sex M/F	Year and county of birth	Please specify <u>any</u> health problems (including problems such as dementia, Parkinson's disease etc)	If deceased, please specify <ul style="list-style-type: none"> • date/year of death • place of death • cause of death
Aunt/Uncle:				
Spouse:				
Children <u>over 18 years of age</u> :				
1.				
2.				
3.				
4.				

Aunt/Uncle Five	Sex M/F	Year and county of birth	Please specify <u>any</u> health problems (including problems such as dementia, Parkinson's disease etc)	If deceased, please specify <ul style="list-style-type: none"> • date/year of death • place of death • cause of death
Aunt/Uncle:				
Spouse:				
Children <u>over 18 years of age</u> :				
1.				
2.				
3.				
4.				

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with FTD)

Section 8: Information about your paternal grandparents (your father's parents)

Last Name (Maiden) First	Sex M/F	Year and county of birth	Please specify <u>any</u> health problems (including problems such as dementia, Parkinson's disease etc)	If deceased, please specify <ul style="list-style-type: none"> • date/year of death • place of death • cause of death
Grandmother:				
Grandfather:				

Section 9: Information about your paternal aunts and uncles (siblings of your father)

Aunt/Uncle One	Sex M/F	Year and county of birth	Please specify <u>any</u> health problems (including problems such as dementia, Parkinson's disease etc)	If deceased, please specify <ul style="list-style-type: none"> • date/year of death • place of death • cause of death
Aunt/Uncle:				
Spouse:				
Children <u>over 18 years of age</u> :				
1.				
2.				
3.				
4.				

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with FTD)

Section 9: Information about your paternal aunts and uncles (siblings of your father)

Aunt/Uncle Two	Sex M/F	Year and county of birth	Please specify <u>any</u> health problems (including problems such as dementia, Parkinson's disease etc)	If deceased, please specify <ul style="list-style-type: none"> • date/year of death • place of death • cause of death
Aunt/Uncle:				
Spouse:				
Children over 18 years of age:				
1.				
2.				
3.				
4.				

Aunt/Uncle Three	Sex M/F	Year and county of birth	Please specify <u>any</u> health problems (including problems such as dementia, Parkinson's disease etc)	If deceased, please specify <ul style="list-style-type: none"> • date/year of death • place of death • cause of death
Aunt/Uncle:				
Spouse:				
Children over 18 years of age:				
1.				
2.				
3.				
4.				

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with FTD)

Section 9: Information about your paternal aunts and uncles (siblings of your father)

Aunt/Uncle Four:	Sex M/F	Year and county of birth	Please specify <u>any</u> health problems (including problems such as dementia, Parkinson's disease etc)	If deceased, please specify <ul style="list-style-type: none"> • date/year of death • place of death • cause of death
Aunt/Uncle:				
Spouse:				
Children <u>over 18 years of age:</u>				
1.				
2.				
3.				
4.				

Aunt/Uncle Five:	Sex M/F	Year and county of birth	Please specify <u>any</u> health problems (including problems such as dementia, Parkinson's disease etc)	If deceased, please specify <ul style="list-style-type: none"> • date/year of death • place of death • cause of death
Aunt/Uncle:				
Spouse:				
Children <u>over 18 years of age:</u>				
1.				
2.				
3.				
4.				

DO NOT INCLUDE INFORMATION ON CHILDREN YOUNGER THAN 18 YEARS OF AGE

"Proband" is the patient (i.e. the individual with FTD)

If there is any information that we did not ask you for, that you would like to share please feel free to include it below. If you have any cousins / distant relatives that you would like to mention this would also be helpful:

If you would like to make any general comments please feel free to do this:

Many thanks again for taking the time to complete this questionnaire. A stamped addressed envelope is provided for you to return this form.

Best wishes,

Taha Omer

Research Doctor, Beaumont Hospital

BEAUMONT HOSPITAL HEADED PAPER

A study of Frontotemporal Dementia in Ireland
Prof. Orla Hardiman, Dr. Taha Omer, Dr. Niall Pender.

Combined Consent Form

page 1/2

Please tick the appropriate answer.



I confirm that I have read and understood the **Patient Information Leaflet** dated _____ attached, and that I have had sufficient opportunity to ask questions all of which have been satisfactorily answered.
Yes No

I have been given a copy of the Patient Information Leaflet and this Consent form for my records.
Yes No

I understand that my participation in this study is entirely **voluntary** and that I may withdraw at any time, without giving reason, and without this decision affecting my future treatment or medical care.
Yes No

I give permission for research personnel and clinical staff to look at my medical records to obtain information. I have been assured that information about me will be kept confidential.
Yes No

I understand that my identity will remain confidential at all times.
Yes No

I agree to my medical details being placed on a database. I understand that this database is web-based and my data will be held for at least ten years.
Yes No

I agree to undergo neuropsychological examinations every six months, for 3 years at the specialist neurodegeneration clinic.
Yes No

Deep phenotyping of FTD and FTD-MND patients Documentation Version 2 January 2013

I agree to give a blood sample to be stored for future research projects. I understand that my DNA will be extracted from this blood sample and future research projects may include genetic research.

Yes No

I agree to give a skin sample to be stored for future research projects. I understand that future research projects may include genetic research.

Yes No

If I require a lumbar puncture for diagnostic purposes, I agree that an extra (1ml) sample of spinal fluid will be stored for future research projects. I understand that future research projects may include genetic research.

Yes No

I agree to attend St. James's Hospital and undergo a 3T MRI Scan on a yearly basis for three years.

Yes No

I agree to attend Trinity College Dublin and undergo a specialist EEG test, every 6 months, for 3 years.

Yes No

I am aware of the potential risks of this research study.

Yes No

FUTURE USE OF INFORMATION COLLECTED:

I give my approval that coded data concerning my person may be stored or electronically processed for the purpose of scientific research and may be used in other studies in the future, subject to approval by a hospital Research Ethics Committee.

STORAGE AND FUTURE USES OF BIOLOGICAL MATERIAL:

I give permission for my coded samples to be stored for possible future research (including DNA or genetic studies) but only if the research is approved by a hospital Research Ethics Committee.

Yes No

Signed _____ -Date _____
Name of research participant (Block Capitals)

Signed _____ -Date _____
Name of family member / witness / next of kin (Block Capitals)

Signed _____ -Date _____
Name of person taking consent (Block Capitals) _____
Qualifications _____

BEAUMONT HOSPITAL HEADED PAPER

A study of Frontotemporal Dementia in Ireland
Prof. Orla Hardiman, Dr. Taha Omer, Dr. Niall Pender.

Combined Next of Kin Assent Form

page 1/2

Please tick the appropriate answer.



I confirm that I have read and understood **Next of kin Information Leaflet** dated _____ attached, and that I have had sufficient opportunity to ask questions all of which have been satisfactorily answered.
Yes No

I have been given a copy of the next of kin Information Leaflet and this assent form for my records.
Yes No

I understand that participation in this study is entirely **voluntary** and that I may withdraw my relative from this study at any time, without giving reason, and without this decision affecting his/her future treatment or medical care.
Yes No

I give permission for research personnel and clinical staff to look at the medical records of my relative to obtain information. I have been assured that information about me will be kept confidential.
Yes No

I understand that the identity of my relative will remain confidential at all times.
Yes No

I agree to the medical details of my relative being placed on a database. I understand that this database is web-based and my data will be held for at least ten years.
Yes No

I agree to my relative undergoing neuropsychological examinations every six months, for 3 years at the specialist neurodegeneration clinic.
Yes No

Deep Phenotyping of FTD and FTD-MND Patients Documentation Version 2 January 2013

I agree to my relative providing a blood sample to be stored for future research projects. I understand that DNA will be extracted from this blood sample and future research projects may include genetic research.

Yes No

I agree to my relative attending St. James's Hospital and undergoing a 3T MRI Scan on a yearly basis for three years.

Yes No

I agree to attending Trinity College Dublin and undergoing a specialist EEG test, every six months, for 3 years.

Yes No

I am aware of the potential risks of this research study for my relative.

Yes No

FUTURE USE OF INFORMATION COLLECTED:

I give my approval that **coded** data concerning my relative may be stored or electronically processed for the purpose of scientific research and may be used in other studies in the future, subject to approval by a hospital Research Ethics Committee.

STORAGE AND FUTURE USES OF BIOLOGICAL MATERIAL:

I give permission for my **coded** samples of my relative to be stored for possible future research (including DNA or genetic studies) but only if the research is approved by a hospital Research Ethics Committee.

Yes No

Signed _____ -Date _____
Name of research participant (Block Capitals)

Signed _____ -Date _____
Name of family member / witness / next of kin (Block Capitals)

Signed _____ -Date _____
Name of person taking consent (Block Capitals) _____
Qualifications _____

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BEAUMONT HOSPITAL
 P. O. Box 1297 Beaumont Road Dublin 9
 Telephone: 809 3000 / 837 7755 Facsimile: 837 6982

Patient Consent Form

Study title: Motor Unit Index Estimation (MUNIX): a quality improvement and comparative study

Dr. Gerard Mullins, Dr. Fiona Molloy, Prof. Orla Hardiman, Dr. Taha Omer.

I have read and understood the Information Leaflet about this research project. The information has been fully explained to me and I have been able to ask questions, all of which have been answered to my satisfaction.	Yes <input type="checkbox"/>	No <input type="checkbox"/>
I understand that I don't have to take part in this study and that I can opt out at any time. I understand that I don't have to give a reason for opting out and I understand that opting out won't affect my future medical care.	Yes <input type="checkbox"/>	No <input type="checkbox"/>
I am aware of the potential risks of this research study.	Yes <input type="checkbox"/>	No <input type="checkbox"/>
I give permission for researchers to look at my medical records to get information. I have been assured that information about me will be kept private and confidential.	Yes <input type="checkbox"/>	No <input type="checkbox"/>
I agree to undergo Motor Unit Index Estimation (MUNIX) test every 3 months for one year.	Yes <input type="checkbox"/>	No <input type="checkbox"/>
I have been given a copy of the Information Leaflet and this completed consent form for my records.	Yes <input type="checkbox"/>	No <input type="checkbox"/>
Storage and future use of information:	Yes <input type="checkbox"/>	No <input type="checkbox"/>
I give my permission for information collected about me to be stored or electronically processed for the purpose of scientific research and to be used in <u>related studies or other studies in the future</u> , subject to approval by a Research Ethics Committee.		

Motor Unit Index Estimation (MUNIX): a quality improvement and comparative study, Version 2, June 2013

Website: www.beaumont.ie

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BEAUMONT HOSPITAL
 P. O. Box 1297 Beaumont Road Dublin 9
 Telephone: 809 3000 / 837 7755 Facsimile: 837 6982

Normal Control Consent Form

Study title: Motor Unit Index Estimation (MUNIX): a quality improvement and comparative study

Dr. Gerard Mullins, Dr. Fiona Molloy, Prof. Orla Hardiman, Dr. Taha Omer.

I have read and understood the Information Leaflet about this research project. The information has been fully explained to me and I have been able to ask questions, all of which have been answered to my satisfaction.	Yes <input type="checkbox"/>	No <input type="checkbox"/>
I understand that my participation in this study is completely voluntary and that I can opt out at any time. I understand that I don't have to give a reason for opting out.	Yes <input type="checkbox"/>	No <input type="checkbox"/>
I am aware of the potential risks of this research study.	Yes <input type="checkbox"/>	No <input type="checkbox"/>
I have been assured that information about me will be kept private and confidential.	Yes <input type="checkbox"/>	No <input type="checkbox"/>
I agree to undergo Motor Unit Index Estimation (MUNIX) test once.	Yes <input type="checkbox"/>	No <input type="checkbox"/>
I have been given a copy of the Information Leaflet and this completed consent form for my records.	Yes <input type="checkbox"/>	No <input type="checkbox"/>
Storage and future use of information: I give my permission for information collected about me to be stored or electronically processed for the purpose of scientific research and to be used in <u>related studies or other studies in the future</u> subject to approval by a Research Ethics Committee.	Yes <input type="checkbox"/>	No <input type="checkbox"/>

Motor Unit Index Estimation (MUNIX): a quality improvement and comparative study, Version 2, June 2013

RESEARCH ARTICLE

Quality Control of Motor Unit Number Index (MUNIX) Measurements in 6 Muscles in a Single-Subject “Round-Robin” Setup

Christoph Neuwirth^{1*}, Christian Burkhardt¹, James Alix², José Castro³, Mamede de Carvalho³, Malgorzata Gawel⁴, Stephan Goedee⁵, Julian Grosskreutz⁶, Timothée Lenglet⁷, Cristina Moglia⁸, Taha Omer⁹, Maarten Schrooten¹⁰, Markus Weber^{1,11}

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

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Abstract

Background

Motor Unit Number Index (MUNIX) is a neurophysiological measure that provides an index of the number of lower motor neurons in a muscle. Its performance across centres in healthy subjects and patients with Amyotrophic Lateral Sclerosis (ALS) has been established, but inter-rater variability between multiple raters in one single subject has not been investigated.

Objective

To assess reliability in a set of 6 muscles in a single subject among 12 examiners (6 experienced with MUNIX, 6 less experienced) and to determine variables associated with variability of measurements.

Methods

Twelve raters applied MUNIX in six different muscles (abductor pollicis brevis (APB), abductor digiti minimi (ADM), biceps brachii (BB), tibialis anterior (TA), extensor dig. brevis (EDB), abductor hallucis (AH)) twice in one single volunteer on consecutive days. All raters visited at least one training course prior to measurements. Intra- and inter-rater variability as determined by the coefficient of variation (COV) between different raters and their levels of experience with MUNIX were compared.

Competing Interests: CN and MW received honoraria from Hänseler AG, Switzerland, and Biogen, USA, as advisory board members. MW received advisory board honoraria from Merz Pharma, Switzerland. TL is consulting for LFB Biomédicaments, France. This does not alter the authors' adherence to PLOS ONE policies on sharing data and materials. The other authors have declared they have no competing interests.

Results

Mean intra-rater COV of MUNIX was 14.0% (± 6.4) ranging from 5.8 (APB) to 30.3% (EDB). Mean inter-rater COV was 18.1 (± 5.4) ranging from 8.0 (BB) to 31.7 (AH). No significant differences of variability between experienced and less experienced raters were detected.

Conclusion

We provide evidence that quality control for neurophysiological methods can be performed with similar standards as in laboratory medicine. Intra- and inter-rater variability of MUNIX is muscle-dependent and mainly below 20%. Experienced neurophysiologists can easily adopt MUNIX and adequate teaching ensures reliable utilization of this method.

Introduction

Motor Unit Number Index (MUNIX) is a novel variant of motor unit number estimation (MUNE) techniques which provides an index of the number of functional lower motor neurons in a muscle. Recent studies have suggested that this technique may serve as a marker of disease progression in diseases with progressive loss of motor units, such as amyotrophic lateral sclerosis (ALS). Several studies have also demonstrated a good test-retest reliability in healthy subjects and ALS patients. [1–7]

Sensitive biomarkers in early phase II ALS trials are sorely needed to reveal potential beneficial effects of therapeutic interventions. [8] Biomarkers directly linked to the fundamental underlying disease process, which in the case of ALS is the loss of motor neurons over time, would be advantageous. An important attribute of any biomarker is not only its sensitivity to change, but also reliability of measurements, which will allow a reduction in sample size and increase power to detect significant differences in ALS trials. [9] In laboratory medicine assessment of inter-centre variability can be relatively easily achieved with so called "round robin" tests. [10] A well-defined sample is sent to different laboratories which then perform a test-retest and compare the results with a reference value.

This kind of quality control is difficult to achieve in outcome measures or biomarkers which are linked to the performance of the test subject and/or rater. However, a pivotal study of the forced vital capacity in a large multi-centre trial clearly showed that after adequate training an excellent inter-rater reliability can be achieved. [11] Such an approach has never been used for neurophysiological measures or neuroimaging. Over the past few years, several European centres have been trained to undertake the novel MUNIX method as part of the SOPHIA (Sampling and biomarker OPTimization and Harmonization In ALS and other motor neuron diseases) project. A refresher course held during the ENCALS meeting in Dublin 2015 offered a unique opportunity to perform a "round robin" test on a single subject. The goal was to evaluate MUNIX variability among 12 raters and to analyse associated factors.

Subjects and Methods

At the ENCALS (European Network for the Cure of ALS) meeting in Dublin 2015, a MUNIX training course was held over 2 days. Neurophysiologists from different European countries already familiar with this method and who had previously attended one or more training courses were invited. Twelve raters were included, 6 of them had passed a qualification process as part of a longitudinal study (SOPHIA). For this qualification process, raters had to perform

MUNIX measurements in 6 muscles (*Mm. abductor digiti minimi, abductor pollicis brevis, biceps brachii, tibialis anterior, extensor digitorum brevis* and *abductor hallucis*) in 4 healthy volunteers in two separate sessions. Raw data and results were sent to one reviewer (C.N.) and raters were certified when measurements showed a coefficient of variation (COV) below 20%.

During the round robin study, all 12 raters measured above mentioned muscles in one healthy subject (M.W.) in two sessions. Test and retest session were separated by one day. No specific sequence of raters was determined but the order was kept the same on the 2 consecutive days. A Dantec Keypoint[®] Focus EMG system was used with clamp cables and self-adhesive Kendall™ Nutab electrodes with 15 mm diameter for recordings. Electrodes and marks were completely removed between each rater. Raters were timed during the recording and allowed a maximum of 5 minutes on a single muscle. MUNIX values of recordings were calculated separately after the recording process.

MUNIX applies a statistical approach, using the area and power of the supramaximal stimulated compound muscle action potential (CMAP) and area and power of the surface electromyography with different force levels of voluntary isometric activation. With these values the 'ideal case motor unit count' is computed to estimate the amount motor neurons, reflected by an index value. The method has been described in detail. [3, 12]

Electrode placement and electrical supramaximal nerve stimulation was performed according to standard neurographic procedures. A mandatory step was to reposition the recording electrode over the muscle belly several times to obtain the highest CMAP amplitude. Details of electrode placements including photo material are available online at <http://www.encals.eu/page/european-collaborative-projects>.

The protocol for MUNIX test-retest measurements in healthy volunteers was approved by the Ethics Committee St.Gallen previously. [6] The single test subject (M.W.) gave written informed consent to participate during the ENCALS meeting and MUNIX training course. According to the Swiss regulations, no separate ethical approval was needed for observational single case studies in a healthy subject.

In advance of the meeting, raters were sent a questionnaire regarding their general experience in electrophysiology, percent of daily time devoted to electrophysiology and nerve conduction studies (NCS), number of prior performed MUNIX measurements, number of MUNIX training sessions undertaken and what they felt would be the two most difficult muscles to measure. Variables assessed during the MUNIX measurements included procedure time and maximum electric stimulation intensity for each single measurement.

Since a systematic error (e.g. non-optimal CMAP amplitude) may not necessarily affect the test-retest reliability but accuracy, in addition a hypothetical reference value was determined for each muscle. For this reference value, the 6 largest CMAPs (mean of test-retest measurements) were determined for each muscle. Of these 6 test-retest measurements, the 3 test-retest measurements with the lowest CMAP variability were selected to calculate the "reference" CMAP amplitudes and the corresponding MUNIX values (mean of 3 measurements).

The muscle-specific difference between real measurements and reference value was determined for all raters (accuracy).

Statistics

To evaluate the reliability of MUNIX and CMAP measurements, the coefficient of variation (COV: $100 \cdot SD/mean$) and variability (VAR: $100 \cdot \text{difference of test-retest}/mean$) were determined for each muscle. Intra-class correlation coefficient values turned out to be unfavourable because of the special situation of only one study subject yielding inter-subject variabilities near zero. Depending on the comparisons Welch's t-test, paired t-test, (nested) linear mixed-

effects models with "Rater" as random effect and linear regressions were performed as indicated in the results.

All analyses were performed using the statistical programme R Version.2.15.2. [12]

Results

MUNIX was well tolerated in the single subject, even when a total of 144 measurements were performed over 2 days. One rater (rater 5) was unsuccessful in obtaining a proper biceps CMAP on the first measurement. As per protocol the recording was aborted after 5 minutes. Otherwise, no major technical issues occurred.

Table 1 shows the characteristics of raters. Raw data are listed in the S1 Table.

All raters specified prior to the study which two muscles they felt to be most challenging. The biceps muscle was mentioned most frequently, followed by the M. abductor hallucis. (Table 1)

Reliability

Test-retest data and coefficients of variation (COV) for MUNIX measurements in individual muscles are summarized in Fig 1 and Tables 2 and 3, respectively.

Intra-rater coefficient of Variation (COV) ranged from 7.4 (APB) to 24.3 (EDB). Range was smaller in the experienced group (8.5 (ADM) and 18.9 (TA)) with a mean of 13.4 (SD ± 4.5) compared to the less experienced group (5.8 (APB) to 30.3 (EDB), mean 14.7 (SD ± 8.4)). The EDB showed comparably low MUNIX values (mean MUNIX 69 ± 16) compared to the MUNIX values of other 5 muscles (mean 192 ± 35), which contributes to a relatively higher COV.

Inter-rater reliability differed muscle-specific considering both measurements and ranged from 8.0 to 31.7 (mean 18.1 ± 5.4) (Table 3). The biceps exhibited the lowest overall inter-rater variability, the AH the largest (means of measurements). With the exception of the AH, all other muscles revealed inter-rater COV equal or below 20%.

Table 1. Characteristics of raters familiar (1 to 6) and less familiar (7 to 12) with the MUNIX method. # = number.

rater	experience	daily time	NCS per	experience	MUNIX	challenging		MUNIX
	neurophys.	neurophy.	week	of MUNIX	/ muscle	muscle 1	muscle 2	courses
	[years]	[%]	[#]	[months]	[#]			[#]
1	12	25–50%	26–50	84	>100	AH	Biceps	4
2	9	25–50%	26–50	30	51–100	AH	TA	3
3	11	>75%	51–100	72	>100	Biceps	AH	2
4	3	>75%	11–25	17	26–50	Biceps	AH	1
5	15	50–75%	26–50	17	>100	Biceps		2
6	3	25–50%	26–50	24	>100	EDB		1
7	25	50–75%	26–50	36	11–25	AH		3
8	15	10–25%	11–25	12	1–10	EDB	Biceps	2
9	8	<10%	11–25	5	11–25	AH	Biceps	2
10	10	50–75%	26–50	36	11–25	Biceps		1
11	4	25–50%	11–25	10	26–50	EDB	Biceps	1
12	10	25–50%	51–100	24	11–25	AH	Biceps	1
mean	10.4	25–50% *	26–50 *	30.6	26–50 *			1.9
SD	(± 6.2)			(± 24.3)				(± 1.0)

* = median

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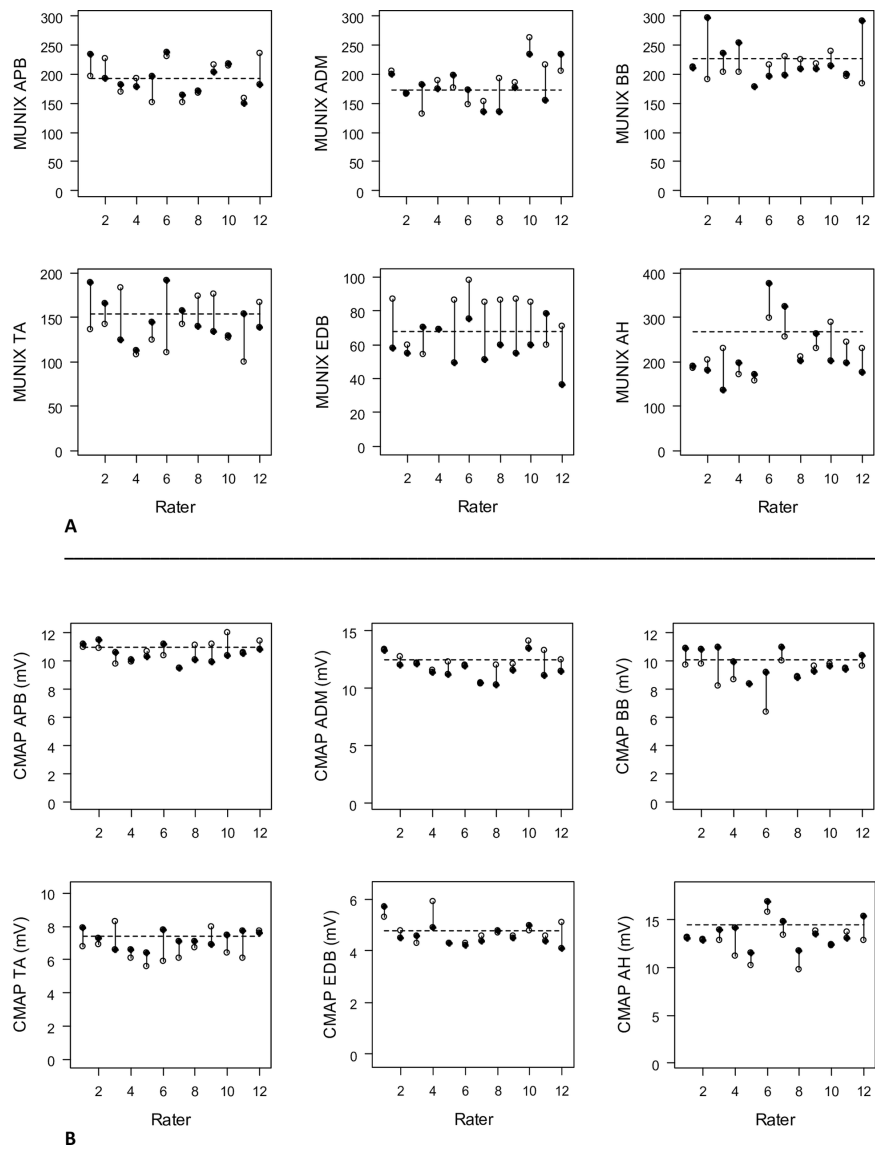


Fig 1. A and B: Test-retest results for MUNIX (A) and CMAP (mV) (B) in individual muscles. The dotted lines indicate the hypothetical reference value. Note the different y-axis scales for each muscle. Open dots = test values, filled dots = retest values.

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Table 2. Coefficient of variation (COV) and variability (σ) for MUNIX measurements in individual muscles in raters.

intra-rater muscle	all raters (n = 12)		exp. raters (n = 6)		non-exp. raters (n = 6)	
	COV MUNIX	COV CMAP	COV MUNIX	COV CMAP	COV MUNIX	COV CMAP
APB	7.4 (10.5)	4.2 (5.9)	9.0 (12.8)	3.3 (4.7)	5.8 (8.2)	5.0 (7.1)
ADM	10.7 (15.1)	4.2 (5.9)	8.5 (12.0)	2.4 (3.3)	12.9 (18.3)	6.0 (8.5)
BB	11.1 (15.6)	8.0 (11.3)	12.6 (17.8)	14.1 (19.9)	8.9 (13.9)	2.9 (4.2)
TA	16.6 (23.5)	9.9 (14.0)	18.9 (26.7)	10.9 (15.4)	14.4 (20.3)	9.0 (12.7)
EDB	24.3 (34.4)	4.7 (6.7)	18.4 (26.0)	4.9 (6.9)	30.3 (42.8)	4.6 (6.5)
AH	13.9 (19.6)	6.2 (8.8)	13.0 (18.4)	6.1 (8.6)	14.8 (20.9)	6.3 (8.9)

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Analysing the difference between MUNIX and CMAP measurements and the arbitrary reference value revealed a high accuracy (relative mean) and good reliability (SD) of measurements. (Fig 2)

Among the 12 individual raters, intra-rater COV of all MUNIX measurements ranged from 6.6 to 22.9 (mean 14.1 ± 4.3, data not shown).

In univariate linear mixed-effects models, no significant influence on the variability of MUNIX and CMAP measurements was present for general neurophysiological experience (years), amount of clinical electrophysiology in daily practice, experience in MUNIX (months) and number of attended MUNIX training courses (data not shown).

Between the experienced and less experienced group, no significant differences were observable for MUNIX, CMAP, time and maximum stimulation intensity determined by Welch's t-tests (p values >0.22, not shown in Table 4). One rater in the experienced group used habitually higher stimulation intensities (up to 85 mA) compared to all other raters, leading to a trend of slightly higher stimulation intensities in the experienced group.

Retests the following day were generally performed 0.6 minutes faster (all 6 muscles together) (p = 0.001, paired t-test). A more detailed analysis by a nested linear mixed effects model revealed that only the AH and TA differed significantly (p = 0.027 and 0.006, respectively).

Longer duration of MUNIX measurements were correlated with higher stimulation intensities. (Fig 3) Linear regression of all measurements revealed a significant correlation (p<0.001) between higher stimulation intensities and longer duration of measurements. Each increase of 10 mA is estimated with 0.34 minutes longer duration. This was also true when excluding all measurements with very high stimulation intensities > 50 mA (p<0.001, 0.46 min per 10mA increase).

Table 3. Inter-rater variability (COV) in individual muscles for the first and second measurement and mean of both values.

inter-rater muscle	1st measurement		2nd measurement		mean of measurements	
	COV MUNIX	COV CMAP	COV MUNIX	COV CMAP	COV MUNIX	COV CMAP
APB	16.4	6.7	14.1	5.6	13.5	4.9
ADM	18.9	7.7	17.8	8.2	15.9	7.4
BB	8.0	11.6	16.8	9.2	8.1	8.5
TA	20.3	12.9	16.4	7.0	10.9	6.9
EDB	18.0	9.8	20.0	9.5	12.2	8.4
AH	19.2	13.0	31.7	11.4	22.8	11.3

doi:10.1371/journal.pone.0153948.t003

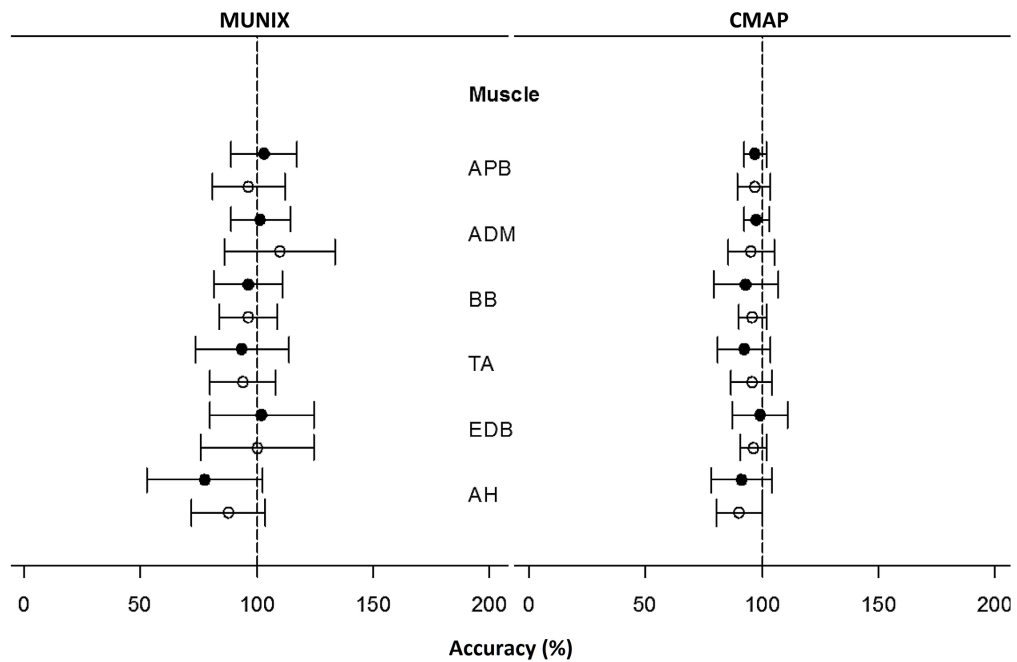


Fig 2. Relative mean and standard deviation of MUNIX and CMAP measurements in individual muscles of the experienced group (filled circles) and less-experienced group (empty circles) compared to the hypothetical reference values, expressed as accuracy (%).

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Table 4. Descriptive results of MUNIX and CMAP measurements in different rater groups.

muscle	all raters (n = 12)				experienced raters (n = 6)				non-experienced raters (n = 6)			
	MUNIX	CMAP mV	time (min)	max. stim. (mA)	MUNIX	CMAP mV	time (min)	max. stim. (mA)	MUNIX	CMAP mV	time (min)	max. stim. (mA)
APB	192 (29)	10.6 (0.7)	3.2 (1.2)	28.3 (17.7)	199 (27)	10.6 (0.5)	3.2 (1.4)	28.9 (18.1)	186 (30)	10.6 (0.8)	3.3 (1.1)	27.6 (18.0)
ADM	183 (33)	12.0 (1.0)	3.6 (1.3)	21.9 (10.7)	176 (22)	12.2 (0.7)	3.5 (1.1)	25.0 (13.0)	190 (41)	11.9 (1.3)	3.8 (1.5)	18.9 (7.1)
BB	218 (30)	9.7 (0.6)	3.5 (0.9)	34.3 (12.2)	218 (33)	9.4 (1.4)	3.8 (1.0)	39.2 (15.1)	218 (28)	9.7 (0.6)	3.3 (0.9)	29.8 (6.5)
TA	145 (26)	7.0 (0.7)	4.3 (1.4)	23.4 (9.2)	144 (31)	6.8 (0.8)	4.0 (1.1)	24.9 (11.2)	145 (22)	7.1 (0.6)	4.7 (1.7)	21.9 (6.8)
EDB	69 (16)	4.7 (0.5)	4.9 (1.6)	43.6 (14.8)	69 (15)	4.8 (0.6)	5.0 (1.9)	46.8 (19.1)	68 (17)	4.6 (0.3)	4.9 (1.4)	40.4 (8.6)
AH	222 (57)	13.1 (1.6)	4.2 (1.4)	34.9 (14.2)	208 (67)	13.2 (1.9)	4.4 (0.9)	37.4 (17.0)	235 (43)	13.1 (1.5)	4.0 (1.8)	32.4 (11.0)

() = SD

doi:10.1371/journal.pone.0153948.t004

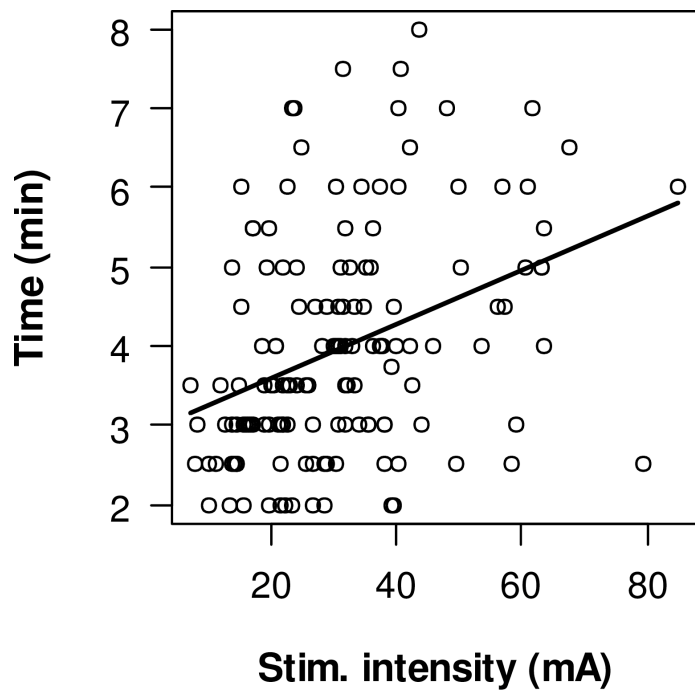


Fig 3. Correlation between duration of measurements and maximum stimulus intensity for all 6 muscles.

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Discussion

Reliability and accuracy of measurements is a key feature of any biological marker of disease. While this is relatively easily determined and common in laboratory medicine, it is much more challenging with physiological measures as these factors depend on both the subject's and rater's performance. Moreover, individual subjects cannot easily be sent to different laboratories. A "round robin test" is commonly used to evaluate reliability of measurements for biochemical and other "wet biomarker" laboratory tests between centres. [10, 13] This study is unique in that for the first time both reliability and accuracy of an electrophysiological measurement (MUNIX) was determined in a "round robin" setup.

The most important finding in this study is that the COV both within raters (intra-rater variability) and between raters (inter-rater variability) is equal or below 20%. The only exceptions are the AH which revealed the highest inter-rater COV for CMAP and MUNIX, and the intra-rater COV for EDB MUNIX. This reliability compares favourably with e.g. "wet biomarkers" of neuronal death and axonal damage like neurofilament (NF) proteins in cerebrospinal fluid, which exhibited an inter-lab COV of 59%. [14] Given that for biomarker qualification processes, as implemented by the FDA and European Medicines Agency (EMA), performance

characteristics are also an important factor (www.fda.gov), we propose that regardless of the character of the biomarker (dry or wet), studies of inter and intra-rater reliability should be mandatory before such measures are taken up into clinical trials. [15, 16] Our study also provides evidence that reliability tests—as part of a quality control process—can be studied with reasonable costs and effort.

Previous data have suggested that intra- and inter-rater test-retest reliability of the MUNIX method is dependent on individual rater's experience. [1, 2, 4–6, 17] In two multicentre MUNIX studies in healthy subjects and ALS patients, test-retest variability decreased in the second study in the same raters. [5, 6] In this study, no significant difference between trained raters less familiar with the MUNIX method and raters with several experiences in MUNIX was observed, suggesting that the method itself is robust and can be easily adopted. General electrophysiological practice seemed not to influence MUNIX reliability. However, all participants had several years of electrophysiological experience and at least one intense whole-day training course (theoretic aspects and hands-on training). This suggests that with appropriate training, MUNIX might be adopted with sufficient reliability in EMG labs.

It would be desirable to perform the same study setup with an ALS patient. However, for ethical reasons it seems inappropriate to perform 144 measurements in a patient over 2 days. From previous studies it is known that test-retest reliability is similar in ALS patients compared to healthy subjects. [5, 18] This suggests that this method can be applied reliably in ALS patients.

The AH muscle showed a tendency of lower CMAP and greater range of MUNIX and CMAP values and therefore lower accuracy when applying a hypothetical reference value. One reason might be that CMAP amplitude over AH is generated by multiple muscles after supra-maximal tibial nerve stimulation and SIP recordings are mostly performed with voluntary toe flexion, as exclusive abduction of the hallucis is rarely obtainable. [19] It has also been demonstrated that MUNIX values are dependent of the direction of movement, which in total makes this muscle comparably unfavourable. [5, 20, 21]

The relative high MUNIX variability of the EDB muscle is most likely caused by comparably low absolute values. The volunteer exhibited a clearly damaged and atrophic EDB on the contralateral side; consequently, a bilateral (and before that date unrecognised) damage of the distal motor branch of the deep peroneal nerve might be the reason.

Single measurements were generally fast to perform in less than 5 minutes, with no significant difference between the experienced and less experienced group. We found a significant correlation of longer duration of measurements with increased stimulation intensities. This was visible in all 6 muscles, particularly in the biceps muscle, as electrical stimulation of the musculocutaneous nerve solely without co-stimulation of adjacent nerves is technically challenging. The most likely explanation is that raters, who had difficulties optimizing electrode position for maximum CMAP amplitude or finding the optimal stimulation electrode placement, tended to use higher electrical stimulation to ensure supramaximal nerve stimulation.

There are some limitations of this study. First, the test subject was not the typical volunteer and already familiar with this method. A "learning effect" seems possible, as MUNIX needs active cooperation of the test subject and so the study volunteer may have provided more consistent recruitment patterns than a typical study participant. Additionally, the environmental conditions were the same for all raters (EMG equipment and software, recording electrodes, filter settings), which might not be always the case in multicentre trials. Furthermore, less experienced raters performed measurements during or immediately after the training session. It is unclear, if the performance of these raters will persist when returning to their own EMG laboratory. In the aforementioned SOPHIA project, several raters failed to pass the qualification process at the first attempt. Therefore, we recommend continuous practice of this method

prior to a reliability qualification process. The same would apply before this method is utilized in clinical trials, like in a previous MUNE study. [22]

Conclusion

In conclusion, quality control of MUNIX shows that this is a reliable and robust electrophysiological method with high accuracy. Our data suggest that experienced neurophysiologists can easily utilize this method after appropriate training. Round robin tests can be implemented with reasonable effort to neurophysiological techniques.

Supporting Information

S1 Table. Raw data. Units of parameter: „time” = minutes; “CMAP” = mV; MUSIX = Motor Unit Size Index (μ V); “stim” = stimulation intensity in mA; yellow fields = missing data. (DOCX)

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Author Contributions

Conceived and designed the experiments: CN MW CB. Performed the experiments: CN MW CB JA JC MdC MG SG JG TL CM TO MS. Analyzed the data: CN MW CB MdC. Contributed reagents/materials/analysis tools: CN MW CB. Wrote the paper: CN MW CB JA JC MdC MG SG JG TL CM TO MS.

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Neuroimaging patterns along the ALS-FTD spectrum: a multiparametric imaging study (under review)

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Abstract

Frontotemporal dementia is associated with remarkable clinical, genetic and pathological heterogeneity. The objective of this study is to characterize the imaging signatures of the main FTD phenotypes along the ALS-FTD spectrum using multiple complementary imaging techniques. A total of 100 participants underwent comprehensive multimodal neuroimaging, genetic testing and neuropsychological evaluation. Seven patients with behavioural variant FTD (bvFTD), eleven patients with non-fluent-variant primary progressive aphasia (nfvPPA), two patients with semantic-variant primary progressive aphasia (svPPA), ten patients with amyotrophic lateral sclerosis and FTD carrying the C9orf72 hexanucleotide repeat (ALS-FTD C9+), ten patients with ALS-FTD without hexanucleotide repeats (ALS-FTD C9-), twenty ALS patients without behavioural or cognitive deficits (ALSnci) and forty healthy controls (HC) were included in a prospective quantitative neuroimaging study. Phenotype-specific patterns of grey matter atrophy were evaluated using both whole-brain voxel-wise statistics as well as region-of-interest analyses. White matter alterations were explored using multiple diffusivity indices; radial diffusivity, axial diffusivity and fractional anisotropy. Our findings demonstrate that the clinical manifestations of FTD are underpinned by phenotype-specific patterns of white and grey matter degeneration. Our study also confirms the descriptive role of multi-parametric quantitative neuroimaging in FTD.

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