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Measuring the progression of upper motor neuron disease using diffusion MRI and its correlation with Clinical phenotype

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Abstract

Background: Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that affects motor and extra-motor systems. Diffusion tensor imaging (DTI) can be used to assess white matter (WM) tracts and has potential to be a marker of disease progression.

Aims: This study aims to assess the abnormalities in brain white and gray matter in patients with amyotrophic lateral sclerosis (ALS) using MRI. It focuses on the white matter changes associated with cognitive change and serial studies in patients with ALS.

Methods: MRI data were acquired in a total of 30 patients and 19 healthy controls. Patients were scanned up to three times with 6–month intervals on a 3T MRI. ALSFRS-R and cognitive testing were administered at each clinical visit. The MRI included a T_1-weighted image and diffusion weighted MRI along 64 directions. MRI data were analyzed using voxel-based morphometry (VBM), and tract-based spatial statistics (TBSS) for all DTI metrics, sub-grouping ALS patients based on their cognitive performance. For the serial studies, TBSS and region-of-interest (ROI) methods were applied to the longitudinal datasets. In the 6–month follow-up study, total number of ALS subjects was 23, FA and MD were measured for the motor and extra-motor pathways and correlated with the revised ALS functional rating scale (ALSFRS–R) and disease duration. TBSS and ROI methods, both manual and atlas-based approaches, were used for the 12–month follow-up study which included only 15 subjects. Results for both ROI approaches were compared.

Results: There was variability in clinical presentation among patients, with a mixture of site of onset and presence/absence of cognitive changes. At the first time-point study, all DTI measures and GM volume differed significantly between ALS subjects and controls in motor and extra-motor regions. Comparing each ALS subgroup to controls, greater DTI changes were present in ALS with cognitive impairment (ALScog) than ALS subjects without cognitive impairment (ALSnon-cog) subjects. In ALS compared to controls, there were changes on the right side and in a small region in the left middle frontal gyrus. Comparing ALS sub-groups, GM results showed reduction in the caudate nucleus volume in ALScog subjects. In the serial scans, when comparing all ALS time-points, the TBSS showed no significant changes between the first two scans or at three time-points. Using ROI method for 6-month follow-up, the average changes in FA and

MD in the selected ROIs were small and not significant after correcting for multiple com parisons. The FA correlated with ALSFRS-R in the genu of corpus callosum (gCC) and bilaterally in the forceps minor and inferior longitudinal fasciculus (ILF) at first scan but not at the second scan. The MD in the hippocampus WM tracts (Hpc), the association fibers and anterior limb of internal capsule (ALIC), gCC and corona radiata (CR) correlated with disease duration and ALSFRS-R. Over 12-month follow-up, when TBSS group comparison were performed for each time-point compared to controls, the changes in serial three time-points were mainly along the cortico-spinal tract (CST) and the whole corpus callosum (CC). ROI methods showed no significant differences in the average FA in the posterior limb of internal capsule (PLIC) or CST at the pons between time-point. There was a significant correlation of the values for the rate of loss in the left PLIC with survival and the correlation approached significance for the right PLIC. Linear regression analysis showed a significant relationship between the manual and atlas based methods for the PLIC but not the pons. However, this study found that manual ROI (mROI) is correlated with the atlas-based ROI (aROI).

Conclusion: The combined DTI and VBM study at the first time point showed changes in motor and extra-motor regions in patients with ALS compared to controls. The DTI changes were more extensive in ALS with cognitive impairment than pure ALS subjects. It is likely that the inclusion of ALS subjects with cognitive impairment in previous studies resulted in extra-motor WM abnormalities being reported in ALS subjects. Our study finds that there was little change in the DTI over time in patients with ALS. However, there was variability in clinical features among patients. For individuals there is some correlation with disease progression, measured by ALSFRS-R and survival. Our results would suggest that better results are obtained from the PLIC than the CST in the pons.

Declaration by author

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Contributions by others to the thesis

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Neurodegeneration

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List of Abbreviations

AD – Axial diffusivity

ALIC – Anterior limb of IC

ALS – Amyotrophic Lateral Sclerosis

ALScog – ALS subjects with cognitive impairment

ALSFRS-R – Revised Amyotrophic Lateral Sclerosis Functional Rating Scale

ALS-FTD-Q – ALS-FTD-Questionnaire

ALSnon-cog – ALS subjects without cognitive impairment

aTR – Anterior thalamic radiation

CBS – Cognitive Behavioral Screen

CC – Corpus callosum

Cg – Cingulum

CST – Corticospinal tract

ECAS – Edinburgh Cognitive and Behavioral Amyotrophic Lateral Sclerosis Screen

FA – Fractional anisotropy

FAB – Frontal Assessment Battery

FTD – Frontotemporal dementia

GM – Gray matter

Hpc – Hippocampal network

ILF – The inferior longitudinal fasciculus

IFOF – The inferior fronto-occipital fasciculus

IC – Internal capsule

MD – Mean diffusivity

PLIC – Posterior limb of IC

ROI – Region–of–interest

RD – Radial diffusivity

SLF – Superior longitudinal fasciculus

TBSS – Tract-based spatial statistics

TOI – tract of interest

UncF –uncinated fasciculus

VBM – Voxel-based morphometry

WM – White matter

Thesis

Chapter 1: Introduction

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a devastating neurological disease characterized by the loss of upper motor neurons of the brain and lower motor neurons of the spinal cord (Kiernan, Vucic et al. 2011). Motor neurons control the muscles in the legs, arms, or those used for speech, swallowing or breathing. The clinical features of ALS result from the loss of these motor neurons (Zarei, Carr et al. 2015). The term Amyotrophic Lateral Sclerosis is used in some parts of the world for this disease, while the term motor neuron disease is often used in Australia and United Kingdom. The term "motor neuron disease" can also be used to encompass a number of other diseases such as spinal muscular atrophy and post-poliomyelitis syndrome, that do not fulfill the criteria for diagnosis of ALS (Miller, Gelinas et al. 2005). In France, ALS is sometimes called Charcot's disease (Maladie de Charcot), as the disease was first described by the French neurologist Jean-Martin Charcot (Charcot 1865, Rowland 2001).

The anatomical pathology of ALS involves loss of upper and lower motor neurons. However, the microscopic pathology of ALS includes the presence of Bunina bodies, which are eosinophilic inclusions. These are considered to be the pathological hallmark of ALS (Okamoto, Mizuno et al. 2008), along with the accumulation of insoluble protein aggregates (Peters, Ghasemi et al. , Leigh, Anderton et al. 1988, Okamoto, Mizuno et al. 2008). The pathological protein aggregations vary among patients and can be categorized into TAR DNA-binding protein (Tdp-43) pathology, Tdp-with C9orf72 pathology, FUS pathology, and superoxide dismutase gene (SOD1) pathology (Saberi, Stauffer et al. 2015).

The possibility of genetic factors being involved in ALS was first suggested in 1850 with reports of cases with a family history (Strong, Hudson et al. 1991). It is now found that approximately 10% of ALS is inherited, which is referred to as familial ALS (fALS) (Camu, Khoris et al. 1999). In all patients, ALS is thought to arise from a combination of genetic susceptibility (Renton, Chio et al. 2014, Marangi and Traynor

2015), and environmental exposure (Fang, Kamel et al. 2009), and may be due to a multi-stage process (Al-Chalabi, Calvo et al. 2014). Causative genes have been identified in fALS, with some of these genetic mutations also found in patients with sporadic ALS (sALS) (Lattante, Conte et al. 2012). It has been estimated that 61% of the variance in risk of developing sALS is due to genetic factors (Al-Chalabi, Fang et al. 2010). The known causative genes usually encode for the proteins that accumulate within cells or are involved in the metabolism of protein aggregates (Chen, Sayana et al. 2013, Renton, Chio et al. 2014).

The environmental factors that contribute to ALS are less well understood. Epidemiological studies have implicated smoking, dietary factors, and pesticide exposure as risk factors for ALS (Okamoto, Mizuno et al. 2008). Environmental neurotoxins have also been implicated in the development of ALS. These include ß-methylaminoalanine (BMAA) and formaldehyde (Juntas-Morales, Pageot et al. 2014, Roberts, Johnson et al. 2015). It is possible that these environmental factors act through direct toxicity on neurons, but an epigenetic effect on gene expression is also possible (Kiernan, Vucic et al. 2011, Martin and Wong 2013, Lam, Chin et al. 2016).

Although ALS was described over 100 years ago, the understanding of the etiology of this disease is still limited. There is disagreement over whether the degeneration and death of the upper motor neurons (UMN) is a consequence of death of lower motor neurons (LMN) or vice versa (Dadon-Nachum, Melamed et al. 2011). Theories of pathogenesis of ALS include excitotoxicity, hyperexcitability, the effects of aggregation of proteins and abnormalities in dealing with misfolded proteins (Peters, Ghasemi et al. , Rothstein 2009).

The incidence of ALS is 1.5–2 per 100,000 people per year (Mitchell and Borasio). The prevalence of ALS is 5–6 subjects per 100,000 (Kurtzke 1981). In an epidemiological analysis combining global ALS studies, the average age of onset was 61.8 years (Chiò, Logroscino et al. 2013), typically between the ages of 50 and 70 years (Al-Chalabi, Calvo et al. 2014). Onset of ALS is rare before age 40 and both the incidence (Durrleman and Alperovitch 1989) and mortality rates (Hudson, Davenport et al. 1986) have been found to increase with age. Men are 1.5 times more likely to develop sporadic

ALS than women (McCombe and Henderson 2010); however, men and women are equally likely to develop familial ALS (fALS) (Camu, Khoris et al. 1999). The mean age at onset is younger in men than in women (Miller, Gelinas et al. 2005, Sabatelli, Madia et al. 2008). There is no cure for ALS. Studies have shown that Riluzole, a neuroprotective and anticonvulsant compound which inhibits the release of glutamic acid from neurons, prolongs the average survival in ALS patients by several months (Amyotrophic Lateral Sclerosis/Riluzole Study, Lacomblez et al. 1996).

Requirements for the diagnosis of motor neuron disease include: (a) the presence of UMN and LMN signs in different body regions, (b) evidence of disease progression with spread of weakness (c) the absence of other underlying pathological conditions. Currently, the diagnosis of ALS is grounded in the clinical assessment of disease features by an experienced neurologist. The criteria for the diagnosis of ALS are the revised El Escorial criteria (Brooks, Miller et al. 2000). These criteria require the combination of upper and lower motor neuron signs (Lomen-Hoerth 2008). There are also neurophysiological criteria for diagnosis (de Carvalho, Dengler et al. 2008).

The majority of patients die from respiratory failure within 3 to 5 years following symptom onset (Bruijn, Miller et al. 2004). However, some patients have longer survival and live beyond five years (Turner, Parton et al. 2003, Mateen, Carone et al. 2010, Pupillo, Messina et al. 2014). Factors that are associated with poor prognosis are older age, bulbar onset, shorter time from onset to diagnosis (del Aguila, Longstreth et al. 2003) and site of onset (Louwerse, Visser et al. 1997).

Clinical heterogeneity in the site of onset, disease progression and pattern of progression is a characteristic yet poorly understood feature of ALS (Baumann, Henderson et al. 2012). Patients can vary in terms of the degree of upper motor neuron and lower motor neuron weakness, but the most obvious difference between patients is the site of onset of weakness and the rate of progression (Beghi, Chiò et al. 2011). Patients with ALS also vary in the presence of extra-motor features, including the presence of cognitive change, as discussed below.

Cognition in ALS

Prevalence of cognitive disturbance in ALS

One of the important causes of heterogeneity in ALS is the presence or absence of cognitive abnormalities. In Charcot's first description of ALS, there was no mention of mental symptoms (Charcot 1865). A clinicopathologic study, published in 1932, was the first study to suggest that psychological abnormalities can occur in ALS (Wechsler and Davison 1932). Studies of the prevalence of cognitive dysfunction in ALS clinics, where most patients have sporadic ALS, indicate a prevalence of cognitive impairment ranging from 21% to 53% (Massman, Sims et al. 1996, Abrahams, Leigh et al. 2005, Ringholz, Appel et al. 2005, Rippon, Scarmeas et al. 2006, Gordon, Goetz et al. 2010, Phukan, Elamin et al. 2012, Oh, Park et al. 2014, Cui, Cui et al. 2015).

Frontotemporal dementia (FTD) is a form of dementia with pathology in the frontal and temporal parts of the brain (van der Zee and Van Broeckhoven 2014). Changes of personality, such as changes in sleep and eating patterns, social functioning and lack of judgment are the common symptoms of FTD. Some patients experience disinhibition and repetitive compulsive behavior and decreased attention (Ferrari, Kapogiannis et al. 2011).

Some patients have both ALS and frontotemporal dementia (FTD) (Burrell, Kiernan et al. 2011, Kiernan 2012). ALS patients with co–existent FTD have a poor prognosis (Mathuranath, Nestor et al. 2000, Ratnavalli, Brayne et al. 2002, Hodges, Davies et al. 2003). Language changes and abnormalities of comprehension are also found in ALS (Bak and Hodges 2004). It is accepted that some symptoms of FTD can be detected in up to 50% of ALS patients (Lomen-Hoerth, Anderson et al. 2002, Liscic, Grinberg et al. 2008, Strong 2008). Conversely, one study of FTD patients found that 14% had definite ALS, while 36% met the criteria for possible ALS (Lomen-Hoerth, Anderson et al. 2002). A number of recent studies emphasize the need to assess the motor dysfunction in FTD patients along with investigating cognition in ALS patients (Irwin, Lippa et al. 2007).

The continuum of the ALS with FTD has gained growing recognition based on epidemiological data and genetic studies (Renton, Majounie et al. 2011). Recent studies have established a genetic link between ALS and FTD by the discovery of the hexanucleotide repeat expansion of GGGGCC in the C90RF72 gene, which is associated with both ALS and FTD (DeJesus-Hernandez, Mackenzie et al. 2011, Renton, Majounie et al. 2011). These cognitive changes may have consequences for the planning of symptomatic care and the design and analysis of therapeutic trials.

Clinic based tools to measure cognition in ALS

Thorough assessment of cognition in ALS requires a full neuropsychological assessment. This is, however, not within the scope of routine clinical practice. Therefore, there is a need for a screening test that provides a measure for cognitive evaluation, which can be administered in the clinic in a relatively short time. The most widely used tests are the Mini mental state examination (MMSE), the revised Addenbrooke's Cognitive Examination (ACE-R) (Davies and Larner 2013), and the Frontal Assessment Battery (FAB) (Strong, Grace et al. 2009). More recently, the Edinburgh Cognitive and Behavioral ALS Screen (ECAS) and the ALS cognitive behavioral screen (ALS-CBS™) were developed. The ECAS aims to determine cognitive and behavioral changes of ALS patients (Abrahams and Bak 2013), while the ALS-CBS™ also aims to detect frontal lobe-mediated cognitive and behavioral changes (Woolley, York et al. 2010). Neither of these tests was available at the start of this project and could not be used here.

The Mini-Mental State Examination (MMSE) is a screening test that provides a brief and objective measure of cognitive function that is used to evaluate memory and cognition in clinical practice to diagnose dementia. Despite the vast literature on MMSE, there are certain limitations, such as (a) poor sensitivity to the early stages of Alzheimer's Disease when it is confined to amnestic syndrome (Feher, Mahurin et al. 1992), (b) inability to differentiate the type of dementia (Mathuranath, Nestor et al. 2000), and (c) inability to isolate linguistic or frontal deficits in early FTD that are critically dependent on executive ability measures, also it fails to detect deficits in memory and language tasks until the deficit is progressed (Hodges, Patterson et al.

1999). It is not effective in evaluating behavior or assessing the executive dysfunction that is common to ALS (Strong, Grace et al. 2009).

The Addenbrooke's Cognitive Examination (ACE) was designed to survey key domains of cognitive function without the need for specialized equipment, and to be sensitive to early stages of Alzheimer's disease and FTD. Sub-scores of the ACE are suggested to differentiate the diagnosis between FTD and Alzheimer's disease (Mathuranath, Nestor et al. 2000, Davies and Larner 2013). The ACE incorporates the Mini-Mental State Examination, which can be derived from the scores generated. Compared to the MMSE, naming in ACE is more difficult. Components such as verbal fluency and visuospatial function (copying a 3-D cube and drawing a clock face) have been added to ACE to detect the impaired constructional ability. Compared to the MMSE, the ACE has expanded memory and language tasks to reflect the impairment of episodic memory in early detection of Alzheimer's disease whereas the letter-based fluency is sensitive to FTD. ACE also incorporates the delayed recall test for the name and address which appears to be a sensitive measure in Alzheimer's disease (Mathuranath, Nestor et al. 2000). The performance of FTD patients in orientation tasks and episodic memory tasks is better than that of Alzheimer's disease patients. However, the latter perform better on verbal fluency and language components (Mathuranath, Nestor et al. 2000).

The Addenbrooke's Cognitive Examination Revised (ACE-R) is the revised version of ACE which incorporates the MMSE with the total possible score of 100 (Mioshi, Dawson et al. 2006). The revised version aimed to be quick to administer and readily acceptable to patients. The authors created three alternative versions of the name and address recall test, thereby preventing recalling from previous clinical visits. ACE-III was subsequently developed to omit the MMSE items due to copy-right restrictions. Modification to ACE is explained further in the methodology section.

The Frontal Assessment Battery (FAB) was designed to assess frontal lobe processes and identify the presence and severity of the dysexecutive syndrome that affects both motor behavior and cognitive function in several neurodegenerative diseases (Dubois, Slachevsky et al. 2000). A global agreement amongst researchers accepted the FAB as

a short and easy bedside battery to measures dysexecutive function and that can follow this dysfunction as the disease progresses (Slachevsky A 2004, Maeshima, Tanemura et al. 2006).

Biomarkers in ALS

The WHO has defined biomarkers as "almost any measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical, or biological. The measured response may be functional and physiological, biochemical at the cellular level, or a molecular interaction" (WHO 1993).

A delay of about 12–18 months from onset of ALS symptoms to diagnosis prevents early treatment with a disease-modifying drug (Zoccolella, Beghi et al. 2006). It has been suggested that the effects of motor neuron loss may be masked by the compensatory mechanism; in which neuronal damage might stimulate neural stem cell activity (Kuhn, Palmer et al. 2001), before the onset of clinical symptoms (Swash and Ingram 1988). Thus, there is a need for sensitive and specific biomarkers to assist with early diagnosis, to test a drug, to monitor progression, and as a surrogate endpoint in clinical trials.

Biomarkers can be relevant to phenotype, disability, or progression with diagnostic value. In ALS, these biomarkers can be from blood, body fluids such as cerebrospinal fluid (CSF), or imaging.

Clinical biomarkers

The ALS Functional Rating Scale (ALSFRS-R) is used to determine the capability and independence of ALS patients in functional activities relevant to their daily routine (Cedarbaum, Stambler et al. 1999). It is widely used to monitor progression of disease.

The extent of respiratory involvement has been reported to be a major prognostic factor in ALS (Haverkamp, Appel et al. 1995). Therefore, studies have moved towards the measurements of respiratory muscle strength such as measurement of the sniff nasal

inspiratory pressure (SNIP), which is a measure of diaphragmatic strength. It has been suggested that SNIP is more accurate than forced vital capacity (FVC) (Wijesekera 2009) because FVC assesses inspiratory muscle strength but not the expiratory muscles. Supine measurements can be used and were found to predict outcome (Baumann, Henderson et al. 2010).

Motor unit number estimation (MUNE) is an electrophysiological technique that assesses loss of lower motor neurons by estimating the number of active motor neurons innervating muscles (Felice 1997). MUNE would be expected to be a useful tool to follow the progression of ALS, but there are technical difficulties in the methods and limitation concerning the estimation of surface motor unit potential (SMUP) (Bromberg 2007).

Body fluid biomarkers

It has been suggested that plasma or serum would be the best source of biomarkers in ALS (Turner, Kiernan et al. 2009). Amino acid concentrations have been investigated, with increased levels of tyrosine (Patten, Harati et al. 1978) and glutamate (Iłżecka, Stelmasiak et al. 2003) being found in ALS patients compared to healthy controls. Moreover, blood studies have led researchers to investigate connective tissue in ALS patients and found that lower levels of fibronectin correlated with disease duration (Ono, Imai et al. 2000). Immune biomarkers were the focus of other researchers who found raised concentrations of T-cells that correlated negatively with ALSFRS-R and positively with the rate of disease progression (Shi, Kawano et al. 2007). Finally, levels of growth factors (Houi, Kobayashi et al. 2002) and lipoprotein (Dupuis, Corcia et al. 2008) have been found to affect progression and survival in ALS.

CSF biomarkers such as higher concentration of neurofilament have been implicated in the pathogenesis of motor neuron degeneration and indicated shorter survival (Beghi, Logroscino et al. 2006, Brettschneider, Petzold et al. 2006). Lower concentrations of vascular endothelial growth factor were found in ALS patients when compared to controls (Moreau, Devos et al. 2006, Xu, Henderson et al. 2016).

Imaging biomarkers

In this study the use of MRI as a biomarker will be the main aim. MRI gives an insight in the understanding of disease pathology and the possible progression of disease to other body regions (Keil, Prell et al. 2012). Since MRI is a non-invasive imaging technique, it has been extensively applied in neuroscience and neurological diseases and has the potential to be a useful biomarker for progression of ALS. The role of MRI in ALS will be discussed in detail in the next chapter.

Aims and hypotheses of this study

In this project I aim to provide more insight not only into the usefulness of diffusion MRI in ALS but also the structural changes associated with cognitive involvement in ALS. The study will concentrate on diffusion tensor imaging (DTI), which is thought to be a promising tool for prognostic, diagnostic or/and monitoring purposes in ALS (Rose, Pannek et al. 2012).

My aims were:

- To review the evidence of WM alterations in both ALS and FTD. Thus, I performed a thorough review of the literature on DTI in these diseases. If 10-15% of ALS patients meet the criteria for FTD, then I hypothesize that both conditions will share similar structural changes in the brain and that this will be evident in the published literature.
- To identify brain regions involved in ALS patients with and without cognitive impairment compared to controls. I used whole brain voxel-wise analyses to investigate the microstructural and macrostructural changes using both DTI and structural MRI data. ALS patients with cognitive impairment will be expected to show more extensive changes in the brain on MRI than those without cognitive impairment due to the involvement of brain regions that play role in cognition. I hypothesized that ALS patients with cognitive impairment would show more widespread alterations in both brain microstructure and macrostructure, particularly in extra-motor regions.
- To determine whether there are WM microstructural changes, either in motor or extra-motor pathways, over 6 months. I performed this analysis

using whole-brain voxel-wise analysis and ROI analysis of DTI. I hypothesize that there would be changes in a wide range of WM tracts over 6 months.

- To investigate the DTI changes of the CST over 12 months to determine if there are significant changes over time and whether the rate of change of DTI over 12 months correlates with clinical progression. This was performed using whole brain and ROI analyses. I hypothesize that the motor pathways will show changes over 12 months with changes in the distal CST, due to dying back of fibers.

Chapter 2: Diffusion MRI and imaging in ALS

History of MRI

The phenomenon of nuclear magnetic resonance (NMR) was first described by Rabi in 1938 (Ai, Morelli et al. 2012). In the ground state, the atomic nuclei have non-zero spin momentum and a dipolar momentum. These moments give the rise to nuclear magnetism (Abragam 1961). The idea behind the use of NMR for imaging was proposed by Damadian in 1971: he found that tissue relaxation times during MR acquisition can discriminate between benign and malignant neoplasms, and concluded that a point-by-point assessment of relaxation times in the body could be used to detect cancerous tissues (Damadian 1971). The first MR images were acquired in 1973 by Lauterbur who reconstructed 2–D images of two tubes in water (Lauterbur 1973). In 1974, Mansfield devised a faster pulsed-sequence method (Mansfield 1977). The first human MRI scans were performed in 1977 by Damadian together with Goldsmith and Minkoff (Damadian, Goldsmith et al. 1977). Since then, different MR techniques, methods of acquisition, and image analysis have been developed to reduce the scan times and to improve image quality. In 1981, ultrafast MR imaging was first used to obtain real-time images (Ordidge, Mansfield et al. 1982).

Diffusion weighted imaging (DWI)

Diffusion refers to the Brownian displacement of molecules, which was initially thought to be due to concentration or thermal gradient. However, microscopic observations by Robert Brown made it clear that diffusion continues even in the absence of concentration or thermal gradients (Brown 1828).

In MRI, diffusion usually considers the movement of water molecules. Diffusion of water molecules can be isotropic, when the diffusion of water molecule is the same in all directions, or anisotropic, when the diffusion of water molecule is hindered or restricted. In the human brain, the presence of macromolecules and cellular membrane cause the diffusion to be

hindered (Gaussian) within the extra-axonal space; and restricted (non-Gaussian) within intra-axonal space. The idea of diffusion weighted MR imaging was introduced in 1986 (Bihan, Breton et al. 1986). To acquire DW sequences, two equal and opposing motion-probing gradients must be applied and this will reduce the signal of the voxel exponentially. The attenuation of the final MR signal occurs when the applied diffusion sensitizing gradient is along the same direction of the diffusion in voxels, i.e parallel to white matter tracts. The strength, duration, and time between the motion-probing gradients is summarized by the b-value. Higher b-values allow better angular resolution of underlying crossing fibers, at the expense of a reduced signal to noise ratio. For diffusion tensor imaging (described below), b-values between 700-1200 sec/mm2 provide the most robust measurements (Alexander and Barker 2005).

Diffusion tensor imaging (DTI) is a promising tool used in diffusion MRI for characterizing the diffusion in brain white matter. The diffusion tensor is a 3 x 3 covariance matrix which describes the diffusion displacements in three dimensions:

$$D = \begin{bmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{xy} & D_{yy} & D_{yz} \\ D_{xz} & D_{yz} & D_{zz} \end{bmatrix}$$

where D is the diffusion tensor and D_{xx}, D_{yy}, D_{zz}, D_{xy}, D_{xz}, D_{yz} are the 6 independent elements of the tensor. The diffusion tensor can be represented as an ellipsoid-shape that is tilted in different directions, based on the local diffusion coefficients, see Figure 2.1. The diffusion tensor has three principal diffusion values (eigenvalues): λ_1, λ_2, and λ_3, and associated eigenvectors, which together determine the orientation and length of the axes of the ellipsoid. The first eigenvector is thought to correspond to the major diffusion direction and reflect the underlying fiber orientation. If the tissue is isotropic and water can diffuse freely, then $\lambda_1 = \lambda_2 = \lambda_3$; if the tissue is anisotropic with the convention that λ_1 is the largest diffusion coefficient, $\lambda_1 > \lambda_2 \geq \lambda_3$ (Taylor, Hsu et al. 2004), Figure 2.1.

The diffusion tensor can be derived from at least seven measurements, including one b0 image and six diffusion weighted images acquired along non-collinear directions (Jones, Horsfield et al. 1999, Hasan, Parker et al. 2001). Ideally, at least 30 diffusion weighted images should be acquired (Jones, Horsfield et al. 1999).

A number of rotationally invariant scalar metrics can be calculated from the diffusion tensor. The most frequently used DTI metric is the fractional anisotropy (FA), which is an index ranging from zero to one (Figure 2.1):

$$FA = \sqrt{\frac{3}{2}} \cdot \sqrt{\frac{(\lambda_1 - \lambda)^2 + (\lambda_2 - \lambda)^2 + (\lambda_3 - \lambda)^2}{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}}$$

Where λ_1, λ_2, λ_3, are the eigenvalues of the diffusion tensor as mentioned above, and λ is the average of the three eigenvalues. FA is high when the diffusion is constrained along the tract such as within the WM and low when the diffusion is less restricted such as GM or cerebrospinal fluid (CSF). Another measure is the mean diffusivity (MD), sometimes also referred to as apparent diffusion coefficient (ADC) (Pyra, Hui et al. 2010). The third of the tensor trace represents the value of MD which describes the total speed of diffusion:

$$\text{MD} = \frac{1}{3}(\lambda_1 + \lambda_2 + \lambda_3)$$

Further diffusion tensor metrics include axial diffusivity (AD), which represents the water diffusivity parallel to the axonal fibers. Diffusion perpendicular to axonal fibers is referred to as radial diffusivity (RD). Both AD and RD can serve as a surrogate markers for the detection of myelin and axonal abnormalities (Song, Yoshino et al. 2005, Klawiter, Schmidt et al. 2011), Figure 2.1. All DTI metrics have been found to reflect microstructural pathology, which is discussed in the next section.

In addition to the abovementioned scalar metrics, the principal direction of diffusion can be determined from the first eigenvector of the diffusion tensor. This principal direction of diffusion can be encoded into a color scheme in which every color is assigned to the major direction– red: left-right, green: anterior-posterior, and blue: superior-inferior (Taber, Pierpaoli et al. 2002). Direction based color coding is an approach for visualization in DT images where the brightness of the image represents the diffusion anisotropy (FA) while the red-green-blue (RGB) color scheme indicates the orientation of the fiber tract (Wakana, Jiang et al. 2004), Figure 2.2. Although the information provided by RGB-color scheme is complex and requires knowledge of 3D architecture of WM, it has proven to be useful in many neurodegenerative disease (Hoon, Lawrie et al. 2002, Canu, Agosta et al. 2011).

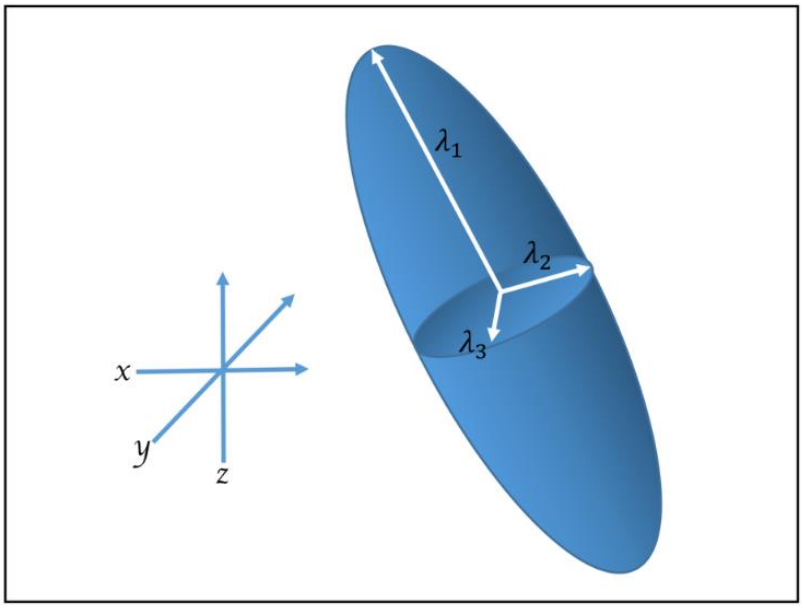

Figure 2.1: Visual -representation of a diffusion tensor with the 3 main axes: the longest axis stands for the primary eigenvector ($\lambda 1$), reflecting the diffusion parallel to the fibers; the two shorter axes represent the second ($\lambda 2$) and third ($\lambda 3$) eigenvectors, whose average provides a measure of diffusivity perpendicular to the fibers.

Some tissues that have gross motion may show artificial increased diffusivity due to loss of signal in diffusion weighted image which can be avoided by reducing the scan time and the use of fast acquisition techniques such as echo-planar imaging (EPI) (Turner, Le Bihan et al. 1991). One would expect artifacts as a result of head motion, physiological noise or even eddy currents and susceptibility effects when applying DTI in scanning patients with neurodegenerative disease. Therefore, to perform DTI analysis, extensive pre-processing (Pannek, Raffelt et al. 2012) is required to deal with these issues.

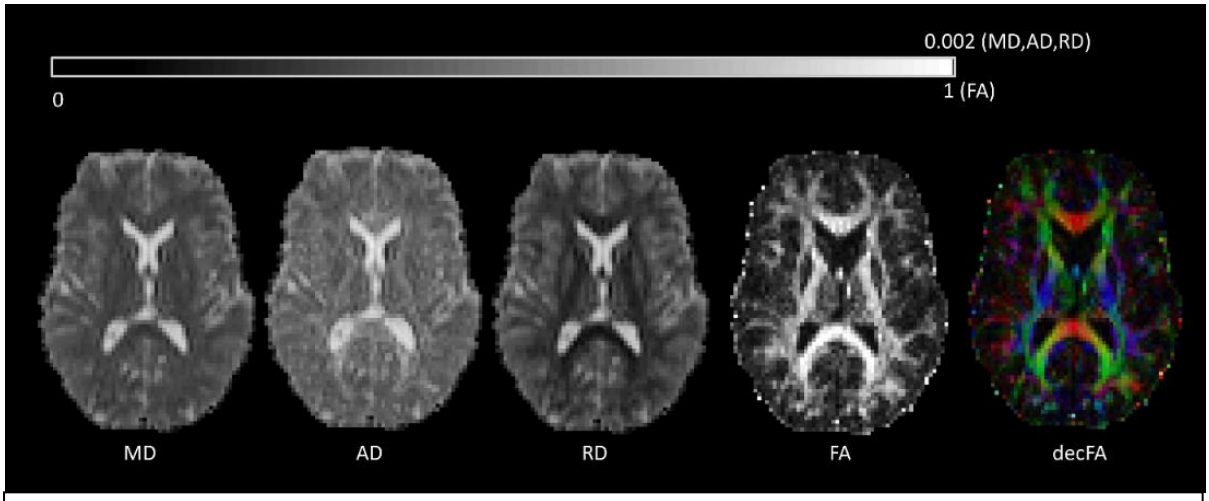

Figure 2.2: Diffusion maps. From left to right: Mean diffusivity (MD) measures the overall speed of diffusion with high MD (brighter) in the cerebrospinal fluid. Axial diffusivity (AD) measure the speed of diffusion along the principal eigenvector, while radial diffusivity (RD) represents the speed of diffusion perpendicular to the principal eigenvector. Fractional anisotropy (FA) is the measure of the local anisotropic diffusion; higher values (closer to 1) represents anisotropic diffusion. FA is high in WM and low in GM and CSF. Directionally encoded color FA maps (decFA) represent the orientation of the principal eigenvector in a red-green-blue (RGB) color scheme.

Radiological pathological correlation of diffusion images

The theoretical basis of diffusion imaging is that it reflects the constraints on the diffusion of water. There have been studies that have investigated the correlation between the DTI appearances and the underlying pathology (Tu, Williams et al. 2016). Alteration of diffusivity occurs as a result of changes in microstructural tissue and fiber organization. Microstructural architecture and increased cellularity were postulated to correlate with gliosis in fractional anisotropy (FA) and mean diffusivity (MD) (Tu, Williams et al. 2016). Quantitative FA measures are believed to reflect changes in the myelination, fiber density and packing (Mädler, Drabycz et al. 2008). Generally speaking, decreased diffusivity and increased diffusion anisotropy reflects more structural organization and better WM structural integrity (Pannek, Guzzetta et al. 2012). What we know about DTI metrics is largely based upon empirical studies that investigate how these measures correlate with the underlying pathology. Evidence suggests that FA is sensitive to the WM integrity but is not very specific and cannot distinguish between different pathological processes (Filippi, Iannucci et al. 2000, Assaf and Pasternak 2008, Sbardella, Tona et al. 2013). MD is suggested to be influenced by free water diffusion in cellular space, such as oedema, which is increased in neurodegenerative diseases (Filippi, Iannucci et al. 2000). Axial diffusivity (AD) is thought to reflect demyelination in white matter tracts. Axial integrity of axons and can be reflected as either increased or decreased AD when WM is destroyed by any other process (Mayer, Ling et al. 2010). It has not yet been well-defined whether RD is specific to demyelination and could also be related to be due to; the occurrence of inflammation, or to axonal damage. RD was found to be high in neurodegenerative disease (Sun, Liang et al. 2006). Radial diffusivity (RD) has been shown to correlate with demyelination and dysmyelination in several pathological models (Song, Sun et al. 2002, Fink, Klein et al. 2010).

Wallerian degeneration (WD) is a pathological event that begins with physical fragmentation of axons within days after injury, followed by breaking down of myelin sheath due to infiltration of macrophages (Thomalla, Glauche et al. 2004). This results in a distal degeneration of the nerve fiber and fibrosis and atrophy (Sawlani, Gupta et al. 1997). This is slower in the central nervous system (CNS) than in the peripheral nervous system (PNS) (Koeppen 2004). MRI can detect Wallerian degeneration in human brain secondary to an insult that correlates to functional disability (Mazumdar, Mukherjee et al. 2003, Grassel, Ringer et al. 2010). Degeneration of neuronal cell bodies and axonal degeneration are separate and

independent processes, and are found to have differing appearances in diffusion studies (Finn, Weil et al. 2000).

Non-conventional MRI techniques are those that are mainly used in the research fields to help in the early diagnosis or as a tool to monitor disease progression and drug trials. One is the diffusion weighted imaging (DWI), which became an established tool for WM integrity and fiber tracking in neurodegenerative diseases.

Role of MRI in ALS

Corticospinal tract degeneration is the hallmark of ALS pathology. Different structural MR imaging modalities can show the changes in CST (Hecht, Fellner et al. 2002, Lee, Markus et al. 2003, da Rocha, Oliveira et al. 2004, Kwan, Jeong et al. 2012). On fluid-attenuated inversion recovery (FLAIR) imaging, signal hyperintensities along the CST were more significant compared to other MR sequences (Hecht, Fellner et al. 2001). Using DTI in ALS, several studies have reported a decrease in the FA in CST earlier than other MR techniques (Pierpaoli, Barnett et al. 2001). In ALS, there is evidence of Wallerian degeneration and atrophy of the myelinated fibers in the CST (Delisle and Carpenter 1984).

DTI in ALS demonstrated abnormalities of the CST at different levels from its origin in the motor cortex to the upper spinal cord (Eisen and Weber 2001). All DTI metrics are found to be increased, except FA, which is reduced (Metwalli, Benatar et al. 2010, Douaud, Filippini et al. 2011, Sarro, Agosta et al. 2011). In ALS, Ellis et al not only demonstrated a positive correlation between the disease duration and the MD but also found a correlation between reduced FA values and the disease severity (Ellis, Simmons et al. 1999).

Many DTI studies have been carried out to compare DTI parameters in different groups and correlate these variables with clinical measures. Since the 20th century, there have been reports suggesting the correlation between reduction in FA values and neuronal damage in neurodegenerative diseases including ALS (Ellis, Simmons et al. 1999, Uluğ, Moore et al. 1999). The DTI changes along corticospinal tract (CST) have been studied in ALS (Cosottini, Giannelli et al. 2005, Wong, Concha et al. 2007, Bartels, Mertens et al. 2008). Other ALS

studies have detected changes on brain stem (BS) and corpus callosum (CC) (Hong, Lee et al. 2004, Ciccarelli, Behrens et al. 2009).

This project used DTI to investigate changes in WM microstructure in patients with or without cognitive impairment, as well as serially. A systematic review of the literature on DTI in ALS is provided in Chapter 4. The image processing and analysis approaches used in this project are described in the following chapter.

Chapter 3: Methods

This chapter describes in detail the methods that were used in the project. The specific analyses used are given in the chapters about each study.

Participants

All participants provided written informed consent for this study. This study was approved by the Human Research Ethics Committee (HREC) at the Royal Brisbane Women's Hospital (RBWH) protocol number 2008/98.

ALS patients

Patients with clinically probable or definite ALS as defined by the revised El Escorial criteria (Brooks, Miller et al. 2000) were recruited for this project from the motor neuron disease (MND) Clinic at the Royal Brisbane and Women's Hospital (RBWH).

Patients were classified according to the site of onset of disease (Limb-onset or bulbar-onset) as well as handedness. The disease duration since onset of symptoms was also recorded. Disease severity was assessed every three months by the Revised ALS Functional Rating Scale (ALSFRS-R) (Cedarbaum, Stambler et al. 1999). MRI was performed at baseline, 6 months, and 12 months. Of 30 recruited patients, 30 successfully underwent baseline MRI. Of these, 23 returned for 6-month follow-up, and 15 returned for 12-month follow-up. Demographics are presented in Table 3.1. Cognitive examination (ACE-III and FAB) was administered every 6 months on the same day as the MRI.

Table 3.1: Details of subjects.

Time-point	Total number of patients	Age (mean, SD)	Gender (M:F)	ALSFRS-R (mean, SD)	ACE-III (mean, SD)	FAB (mean, SD)	Controls
Base-line	30	61.7 (1.4)	19:11	38 (5)	88.5 (7.5)	15.5 (2.5)	19
6–month follow-up	23	59.5 (11)	16:7	38 (4.5)	NA	NA	NA
12–month follow-up	15	60 (13)	10:5	38.7 (4.5)	88 (7)	15 (3)	13

Healthy controls

Nineteen neurologically normal subjects with no current or previous history of neurological, medical or surgical conditions were recruited from the patients' relatives and from the The university of Queensland Centre for clinical research (UQ-CCR) volunteer scheme. Controls had only a single MRI.

Clinical Measures

Amyotrophic Lateral Sclerosis Functional Rating (ALSRFS-R)

Motor function was assessed with the revised Amyotrophic Lateral Sclerosis Functional Rating (ALSRFS-R) (Cedarbaum, Stambler et al. 1999). ALSFRS-R a 12-item scale evaluating motor skills of ALS patients, including speech, breathing, swallowing, and walking and so on) (Cedarbaum, Stambler et al. 1999). Each item is scored from 0 to 4 (worst to best respectively).

Addenbrooke's Cognitive Examination (ACE)

Addenbrooke's Cognitive Examination (ACE), discussed in Chapter 1, was designed to survey key domains of cognitive function without the need for specialized equipment and to be sensitive to early stages of Alzheimer's disease and FTD. It has been proven

that it detects cognitive impairment that causes cognitive decline. The sub scores are suggested to differentiate diagnosis between FTD and AD (Mathuranath, Nestor et al. 2000, Davies and Larner 2013).

Addenbrooke's Cognitive Examination Revised (ACE-R) is the revised version of ACE which incorporates the MMSE and is intended to be quick to administer and acceptable to subjects. The overall score is 100 (Mioshi, Dawson et al. 2006). The authors created three different alternative versions of the name and address recall preventing recalling from previous clinical visits. However, ACE-III was developed to omit the MMSE items due to copy rights restrictions and modification is explained further in the methodology section.

Frontal Assessment Battery (FAB)

FAB is a test that was designed mainly to assess frontal lobe processes and identify the presence and severity of dysexecutive syndrome that affect both motor behaviour and cognitive function in several neurodegenerative diseases (Dubois, Slachevsky et al. 2000). A global agreement amongst researchers validated FAB as a fast, short, and easy bedside battery that measure dysexecustive function (Slachevsky, Villalpando et al. 2004, Maeshima, Tanemura et al. 2006).

Systematic review

A systematic review is a high level of evidence designed to summarizes the results of the available studies in the literature to provide judgments and recommendations for healthcare and research (Collaboration 1996). In this study, a research question was developed to investigate the WM tracts that were studies in ALS and FTD and pool the significant findings using DTI to report the shared tracts in both ALS and FTD. Therefore, a thorough search of the literature from five databases was performed on Dec 2015 with search terms that are discussed in detail in Chapter 4. The result papers were evaluated for inclusion criteria and eligibility by two raters (A.A and M.D) who also extracted the data.

MRI acquisition

MRI data were acquired using a 3 T Siemens TimTrio, using the commercial sequences from VB17 Neuro application and Diffusion Tensor Imaging (DTI) and a 12–channel head coil. Along with a number of clinical radiological sequences, (a) a high resolution structural image using 0.44 mm^3 isotropic 3D T1 MPRAGE (TR=1900 ms, TE= 2.4 ms, TI= 900 ms, FOV 24 × 25.6 × 17.6 cm, flip angle=9º), (b) diffusion images using the following parameters: 60 axial slices, TR=3200ms, TE=91ms, FOV 30x30 cm, acquisition matrix 128x128 with in-plane resolution=2.3 mm, slice thickness=2.5 mm, acceleration factor of 2 employing GRAPPA (Griswold, Jakob et al. 2002) were also acquired. Sixty-four non-collinear diffusion weighted images were acquired at *b*=3000 s mm^{-2}, along with one non-diffusion weighted image, and c) a field map was acquired using two 2D gradient-recalled echo images with TE1/TE2=4.92/7.3 and TR= 488 ms to assist in the correction of geometric distortions. The total scan time was 30–40 minutes while subjects lie comfortably in supine position with supporting cushions and knee rest. The b value was chosen to allow detection of the crossing fibres. These parameters have been used in our previous DTI studies of MND (Rose, Pannek et al. 2012).

Preprocessing of diffusion images

To obtain accurate diffusion results, adequate pre-processing steps for diffusion data is required. Figure 3.1 summarizes the workflow for diffusion data pre-processing suggested by Pannek et al (Pannek, Guzzetta et al. 2012), which was followed in this study. While this pre-processing workflow was initially developed for use with neonates, the same approach was followed here because motion artefacts are frequently observed in ALS patients who are often restless, due to their disease symptoms.

Correction of image artefacts

The following issues were considered and resolved:

a) Quality of raw diffusion images was assessed visually.

b) Misalignment of the odd and even sub–volumes was detected using the discontinuity index (Nam and Park 2011). Affected volumes were removed from further analysis.
c) Intensity inhomogeneity caused by magnetic field inhomogeneity in the static magnetic field B_0 and the radiofrequency (RF) field B_1 was corrected. The bias field was calculated from the non-diffusion weighted images using N3 and applied to unregistered diffusion images (Vovk, Pernus et al. 2007).
d) Susceptibility distortions, which occur in echo planar imaging (EPI), were reduced using parallel imaging (GRAPPA). Residual distortions were corrected using a field map (http://www.fmrib.ox.ac.uk/fsl) (Jenkinson 2004, Wu, Chang et al. 2008).
e) Misalignments due to physical motion were corrected by employing a rigid-body registration using the fit model to all measurements (FMAM) method (Bai and Alexander 2008). The *b*-matrix was rotated to account for head motion (Leemans and Jones 2009).
f) Outliers caused by bulk head motion and cardiac pulsation were corrected using a combination of higher order model outlier rejection (HOMOR) (Tournier, Calamante et al. 2012) and detection and replacement of outliers prior to resampling (DROP-R) (Morris, Nossin-Manor et al. 2011).
g) The diffusion tensor was fit to the corrected diffusion images, and maps of FA, MD, AD, and RD were calculated.

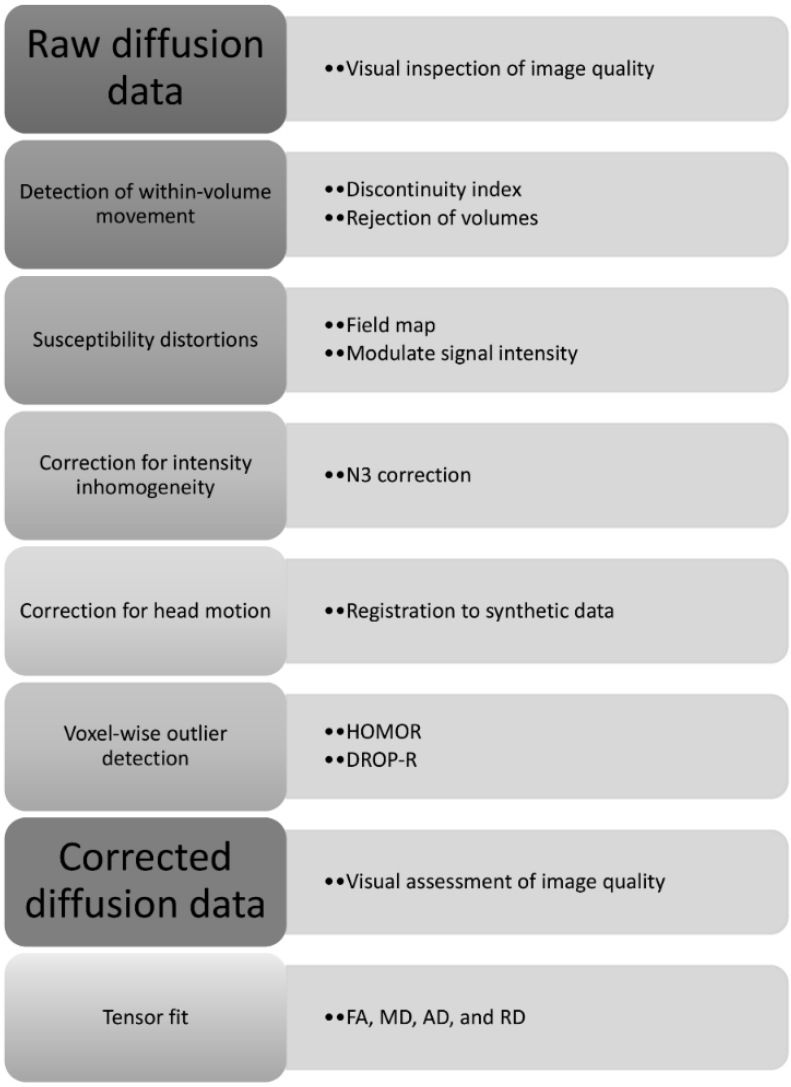

Figure 3.1: Preprocessing pipeline of diffusion data

MRI analysis

WM analysis

Region of interest (ROI)

Region of interest (ROI) analysis involves defining an area the WM which is used to obtain DTI measurements for this specific site. Typically, the structure in question is outlined manually. ROIs are usually defined on the colored FA maps or structural MR images and the structure is visualized to be at its thickest. Manual delineation of ROIs is operator-dependent, which can be time consuming in large cohort studies or when a large number of regions is examined. For reproducibility of results, the ROIs have to be drawn several times by the same operator, and ideally also by an independent operator.

The placement of the ROI can have a significant effect on the results. Thus, one has to understand that ROI has several drawbacks which can be due to user bias and the low resolution. Moreover, there is significant variability in the diffusion measurements as a result of orientation of the plane and the positions of the slices in small structures (Snook, Plewes et al. 2007). It has been suggested that ROIs drawn following the shape of the structure (Schneider, Il'yasov et al. 2004, Snook, Paulson et al. 2005) may be preferred to placing circles or squares over ROI, especially when the area is large (Shimony, McKinstry et al. 1999, Abe, Aoki et al. 2002, Suzuki, Matsuzawa et al. 2003, Yoshiura, Mihara et al. 2005). The major limitation of ROI is the practicality of measuring many ROIs of the brain which introduces time constraints. Although previous studies suggest that voxel placement differs by less than a voxel (Ciccarelli, Parker et al. 2003), the manual method still suffer variability in the placement of ROIs. ROI placement relies on the operator's level of anatomical knowledge and judgment which can be a concern about the intra- and inter-operator reproducibility (Heiervang, Behrens et al. 2006). To overcome this limitation, the automated ROI approach can be employed. Atlas-based ROI demonstrates a significant time savings with minimal difference in reproducibility over manual methods (Chow, Takeshita et al. 2007, Zhang, Zhang et al. 2010). In this study we used both manually outlined ROIs and atlas-based ROIs.

TBSS

Tract-based spatial statistics (TBSS) (Smith, Jenkinson et al. 2006) is a "whole-brain" analysis method that was introduced by Smith et al as part of the FSL package. TBSS has gained interest due to the simplicity and user-friendly input. In contrast to typical voxel-based analyses, TBSS does not require smoothing of the data to improve signal to noise ratio, which can lead to arbitrariness. TBSS creates a skeleton which represents the center of the WM. This step allows any residual misalignment to be removed, which adds the advantage that TBSS does not require perfect registration. TBSS has been shown to enhance specificity (Bach, Laun et al. 2014).

The steps required for running TBSS can be found at (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/TBSS/UserGuide). Briefly, following preprocessing of diffusion images, all individual FA maps are aligned to the FMRIB58 FA template. Registration accuracy is assessed visually. An FA "skeleton", representing the centers of the WM tracts common to all subjects, is created. In this study, we employed an FA threshold of 0.2 for the skeletonisation, which is also the threshold recommended in the User Guide. FA values from all subjects are then projected onto this skeleton for statistical analysis. Although the skeleton is derived from FA values, other DTI measures such as MD, AD and RD can still be employed in the statistical analysis by applying the projection calculated for FA maps.

Statistical analyses are performed for each voxel of the skeleton, with appropriate correction for multiple comparisons across space as described below (Winkler, Ridgway et al. 2014).

GM analysis using Voxel–based morphometry (VBM)

Voxel-based morphometry (VBM) is an automated method for analyzing structural MR images. VBM allows whole brain analysis without a priori hypothesis regarding the brain regions involved (Douaud, Smith et al. 2007). This standard approach was developed by Ashburner and Friston to compare GM volumes between two groups (Ashburner and Friston 2000). It has high sensitivity to GM atrophy and is available in different image analysis packages.

In this study, VBM is performed on T1–weighted images using the FSL–VBM toolbox (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLVBM). Briefly, brain was extracted from all subjects' data to exclude all non-brain tissue followed by a visual check of the extraction for every subject. GM was segmented before applying non-linear registration to the MNI 152 standard space which results in study-specific GM template in standard space (Andersson, Jenkinson et al. 2007). GM maps were averaged and flipped along the x-axis to create a left-right symmetric GM template. All native grey matter images were non-linearly registered to this study-specific template and "modulated" to correct for contraction or expansion. The normalized GM images were then smoothed with an isotropic Gaussian kernel which is developed to reduce Type 1 error (Worsley, Evans et al. 1992). VBM makes a simple and relatively fast approach to assessing small structural differences that is within the capabilities of most research units.

Statistical analysis for TBSS and VBM

To identify group differences in diffusion metrics using TBSS and VBM, we used the "randomise" tool of FSL (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Randomise/UserGuide). With the assumption that each voxel represents the same anatomic location between subjects, VBM and TBSS perform a voxel-wise general linear model (GLM) statistical analysis across space is performed using permutation-based non-parametric testing. Correction for multiple comparisons to localize and make inferences about group differences was performed using threshold-free cluster enhancement (TFCE, (Smith and Nichols 2009)). TFCE is an approach to avoid the problem of thresholding selection in the context of cluster-based statistics. P-values were determined using 5000 random permutations (Nichols and Holmes 2002). Results were considered significant at $p<0.05$, corrected for multiple comparisons using TFCE. The end result shows regions where WM tracts and GM volume differs significantly between groups.

The following chapters are written following the “paper-style”. Most of the chapters are either submitted and under review (Chapter 5), or manuscripts ready for submission (Chapters 4, 6 and 7). These chapters contain further detail about the methods that were used.

Chapter 4: White matter tracts shared in ALS and FTD using DTI

Preamble

This chapter consist of a manuscript titled “A Systematic Review of diffusion tensor imaging in amyotrophic lateral sclerosis and frontotemporal dementia: a study of white matter tracts”. Here, I address the first aim of this thesis, which is to determine the evidence of WM alterations in both ALS and FTD. Thus, the steps included developing the research question, setting the inclusion and exclusion criteria. Next, designing the searches of the databases, screening all titles and abstracts, and inspecting eligibility of the papers. Then extracted the required data and systematically summarized, interpreted and discussed the findings.

A Systematic Review of diffusion tensor imaging in amyotrophic lateral sclerosis and frontotemporal dementia

Introduction:

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the progressive death of upper and lower motor neurons. ALS is thought to arise from a combination of genetic susceptibility and environmental exposure (Fang, Kamel et al. 2009, Sutedja, Veldink et al. 2009). Patients with ALS develop weakness of the limbs, the bulbar muscles and the diaphragm (Kiernan, Vucic et al. 2011). There are clinical signs of dysfunction of both upper motor neurons (UMN) and lower motor neurons (LMN) (Miller, Gelinas et al. 2005).

Frontotemporal dementia (FTD) is the term for progressive dementias characterized by cognitive and behavioral difficulties, with degeneration of the frontal and temporal lobes. FTD can be divided into behavioral variant, primary progressive aphasia and semantic dementia (Warren, Rohrer et al. 2013). Pathological studies allow the division of FTD into tauopathy and ubiquinopathy (Kertesz and McMonagle 2011) .

A proportion of patients with ALS show cognitive impairment with subtle executive function deficits (Raaphorst, De Visser et al. 2010, Phukan, Elamin et al. 2012) and 10-15% fulfill the criteria for diagnosis of frontotemporal dementia (FTD) (Ringholz, Appel et al. 2005). It is now considered that ALS and FTD lie on a pathological and clinical spectrum (Von Braunmühl 1932). Some causative genes are found in both ALS and FTD (Siddique and Deng 1996, Al-Chalabi, Jones et al. 2012, Pan and Chen 2013), with the most important so far identified being C9orf72 (Mori, Weng et al. 2013). Some pathological features are common to FTD, ALS-FTD and ALS (Ferrari, Kapogiannis et al. 2011), particularly TDP-43 pathology and possibly dipeptide repeats (Al-Chalabi and Hardiman 2013), which are found in many patients with ALS and patients with FTD with ubiquinopathy. An anatomical study has shown that behavioural disturbance

in patients with ALS is significantly associated with frontal lobe involvement (Tsujimoto, Senda et al. 2011).

Pathological studies on autopsy material are done at the end stage of disease, whereas imaging studies allow the assessment of earlier stages of disease. Various neuroimaging modalities have been used to study ALS and FTD (Wang, Poptani et al. 2006) including diffusion tensor imaging (DTI), which is able to detect microstructural changes in fiber tracts(Uluğ, Moore et al. 1999, Hong, Lee et al. 2004). DTI is increasingly being used to show changes in fiber tracts in neurodegenerative diseases. Generally speaking, decreased diffusivity and increased diffusion anisotropy reflect greater structural organization and white matter (WM) structural integrity (Pannek, Guzzetta et al. 2012).

There have been previous meta-analyses of DTI abnormalities in ALS. One study reported a voxel based meta-analysis of FA of 143 non-demented ALS patients from 7 studies (Li, Pan et al. 2012). Another reported 170 patients from 9 studies, to determine a threshold for FA abnormality in the corticospinal tract (Rosskopf, Müller et al. 2015).

Because some patients with ALS also have FTD or less severe cognitive defects, we expect there will be overlap of pathology (Yamada, Sakai et al. 2009). Brain regions that show abnormal DTI changes in both ALS and FTD may provide clues to elucidating the pathogenesis of ALS-FTD overlap. Here I aim to review the literature regarding the location and extent of white matter tract degeneration in ALS patients with and without FTD, and patients with pure FTD, to determine if there is overlap of the fiber tract involvement in these conditions. The studies in this review use various MRI measures making a meta-analysis impossible. This systematic review is a therefore a descriptive study. We also review the correlations of DTI measures with clinical status.

Methods:

Search strategy and key words

In December 2015, a systematic search was conducted of the databases; Pubmed Embase, CINAHL, Scopus and Web of Science.

The search strategy used the medical subject heading (MeSH) terms and the keywords: "amyotrophic lateral sclerosis", "ALS", "frontotemporal dementia", "FTD", "cognitive impairment", "diffusion tensor imaging", "DTI", "tractography", and "white matter tracts". These were then combined to limit the findings: ["amyotrophic lateral sclerosis" AND diffusion tensor imaging], ["Frontotemporal dementia" AND diffusion tensor imaging], [amyotrophic lateral sclerosis" AND tractography], ["Frontotemporal dementia" AND tractography], ["Frontotemporal dementia" AND white matter tracts], [amyotrophic lateral sclerosis" AND white matter tracts].

Selection criteria:

Inclusion criteria: 1) studies were carried out in human subjects equal to or older than 18 years of age; 2) subjects diagnosed with amyotrophic lateral sclerosis met the accepted El Escorial diagnostic criteria (Brooks, Miller et al. 2000); 3) subjects diagnosed with ALS-FTD or FTD fulfilled the Neary criteria for diagnosis of frontotemporal dementia (Neary, Snowden et al. 1998); 4) studies provided an adequate descriptions of the patient sample such as male: female ratio, age and whether the subjects had other neurological disorders or disease; 5) studies used diffusion tensor imaging (DTI) region of interest (ROI), tractography, or voxel-based analysis to assess white matter tracts by comparing DTI measures between patients and healthy controls; 6) studies provided adequate description of the imaging protocol (MR sequence, b-value, diffusion directions, threshold) and statistical data such as mean, and SD; 7) studies measured DTI metrics in specific white matter regions with defined coordinates.

Exclusion criteria: 1) studies which included children and adolescents younger than 18 years; 2) subjects who had associated neurological disorders or pathologic changes such as brain tumor, epilepsy or brain injury; 3) studies with fewer than 7 participants, 4) studies without healthy controls participants; 5) studies not published in English. 6) studies with missing data such as details about participants or imaging sequence. 7) studies assessed as low quality studies.

Two independent raters (A.A and M.D) assessed eligibility based on the title and abstract of each study. Then a full screening of references was performed for inclusion.

Assessment of methodological quality

Two raters (Ashwag Alruwaili, Mathew Devine) independently assessed the quality of each study according to the quality of diagnostic accuracy studies (QUADAS) criteria (Whiting, Rutjes et al. 2003). Each criterion was scored “yes”, “no” or “unclear”. A score of 7-10 of 14 is considered to be a high quality study.

Data extraction

The details of data extracted from all eligible studies included: a) total number of participants, 2) the imaging sequences (field strength, b-value, number of directions, smoothing and FA threshold), 3) WM analysis type (ROI, VBA, tractography), 4) DTI metrics (FA, MD, AD, RD) and, 5) the brain regions studied.

Selection of tracts for inclusion in figures

To create a visual representation of the tracts that are abnormal in ALS and FTD, we selected tracts that were reported as being significantly abnormal in more than 66% of the studies that assessed them. These are the tracts for which the evidence is strongest. Selection of these tracts was based on results of FA, because this measure was used most consistently across the different studies.

Results

Overall summary and participant characteristics

As shown in Figure 4.1, of 233 papers, a total of 64 studies met the criteria for full review. These papers reported on a total 1674 patients (ALS N=1192, FTD N=458 and ALS-FTD N=24) and 1411 healthy controls. The extracted data are given in Supp Table 4.1. Table 4.1 summarizes the information about the number of participants in each clinical category. For the studies of ALS, the total patient number was 1192, with 991 controls. For the studies of FTD there were 458 subjects and 420 controls. In addition, two paper studied ALS with FTD (n=24).

Table 4.2 summarizes the use of different techniques to analyze white matter changes in ALS, FTD and ALS-FTD. These were regions of interest (ROI, n=23), tractography (n=15), tract-based spatial statistics (TBSS, n=22), and tract of interest (TOI, n=2). There were also studies that used mixed analysis (n=14).

Table 4.1: Details of participants.

Disease studied	No of papers	No of patients	No of controls
ALS	43	1192	991
FTD	20**	458	420
ALS and FTD	2	24	*

* Controls included in the main ALS studies controls column.

** Three ALS papers have included ALS-FTD subjects (so total of 23 studies).

Table 4.2: Summary of WM analysis.

Disease studied	No. of studies using each technique				
	ROI	Tractography	TBSS	TOI	Mixed
ALS	16	12	15	1	11*
FTD	7	3	6	1	3**
ALS and FTD	-	-	1	-	-

***1 ROI+TBSS+ Tractography, 3 ROI+TBSS, 3 TBSS+Tractography, 1 ROI + Tractography, 1TOI+ROI**
**** 1 TOI+VWA, 1 canonical correlation analysis, 1 ROI+TBSS**

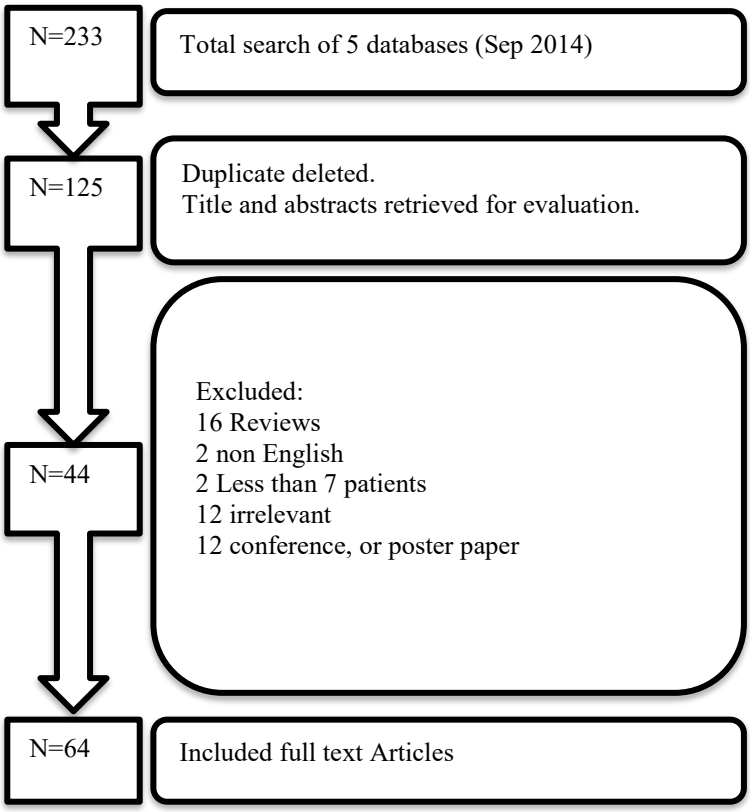

Figure 4.1: Pipeline of inclusion of studies.

DTI in ALS:

A summary of the number of papers that studied each tract, and the number of these papers that found significant abnormalities in each tract using FA is provided in Table 4.3. The full details of the extracted data are presented in Supp Table 4.1. In the following sections, we mostly describe the studies that found significant results from DTI measures. The non-significant findings are presented in each table but not within the text. The full details of the studies with both positive and negative results, for each class of white matter fibers, are presented in Tables 4.4–4.7. A visual summary of the WM tracts that were found to be significant in ALS studies is presented in Figure 4.2. The details of the clinical correlations of the DTI results for each MRI metric are given in Supp Tables (4.2–4.6).

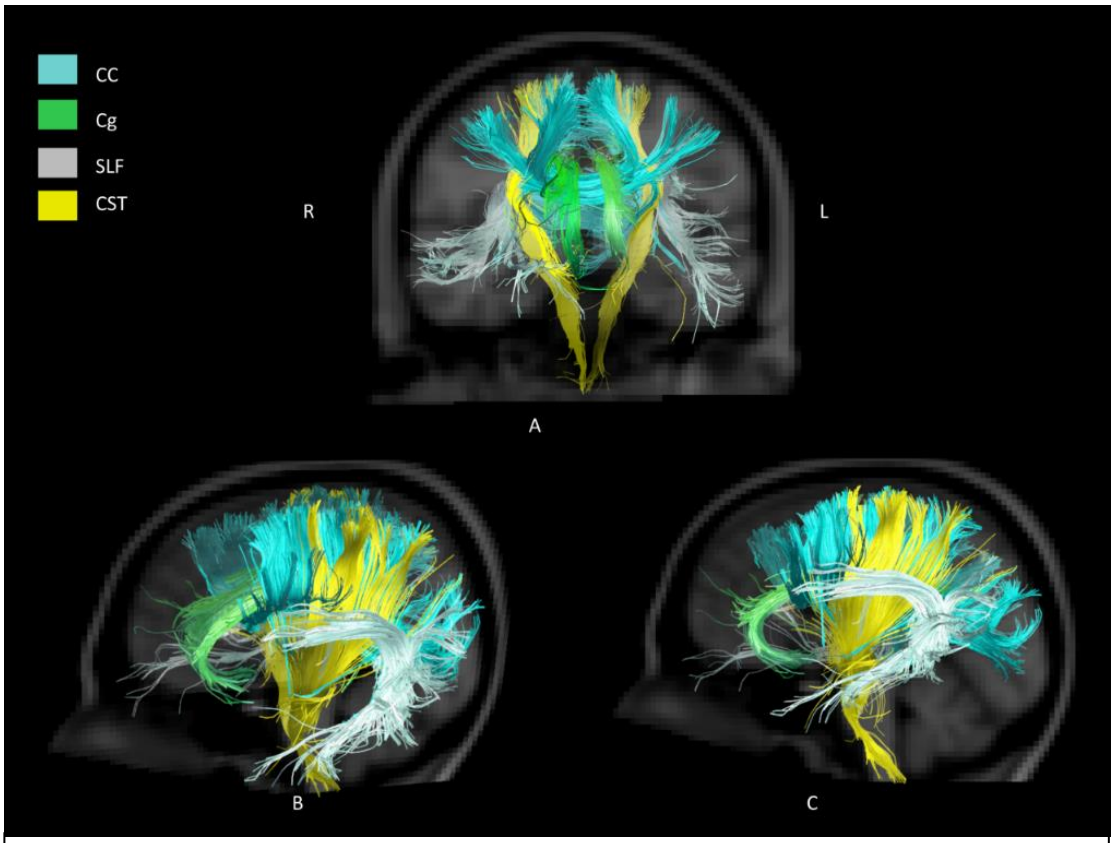

Figure 4.2: Visual representation of WM tracts that were found significant in ALS studies.

Table 4.3: Numbers of FA studies with significant results.

Tracts	ALS Number of positive/total number of studies	%	FTD Number of positive/total number of studies	%
Association fibers				
Superior longitudinal fasciculus	10/13	77	7/7	100
Inferior longitudinal fasciculus	6/10	60	7/8*	87
Inferior fronto-occipital fasciculus	5/7	71	6/7*	86
Uncinate fasciculus	5/10	50	12/13*	92
Fronto-occipital fasciculus	1/1	-	1/1	-
Arcuate fasciculus	1/1	-	1/1	-
Commisural fibers				
Corpus callosum	15/19	79	9/13	69
Forceps minor	1/3	33	3/4	75
Forceps major	0/2	0	1/1	-
Limbic system tracts				
Thalamus	6/10	60	4/5	80
Cingulum	4/6	66	9/11	81
Fornix	1/2	50	1/2	50
Hippocampus	-		1/2	50
Brainstem tracts				
Cerebral peduncle	4/7	57	-	
Cerebellar	1/3	33	1/1	-
Medulla oblongata	2/3	66	-	
Cortico-bulbar tract	1/1	-	-	
Projection fibers				
Cortico-striatal tracts	1/1	-	-	
Cortico-rubral tracts	1/1	-	-	
Cortico-pontine tracts	1/1	-	-	
Cortico-spinal tracts (averaged)	25/33	76	5/8	62
Cortico-spinal tracts (corona radiata)	11/11	100	2/2	100
Cortico-spinal tracts (internal capsule)	12/16	75	1/2	50
Cortico-spinal tracts (pons)	4/6	66	-	

Whole brain analysis

Six studies performed whole brain analysis (Lillo, Mioshi et al. 2012, McMillan, Brun et al. 2012, Pettit, Bastin et al. 2012, Prudlo, Bissbort et al. 2012, Sarica, Cerasa et al. 2014, Trojsi, Caiazzo et al. 2015). Whole brain TBSS found significant changes in FA and MD in the SLF bilaterally (Sarica, Cerasa et al. 2014, Trojsi, Caiazzo et al. 2015). Based on the clinical staging system for ALS, proposed by Roche et al. (Roche, Rojas-Garcia et al. 2012) (refer to appendix for details), a longitudinal study revealed that the body of CC shows reduced FA in stage 2A, and reduced FA and increased MD and RD in stage 3, compared to controls (Trojsi, Caiazzo et al. 2015). A different study found that the FA in the SLF, ILF and IFOF was reduced in ALS patients compared to controls (Prudlo, Bissbort et al. 2012). In both bulbar and limb onset ALS compared to controls, CST showed reduced FA along the tract (Cardenas-Blanco, Machts et al. 2014). One study reported that ALS patients showed more degeneration in the CST than in callosal and association tracts compared to controls (Lillo, Mioshi et al. 2012). While this was not the case when the same ALS subjects were compared to FTD and ALS-FTD patients, TBSS results shows less CST involvement than extra-motor tracts (Lillo, Mioshi et al. 2012).

ROI and Tractography analysis

Projection Fibers

Corticospinal tract (CST) degeneration is a hallmark of ALS and hence the CST was studied in ALS frequently. The studies that found significant and non-significant results are given in Table 4.4 and a summary in Figure 4.2.

Apart from the study of Grapperon et al who reported an increase in FA (Grapperon, Verschueren et al. 2014), all other studies found reduction of the average FA over the entire CST(Abe, Yamada et al. 2004, Karlsborg, Rosenbaum et al. 2004, Iwata, Aoki et al. 2008, Sarro, Agosta et al. 2011, Tsujimoto, Senda et al. 2011, Lillo, Mioshi et al. 2012, Pettit, Bastin et al. 2012, Prudlo, Bissbort et al. 2012, Agosta, Caso et al. 2013, Bastin, Pettit et al. 2013, Rajagopalan, Yue et al. 2013, Trojsi, Corbo et al. 2013, Crespi, Cerami et al. 2014, Floeter, Katipally et al. 2014, Kassubek, Müller et al. 2014, Prokscha, Guo et al. 2014) as well as at different levels along the tract (Toosy, Werring

et al. 2003, Karlsborg, Rosenbaum et al. 2004, Mitsumoto, Ulug et al. 2007, Rajagopalan, Yue et al. 2013, Hübers, Müller et al. 2015). This was more apparent in the right CST (Sarica, Cerasa et al. 2014) with RD increase (Zhang, Schuff et al. 2009), superiorly (Bede, Bokde et al. 2013) caudally (Zhang, Yin et al. 2014, Hübers, Müller et al. 2015) and rostrally (Filippini, Douaud et al. 2010) and with MD increases (Toosy, Werring et al. 2003, Prudlo, Bissbort et al. 2012, Floeter, Katipally et al. 2014) at the level of the internal capsule (Sach, Winkler et al. 2004). One study found changes in CST in familial ALS (fALS) but not in sporadic ALS when compared to controls (Mitsumoto, Ulug et al. 2007). In both bulbar and limb onset ALS compared to controls, CST showed reduced FA and exhibited in higher RD and MD when using ROI (Cardenas-Blanco, Machts et al. 2014). Some other groups reported significant changes in RD (Menke, Körner et al. 2014, Zimmerman - Moreno, Ben Bashat et al. 2015, Vora, Kumar et al. 2016) and AD (Menke, Körner et al. 2014) along the CST (Kassubek, Müller et al. 2014, Menke, Körner et al. 2014). Steinbach et al found connectivity indices[1] to be significantly reduced along the tract (Steinbach, Loewe et al. 2015). The motor pathway was also studied at the level of internal capsule (PLIC) (Kasper, Schuster et al. 2014, Vora, Kumar et al. 2016) and corona radiata where it was found to have significantly reduced FA (Agosta, Caso et al. 2013, Agosta, Galantucci et al. 2015) and increased RD (Sarica, Cerasa et al. 2014) and MD (Pettit, Bastin et al. 2012, Sarica, Cerasa et al. 2014, Agosta, Galantucci et al. 2015). Some studies investigated the CST at the level of pons (Toosy, Werring et al. 2003, Iwata, Aoki et al. 2008, Sheelakumari, Madhusoodanan et al. 2015) and found significantly reduced FA and increased MD. In the CST, Romano et al found significantly reduced FA and increased MD and RD, but no changes in AD (Romano, Guo et al. 2014). Another group classified ALS participants based on a staging system (Roche, Rojas-Garcia et al. 2012) and found reduced FA in left rostral CST in stages 2A; at diagnosis, and 3; when a third body region was involved (Trojsi, Caiazzo et al. 2015). The changes in the latter stage involved more DTI metrics such as higher MD and RD (Trojsi, Caiazzo et al. 2015).

[1] Index of structural connectivity (CI) is the proportion of plausible paths connecting two ROIs, averaged across tractography directions.

The correlations of DTI measures with quantitative clinical measures are shown in Supp Tables 4.2–4.6. In ALS, increased AD (Menke, Körner et al. 2014), MD (Menke, Körner et al. 2014) and RD (Menke, Körner et al. 2014) and decreased FA along the CST were associated with higher UMN scores (Abe, Yamada et al. 2004, Iwata, Aoki et al. 2008, Filippini, Douaud et al. 2010, Trojsi, Corbo et al. 2013, Menke, Körner et al. 2014, Woo, Wang et al. 2014). Longer disease duration correlated with increased FA (Filippini, Douaud et al. 2010) and AD (Rajagopalan, Yue et al. 2013). Reduced FA along the CST correlated positively with delayed central motor conduction time (CMCT) and cortical-brainstem conduction time (CTX-BS) (Iwata, Aoki et al. 2008), but negatively with disease progression (Bastin, Pettit et al. 2013). Disease progression correlated with increased RD when using ROI (Menke, Körner et al. 2014) and correlated negatively with reduced connectivity indices (Steinbach, Loewe et al. 2015). In many studies (Furtula, Johnsen et al. 2013, Grapperon, Verschueren et al. 2014, Woo, Wang et al. 2014) there was no significant correlation between DTI metrics and clinical features such as forced vital capacity (FVC) and site of onset (Pettit, Bastin et al. 2012). Changes in FA (Woo, Wang et al. 2014), RD and MD were negatively correlated with ALSFRS-R (Sarica, Cerasa et al. 2014) in the right CST. The latter study found that there was a trend towards significant correlation between ALSFRS-R scores and FA (Sarica, Cerasa et al. 2014). Other studies found no correlation between FA and ALSFRS-R and disease duration (Furtula, Johnsen et al. 2013, Woo, Wang et al. 2014) or disease progression (Trojsi, Corbo et al. 2013).

When using cognitive examination, it was found that reduced FA correlated with reduced letter fluency (Pettit, Bastin et al. 2012) and negatively with frontal system behavior scale (FrSBe) score at the level of pontomesencephalic junction (Trojsi, Corbo et al. 2013). Reduced FA along CST correlated with Trail-making test scores (Sarro, Agosta et al. 2011) and Stroop test scores (Sarro, Agosta et al. 2011).

Table 4.4: Studies of FA in the projection fibers, which studied the projection fibers.

	cortico-striatal		CRT		CPT		CST			Pons			IC			CR	
	Sig	p	Sig		Sig	p	Sig	p	Non-sig	Sig	p	Non-sig	Sig	p	Non-sig	Sig	p
ALS	Kassubek (2014)	** *	Kassubek (2014)	^^	Kassubek (2014)	^^	Lillo (2012)	^^	Toosy (2003)	Karlsborg (2004)	*	Floeter (2014)	Agosta (2013)	*p	Karlsborg (2004)	Sarica (2014)	*
							Menke (2014)	*	Abe (2004)	Toosy (2003)	*	Steinbach (2015)	Agosta (2011)	*	Toosy (2003)	Sach (2004)	* * *
							Tsujimoto (2011)	##	Karlsborg (2004)	Iwata (2008)	++ +		Sach (2004)	$$$	Furtula (2013)	Prell (2012)	*
							Trojsi (2013)	*	Prudlo (2012)	Skeelakumari (2015)	##		Prudlo (2012)	##	Mitsumoto (2007)	Zhang (2014)	*
							Bede (2013)	*	Zhang (2014)				Zhang (2014)	*		Pettit (2013)	# # A
							Prokscha (2014)	+	Grapperon (2013)				Rajagopalan (2013)	*		Agosta (2013)	*
							Agosta (2013)	*	Rajagopalan (2013)				Floeter (2014)	*		Iwata (2008)	+ + +
							Crespi (2014)	*	Furtula (2013)				Iwata (2008)	+++ p		Agosta (2011)	*
							Sarica (2014)	*					Prell (2012)	*		Agosta (2015)	*
							Bastin (2013)	###					Kasper (2014)	*		Agosta (2015)	
							Pettit (2013)	^^					Vora (2016)	*		Tang (2015)	*
							Woo (2014)	$$$					Agosta (2015)	*			
							Kassubek (2014)	^^									
							Cardenas-Blanco (2014)	*									
							Iwata (2008)	+++									
							Filippini (2010)	*									

Cont Table 4.4: Studies of FA in the projection fibers, which studied the projection fibers.

	cortico-striatal		CRT		CPT		CST			Pons			IC			CR		
	Sig	p	Sig	Sig	p	Sig	p	Non-sig	Sig	p	Non-sig	Sig	p	Non-sig	Sig	p		
ALS						Romano (2014)	$$$											
						Steinbach (2015)	###											
						Trojsi (2015)	+											
						Zimmerman-Moreno (2015)	*											
						Skeelakumari (2015)	##											
FTD						Lillo (2012)	*	Matsuo (2008)				Agosta (2015)	*	Tovar-Moll (2014)	Avants (2010)			
						Whitwell (2010)	*R	Zhang (2009)							Tovar-Moll (2014)	*		
						Avants (2010)	*R	Daianu (2015)										
						McMillan (2012)	$$$											
						Bae (2015)	+											

***p < 0.05, ** at p < 0.005 uncorrected, ***at p≤0.03, $p=0.07, #p=0.02, ##p≤0.001, ###p<0.04, $$p=0.002, $$$p≤0.01, ^p<0.01 uncorrected, ^^p<0.001 uncorrected, +p<0.002, ++P<0.003, +++p<0.0001**

Rright, Lleft, Aanterior, Pposterior, Mmid

Association Fibers

Studies finding both significant and non-significant results for each of the association fiber tracts in ALS are presented in Table 4.5 and Figure 4.2.

There were widespread changes in the association fibers in ALS. Sarro et al found increased MD, AD and RD bilaterally in the SLF but no changes in FA (Sarro, Agosta et al. 2011). ROI analysis revealed changes in DTI metrics such as MD in the uncF and decreased FA in ILF (Pettit, Bastin et al. 2012) and right SLF with increased RD and MD (Menke, Körner et al. 2014). Many studies found that reduced FA was greatest in SLF (Agosta, Caso et al. 2013) and IFOF (Borroni B and et al. 2007). When cognitively impaired ALS (ALSci) were compared to controls and non-impaired counterparts, increased RD and MD were observed in the SLF, IFOF and uncF bilaterally while reduced FA were seen only when ALSci were compared to controls (Kasper, Schuster et al. 2014). Tractography in ALS showed increased AD in uncF compared to controls (Christidi, Zalonis et al. 2014). In later stages of ALS, more association fibers were involved in DTI changes than in the early stages (Trojsi, Caiazzo et al. 2015).While one study found reduced generalized FA[2] in the left SLF (Trojsi, Corbo et al. 2013), another study also studied the arcuate fasciculus but found no significant changes in FA, MD or RD (Bastin, Pettit et al. 2013).

The correlations of DTI measures with quantitative clinical measure in ALS are summarized in Supp Tables 4.2–4.6. Using ROI, RD in the right SLF correlated with ALSFRS while both FA and RD correlated with disease progression (Menke, Körner et al. 2014). In regression analysis, reduced FA in bilateral IFOF and ILF was shown to negatively correlate with Trail-making test scores while the right IFOF and ILF associated with Stroop test scores (Sarro, Agosta et al. 2011). Negative correlation of DTI metrics with UMN scores and Frontal Systems Behavior Scale (FrSBe) was found in the SLF (Trojsi, Corbo et al. 2013). Crespi found an association between specific

[2] The generalized fractional anisotropy (GFA) was calculated as GFA = std(ψ)/rms(ψ), where std is the standard deviation and rms is the root-mean-square.

emotional recognition and FA, MD and mode of anisotropy [3] along the right ILF and IFOF but positive correlation with the cumulative scores of single negative emotions (Crespi, Cerami et al. 2014).

[3] Mode of anisotropy is a recently developed measure of anisotropy providing information about the shape of the tensor.

Table 4.5: Studies of FA in the association fibers, which studied the association fibers.

	SLF			ILF			IFOF			Unc.F			FOF		Arcuate F
	Sig	P	Non-sig	Sig	P	Non-sig	Sig	P	Non-sig	Sig	P	Non-sig	Sig	P	Non-sig
ALS	Prudlo (2012)	*	Zhang (2014)	Prudlo (2012)	*	Bastin (2013)	Prudlo (2012)	*	Sarro (2011)	Prudlo (2012)	*	Pettit (2013)	Trojsi (2013)	*	Bastin (2013)
	Menke (2014)	*R	Sarro (2011)	Lillo (2012)	*	Sarica (2014)	Crespi (2014)	*	Agosta (2011)	Trojsi (2013)	*L	Sarica(2014)			
	Sarica (2014)	*	Agosta (2015)	Crespi (2014)	*	Sarro (2011)	Trojsi (2013)	*L		Bastin (2013)	$	Sarro (2011)			
	Trojsi (2013)	*L		Pettit (2013)	$$$	Agosta (2011)	Kasper (201	*L		Kasper (201	*L	Agosta (2011)			
	Crespi (2014)	**		Kasper (201	*L		Trojsi (2015)	*		Trojsi (2015)	*L	Christidi (2014)			
	Sarica(2014)	*		Trojsi (2015)	*										
	Agosta (2013)	*													
	Crespi (2014)	*													
	Trojsi (2015)	*													
	Kasper (201	*L													
FTD	Whitwell (2010)	*R,L, A		Lillo et al (2012)	*A	Avants (2010)	McMillan (2012)	$$$A	Hornberger (2011)	Zhang (2013)	**	Zhang (2011)	Tovar-Moll (2014)	*	Matsuo (2008)
	Borroni (2007)	*		Whitwell (2010)	*R,A		Borroni (2007)	*		Zhang (2009)	##L				
	Avants (2010)	*R		Borroni (2007)	*		Avants (2010)	*		Whitwell (2010)	*				
	McMillan (2012)	$$$A		Matsuo (2008)	$$$		Daianu (2015)	** *		Hornberger (2011)	##				
	Daianu (2015)	***		McMillan (2012)	$$$A		Tovar-Moll (2014)	*		Matsuo (2008)	$$$				
	Tovar-Moll (2014)	*		Tovar-Moll (2014)	*		Lam (2014)	*		Avants (2010)	*R				
	Lam	*		Lam	*					McMillan	$$$A				

Cont Table 4.5: Studies of FA in the association fibers, which studied the association fibers.

	SLF			ILF			IFOF			Unc.F			FOF		Arcuate F
	Sig	P	Non-sig	Sig	P	Non-sig	Sig	P	Non-sig	Sig	P	Non-sig	Sig	P	Non-sig
FTD	Lam (2014)	*		Lam (2014)	*					McMillan (2012)	$$$A				
										Daianu (2015)	***				
										Tovar-Moll (2014)	*				
										Lam (2014)	*				
										Downey (2015)	*				
										Mahoney (2014)	*				

***p < 0.05, ** at p < 0.005 uncorrected, ***at p≤0.03, $p=0.07, #p=0.02, ##p≤0.001, ###p<0.04, $$p=0.002, $$$p≤0.01, ^p<0.01 uncorrected, ^^p<0.001 uncorrected, +p<0.002, ++P<0.003, +++p<0.0001**
R right, L left, A anterior, P posterior, M mid

Subcortical WM (Limbic tracts)

Studies showing significant and non-significant changes in DTI metrics in limbic tracts are presented in Table 4.6 and Figure 4.2.

There were widespread changes in the limbic tracts in ALS. There was reduced FA in the thalamus (Pettit, Bastin et al. 2012, Heimrath, Gorges et al. 2014), more significant on the right side (Sach, Winkler et al. 2004) and anteriorly (Trojsi, Corbo et al. 2013, Sarica, Cerasa et al. 2014). The hippocampus, thalamus and anterior cingulum were found to have increased RD (Cardenas-Blanco, Machts et al. 2014) and MD (Barbagallo, Nicoletti et al. 2014) in ALS specifically when using ROI-based methods (Kasper, Schuster et al. 2014), which was found to be correlated with reverse digital span (Pettit, Bastin et al. 2012). The average FA of the fornix was also reported to be reduced (Trojsi, Corbo et al. 2013). The amygdala was found by one group to have increased MD, which was negatively correlated with ALSFRS-R (Barbagallo, Nicoletti et al. 2014). ROI analysis showed significant changes in FA and MD in the thalamic radiation in the frontal region (Pettit, Bastin et al. 2012). Agosta et al reported similar changes when comparing ALS versus primary lateral sclerosis (PLS) (Agosta, Caso et al. 2013). One study found widespread reduction in FA and increase in MD in WM subcortical structures (Floeter, Katipally et al. 2014). In ALS patients at stage 3, where a third body region is involved, DTI metrics showed significant changes in thalamic radiation (ThR) (Trojsi, Caiazzo et al. 2015). When comparing ALS with their counterparts who had cognitive impairment, increased RD and MD were seen in ThR and Cg in ALSci (Kasper, Schuster et al. 2014).

The results of clinical correlation of DTI measures with clinical features of ALS in subcortical white matter tracts are summarized in Supp Tables 4.2–4.6. Modified Card Sorting Test (MSCT) and frontal assessment battery (FAB) scores were found to correlate with MD in WM tracts in caudate nucleus, amygdala and hippocampus in ALS (Barbagallo, Nicoletti et al. 2014). FA and MD in the fornix correlated with verbal learning and memory test scores (Filippi, Agosta et al. 2011). In the parahippocampal tract and the left thalamic radiation, increased FA correlated with increased apathy

score on Frontal Systems Behavior Scale (FrSBe) (Tsujimoto, Senda et al. 2011). MD in the thalamus correlated positively with disease duration and negatively with ALSFRS-R (Barbagallo, Nicoletti et al. 2014).

Table 4.6: Studies of FA in the subcortical (limbic) tracts, which studied the subcortical tracts.

	Thalamus			Cingulum			Fornix			hippocampus	
	Sig	P	Non-sig	Sig	P	Non-sig	Sig	P	Non-sig	Sig	Non-sig
ALS	Sach (2004)	^	Prudlo (2012) ATR	Trojsi (2013)	*A	Sarro (2011)	Trojsi (2013)	*	Sarro (2011)		
	Trojsi (2013)	*	Barbagallo (2014)	Agosta (2011)	*	Pettit (2013)					
	Pettit (2013)	$$A	Floeter (2014)	Sarica(2014)	*						
	Heimrath (2014)	*	Bede (2013)	Prell (2012)	*R,A						
	Sarica (2014)	*									
	Kasper (2014)	*									
FTD	Daianu (2015)	*RA	Zhang (2011)	Zhang (2011)	^^A	Zhang (2013)	Mahoney (2014)	*	Zhang (2013)	Mahoney (2014)	Irish (2014)
	Downey (2015)	*RA		Whitwell (2010)	*A	Hornberger (2011)					
	Irish (2014)	*		Zhang (2009)	++						
	Lam (2014)	*A		Daianu (2015)	*						
				Downey (2015)	*						
				Frizell Santillol (2013)	^^A						
				Irish (2014)	*						
				Lam (2014)	*						
				Tovar-Moll (2014)	*						

p < 0.05, ** at p < 0.005 uncorrected, ***at p≤0.03, $p=0.07, #p=0.02, ##p≤0.001, ###p<0.04, $$p=0.002, $$$p≤0.01, ^p<0.01 uncorrected, ^^p<0.001 uncorrected, +p<0.002, ++P<0.003, +++p<0.0001
[R]right, [L]left, [A]anterior, [P]posterior, [M]mid

Commissural fibers

Studies showing both significant and non-significant changes in DTI metrics in commissural fiber tracts are presented in Table 4.7 and Figure 4.2.

Widespread changes in commissural fibers were reported. Reduced FA in the CC was found when comparing ALS and ALS-FTD group with controls (Lillo, Mioshi et al. 2012). Others reported the reduction in FA to be more dominant in the genu (Bastin, Pettit et al. 2013, Bede, Bokde et al. 2013) of the CC, forceps minor and major (Prudlo, Bissbort et al. 2012) but the body of the CC showed more changes when classic ALS patients were compared with controls (Prudlo, Bissbort et al. 2012, Agosta, Galantucci et al. 2015, Tang, Chen et al. 2015). Most studies found reduced FA in the CC (Sach, Winkler et al. 2004, Kasper, Schuster et al. 2014).

Increased MD (Sarro, Agosta et al. 2011, Trojsi, Caiazzo et al. 2015, Vora, Kumar et al. 2016) and RD (Kasper, Schuster et al. 2014, Zimmerman - Moreno, Ben Bashat et al. 2015) were reported in few studies. Prudle et al found that both forceps major and minor showed reduced FA in all ALS subtypes when using ROI analysis (Prudlo, Bissbort et al. 2012). When ROI is used, the genu and splenium of CC reflected significant reduction in the FA in ALS group (Vora, Kumar et al. 2016).

When not corrected for multiple comparisons, reduced FA showed weaker correlation with ALSFRS-R, but higher RD correlated with clinical scores such as UMN scores and ALSFRS-R (Filippini, Douaud et al. 2010). No correlations was found in the CC with ALSFRS-R or UMN scores (Filippini, Douaud et al. 2010). Sarro et al found that the changes in FA and MD in the CC correlated with the results of neuropsychological tests such as the Trail-making test scores and Stroop test which also correlated with the FA in the CC (Sarro, Agosta et al. 2011).

Table 4.7: Studies of FA in the callosal radiation, which studied the callosal tracts.

	Corpus callosum			Forceps Minor			Forceps major		
	Sig	P	None	Sig	P	None	Sig	P	None
ALS	Lillo (2012)	*	Menke (2014)	Hornberger (2011)	##	Lillo (2012)	-		Woo (2014)
	Sach (2004)	^	Pettit (2013)			Crespi (2014)	-		Sarica(2014)
	Zhang (2014)	*	Iwata (2008)						
	Kim (2014)	*	Sarro (2011)						
	Trojsi (2013)	*							
	Bastin (2013)***								
	Agosta (2011)	*							
	Floeter (2014)	*							
	Agosta (2013)	*M							
	Crespi (2014)	*							
	Sarica(2014)	*							
	Bede (2013)	*							
	Filippini (2010)	*							
	Agosta (2015)	*P							
	Kasper (2014)	*							
FTD	Lillo (2012)	*A	Chen (2009)	Lillo (2012)	*	Hornberger (2011)	Irish et al (2014)	*	
	Zhang (2013)		Whitwell (2010)	Irish et al (2014)	*				
	Zhang (2009)	+A	Bozzali (2013)	Tovar-Moll et al (2014)	*				
	Avants (2010)	*A	Hornberger (2011)						
	Borroni (2007)	*							
	Zhang (2011)	^^A							
	McMillan (2012)	$$$A							
	Matsuo (2008)	$$$							
	Agosta (2015)	*							

p < 0.05, ** at p < 0.005 uncorrected, ***at p≤0.03, $p=0.07, #p=0.02, ##p≤0.001, ###p<0.04, $$p=0.002, $$$p≤0.01, ^p<0.01 uncorrected, ^^p<0.001 uncorrected, +p<0.002, ++P<0.003, +++p<0.0001
Rright, Lleft, Aanterior, Pposterior, Mmid

Brain stem tracts

Studies showing significant and non-significant results in DTI metrics in brain stem tracts are presented in Table 4.8 and Figure 4.2.

Significant changes in FA were found in most of brainstem tracts; medulla oblongata (MD but not FA) (Sheelakumari, Madhusoodanan et al. 2015), cerebral peduncle (Sheelakumari, Madhusoodanan et al. 2015, Tang, Chen et al. 2015), and basis pontis (Iwata, Aoki et al. 2008). Reduced FA was reported in the left medullary pyramid (Vora, Kumar et al. 2016) and bilaterally in cerebellar peduncle (CP) (Tang, Chen et al. 2015) in ALS compared to controls. Bede et al divided ALS subjects based on results of testing for C9orf72 hexanucleotide repeats (C9+ve and C9–ve) and found that WM exhibited changes in the cerebellar pathways in both groups (Bede, Bokde et al. 2013). In another study comparing ALS versus primary lateral sclerosis (PLS), increased MD was found in the bilateral middle CP (Sheelakumari, Madhusoodanan et al. 2015) and transverse pontine fibers (Floeter, Katipally et al. 2014). Reduced FA in the medulla oblongata correlated with higher UMN scores in ALS patients (Iwata, Aoki et al. 2008).

Table 4.8: Studies of FA in the brain stem tracts, which studied the brain stem tracts

	CP			Cerebellar			Medulla oblongata			Cortico-bulbar	
	Sig	P	Non-sig	Sig	P	Non-sig	Sig	P	Non-sig	Sig	P
ALS	Toosy (2003)[51]	###	Floeter (2014)[38]	Trojsi (2015)	^^L	Iwata (2008)[27]	Iwata (2008)[27]	+++	Sheelakumari (2015)	Sach (2004)[49]	*
	Zhang (2014)[47]	*	Rajagopalan (2013)[34]	-		Bede (2013)[46]	Vora et al (2016)	$L			
	Iwata (2008)[27]	+++	Iwata (2008)[27]								
	Agosta (2010)	*									
FTD	Mahoney (2014)	***		Bae (2015)	*						
	Agosta (2011)	*									

*p < 0.05, ** at p < 0.005 uncorrected, ***at p≤0.03, $p=0.07, #p=0.02, ##p≤0.001, ###p<0.04, $$p=0.002, $$$p≤0.01, ^p<0.01 uncorrected, ^^p<0.001 uncorrected, +p<0.002, ++P<0.003, +++p<0.0001

[R]right, [L]left, [A]anterior, [P]posterior, [M]mid

DTI in FTD

A summary of the number of papers that studied each tract, and the number of these papers that found significant abnormalities in each tract using FA is provided in Table 4.3. A visual summary of the WM tracts that found to be significant in FTD studies is presented in Figure 4.3.

Whole brain analysis

Nine whole-brain studies of FTD were included (Zhang, Schuff et al. 2009, Agosta, Scola et al. 2011, Hornberger, Geng et al. 2011, Lillo, Mioshi et al. 2012, Zhang, Tartaglia et al. 2013, Lam, Halliday et al. 2014, Tovar-Moll, de Oliveira-Souza et al. 2014, Agosta, Galantucci et al. 2015, Downey, Mahoney et al. 2015). When FTD patients were compared to ALS and ALS-FTD patients, TBSS revealed significant changes involving the callosal tracts; the forceps minor, and ILF than in CST (Lillo, Mioshi et al. 2012). Reduced FA and increased MD were also found in CC, orbitofrontal, occipital and frontoparietal WM in behavioral variant FTD (bvFTD) subjects when compared to controls (Agosta, Scola et al. 2011, Agosta, Galantucci et al. 2015). The most pronounced white matter degeneration in FTD patients was seen in the anterior callosal region (Zhang, Schuff et al. 2009). Increases in RD and AD were similar and shown to be sharing the same regions when FTD were compared to controls. These regions included; anterior callosal region, fornix (Agosta, Scola et al. 2011) and bilateral uncF (Zhang, Schuff et al. 2009). Changes in FA and AD in uncF were found when comparing FTD versus healthy controls (Zhang, Schuff et al. 2009). It has been reported that FA changes were mostly dorsal and ventral when comparing bvFTD versus controls in voxel-wise analysis (Tovar-Moll, de Oliveira-Souza et al. 2014) and whole brain TBSS (Downey, Mahoney et al. 2015). Longitudinal study have documented that DTI changes involved the callosal, association and cingulate tracts in bvFTD and that WM changes overlap GM atrophy (Lam, Halliday et al. 2014). Reduced FA correlated negatively with Hayling task and neuropsychiatric inventory disinhibition in UncF (Hornberger, Geng et al. 2011). Abnormalities in CR and ALIC were also reported when using voxel-wise analysis (Tovar-Moll, de Oliveira-Souza et al. 2014). External capsule and IC were found to have reduced FA but increased RD (Agosta, Scola et al. 2011). Some studies reported that RD shows more regions than other DTI metrics in FTD group (Zhang, Schuff et al. 2009, Lam, Halliday et al. 2014).

ROI and Tractography analysis

Projection Fibers

Table 4.4 shows all the studies that involved projection fibers. Decreased FA bilaterally in the overall CST was reported in two studies (Lillo, Mioshi et al. 2012, McMillan, Brun et al. 2012), but more specifically in the left in one study (Borroni B and et al. 2007). FA was found to be reduced at the level of both cerebral peduncles (CP) (Agosta, Scola et al. 2011, Mahoney, Beck et al. 2012) and corona radiata (CR) with increased AD (Borroni B and et al. 2007, Agosta, Scola et al. 2011) and RD on the right CST (Zhang, Schuff et al. 2009). However, other studies reported increased FA along the whole CST (Avants, Cook et al. 2010, Whitwell, Avula et al. 2010).

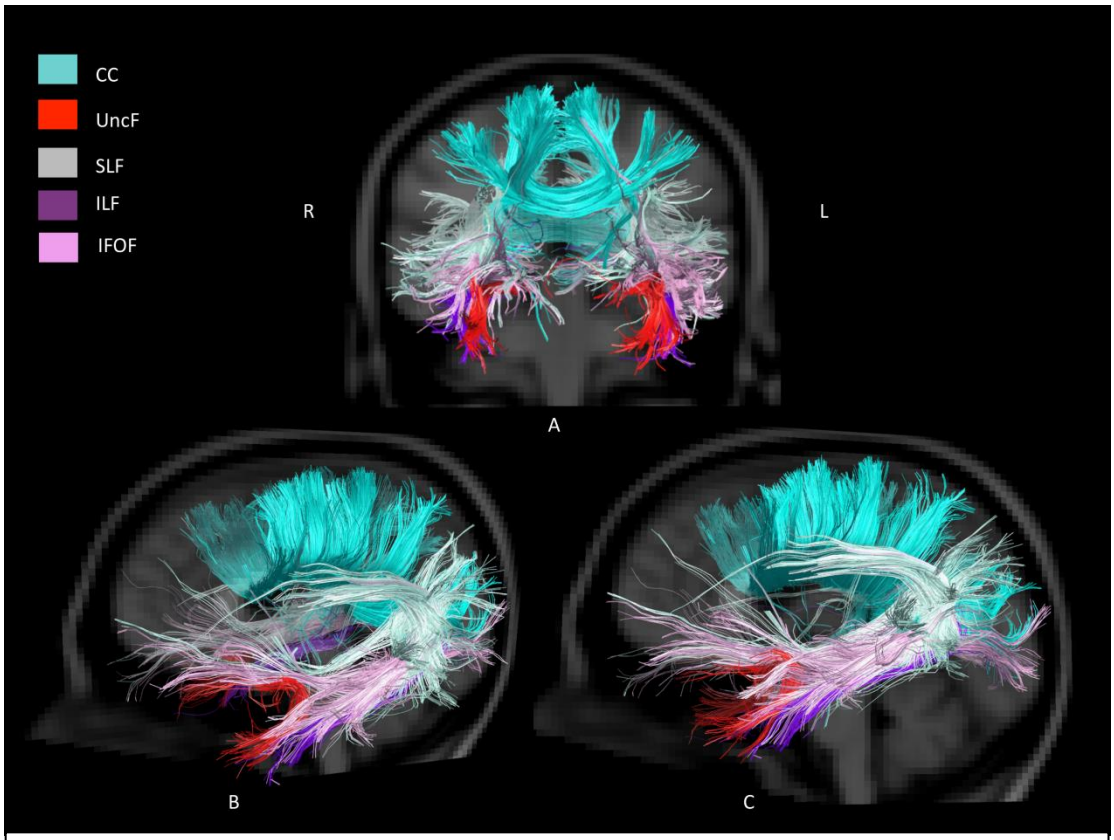

Figure 4.3: Visual representation of WM tracts that were found significant in FTD studies. (CST was removed for better visualization of other tracts).

Although severe degeneration across the motor system was found when comparing bvFTD with controls and ALS patients (Bae, Ferguson et al. 2016), another study reported the CST to be the least affected tract in bvFTD (Daianu, Mendez et al. 2015).The only study that addressed DTI correlations showed that in FTD, reduced FA correlated with cortical thickness (Avants, Cook et al. 2010).

Association Fibers

Table 4.5 shows FTD studies that included association fibers. Reduced FA was reported in all papers that investigated the SLF (Borroni B and et al. 2007, Avants, Cook et al. 2010, Whitwell, Avula et al. 2010, McMillan, Brun et al. 2012). This reduction in FA was associated with increased RD in both the SLF and ILF particularly in the anterior regions (Whitwell, Avula et al. 2010). Reduced FA was also reported in the right SLF in fontal variant FTD (fvFTD) and left SLF in temporal variant FTD (tvFTD), bilaterally in ILF and IFOF when compared to controls while changes were bilateral in the ILF and IFOF (Borroni, Brambati et al. 2007). Changes in uncF were mostly frontal and temporal when comparing bvFTD versus controls in ROI (Tovar-Moll, de Oliveira-Souza et al. 2014).

When comparing bvFTD versus controls, DTI revealed abnormalities in most of the association fibers bilaterally (Irish, Hornberger et al. 2014, Tovar-Moll, de Oliveira-Souza et al. 2014, Daianu, Mendez et al. 2015). This was noted even with longitudinal studies that showed changes of DTI metrics at base line in regions of ILF, IFOF and uncF (Mahoney, Simpson et al. 2015). SLF and ILF had increased MD and AD after 12 months (Lam, Halliday et al. 2014). Rate of changes[4] in DTI metrics in uncF were significant when bvFTD compared to controls in a longitudinal study (Mahoney, Simpson et al. 2015).

WM changes in the SLF, IFOF, uncF and ILF correlated with cortical thickness in FTD (Avants, Cook et al. 2010). A negative correlation was reported between FA in SLF and behavioural and neuropsychological examinations (Borroni, Brambati et al. 2007).

[4] Was calculated based on the time from baseline scan in years, and interaction between disease group and time included to provide estimates of differences in the rate of change as a percentage per year.

Remote ABM retrieval correlated significantly with FA in the left uncF (Irish, Hornberger et al. 2014). DTI measures in the uncF correlated with NPI-aberrant motor behavior sub-score especially in the frontal part (MD and FA) and temporal part (FA) (Tovar-Moll, de Oliveira-Souza et al. 2014), Supp Table 6.

Subcortical WM (Limbic tracts)

All FTD studies that included limbic tracts are shown in Table 4.6. Reduced FA and increased MD were found in the frontotemporal subcortical WM structures such as fornix, anterior ThR (Irish, Hornberger et al. 2014) and cingulum (Floeter, Katipally et al. 2014, Irish, Hornberger et al. 2014, Tovar-Moll, de Oliveira-Souza et al. 2014), but significant changes in the latter were seen only in FA in the bvFTD group when compared to controls (Agosta, Scola et al. 2011).

When using TOI, the fornix exhibited changes in FA, AD and MD (Zhang, Tartaglia et al. 2013). One study reported increased RD and AD in parahippocampal cingulum (Zhang, Tartaglia et al. 2013). Right parahippocampal cingulum showed changes in all assessed DTI metrics in a cross-sectional DTI study that compared bvFTD and controls (Mahoney, Simpson et al. 2015). MD was reported to be increased in ThR and Cg in bvFTD group compared to controls (Daianu, Mendez et al. 2015). At baseline, when comparing bvFTD and controls, all DTI measures reflected significant changes in the anterior ThR and Cg but this was not reported after 12 months (Lam, Halliday et al. 2014). FA changes in the right anterior ThR and fornix had positive correlation with cognitive performance in bvFTD patients (Downey, Mahoney et al. 2015). In bvFTD, DTI changes in the posterior Cg, the parahippocampal part (paraHpc), correlated with NPI-apathy subscores (MD, $p<0.01$), and Mattis total scores (MD and FA, $p<0.05$) (Tovar-Moll, de Oliveira-Souza et al. 2014).

Commissural fibers

FTD studies that investigated commissural fibers are shown in Table 7. There was reduced FA in the CC (Matsuo, Mizuno et al. 2008, Avants, Cook et al. 2010, Whitwell, Avula et al. 2010, Lillo, Mioshi et al. 2012, McMillan, Brun et al. 2012) and more anteriorly when not corrected for multiple comparisons (Zhang, Schuff et al. 2011).

Increased MD was reported when comparing a small group of seven FTD subjects with controls (Chen, Lin et al. 2009). Changes in DTI metrics were found in the CC in most of the fronto-temporal lobar degeneration (FTLD) subgroups (Agosta, Scola et al. 2011). However, in the bvFTD group, when compared to controls, there were changes in FA, MD and AD (Agosta, Scola et al. 2011). Reduced FA in left CC was reported in tvFTD when compared with healthy controls (Borroni, Brambati et al. 2007). At baseline, all DTI metrics exhibited significant changes in the genu of CC, while after 12 months the significant changes in FA and RD extended to bilateral splenium of CC (sCC) (Lam, Halliday et al. 2014). Similar results were reported in bvFTD with all DTI measures in the frontal WM including gCC while this was not observed in sCC (Lu, Lee et al. 2014). Others reported the DTI changes to be in the bCC, bilaterally, in both cross-sectional and longitudinal DTI studies (Lam, Halliday et al. 2014) but the rates of change in FA and MD at later stage involved the bCC for FA, RD, MD and AD only in the c9orf72 group. All assessed DTI metrics showed significant changes in the sCC (Mahoney, Simpson et al. 2015). Using tractography in bvFTD subjects, the genu of CC shows altered diffusion anisotropy (Daianu, Mendez et al. 2015). DTI changes in CC were mostly dorsal and ventral in bvFTD compared to controls (Downey, Mahoney et al. 2015). With ROI, FA and MD changes in sCC were found to be significant in FTD group compared to controls. Similar results were seen when using voxel-wise analysis (Tovar-Moll, de Oliveira-Souza et al. 2014).

Behavioral variables were found to be associated with DTI measures in gCC (Lu, Lee et al. 2014). Lillo et al found that FA changes in the anterior CC and forceps minor occurred in the bvFTD and ALS-FTD subjects when compared to other patient subgroups and controls but minimal changes in these regions were evident when comparing the pure ALS patients and healthy controls (Lillo, Mioshi et al. 2012). Within bvFTD group, impairment of emotion identification impairment was associated with WM alteration in CC (Downey, Mahoney et al. 2015).

Brain stem tracts

A total of three FTD studies examined DTI changes in brain stem, Table 4.8. Significant changes in FA was found in the bvFTD group in the CP when compared with healthy

controls only with FA but not with other DTI metrics (Agosta, Scola et al. 2011). The FTD group, who was C9ORF72 positive, had reduced FA in the superior CP compared to controls (Mahoney, Simpson et al. 2015).

Overlap between ALS and FTD

This review aimed to determine the overlap between ALS and FTD, and also to demonstrates the differences between ALS and FTD. The SLF, ILF, IFOF, UncF, ThR, Cg, Fx and CC were found to be abnormal in 50% or more of the studies in ALS and FTD. There was strong evidence of involvement of uncF in FTD, but only a minority of studies found abnormality of uncF in ALS. There were abnormalities in the cingulum and IFOF in both ALS and FTD, and forceps minor was reported to be abnormal in minority of studies of ALS but more evident in FTD studies. As shown in Table 4, there is extensive evidence that the CST was abnormal in ALS, and less evidence in FTD, where there were fewer studies that only examined the average FA of the whole CST. However, two studies have reported CR to show significant changes in FTD.

Figures 4.2–4.4 show the tracts that were abnormal in ALS, FTD and in both ALS and FTD, respectively, using tracts that were abnormal in more than 66% of studies, where the evidence is strongest.

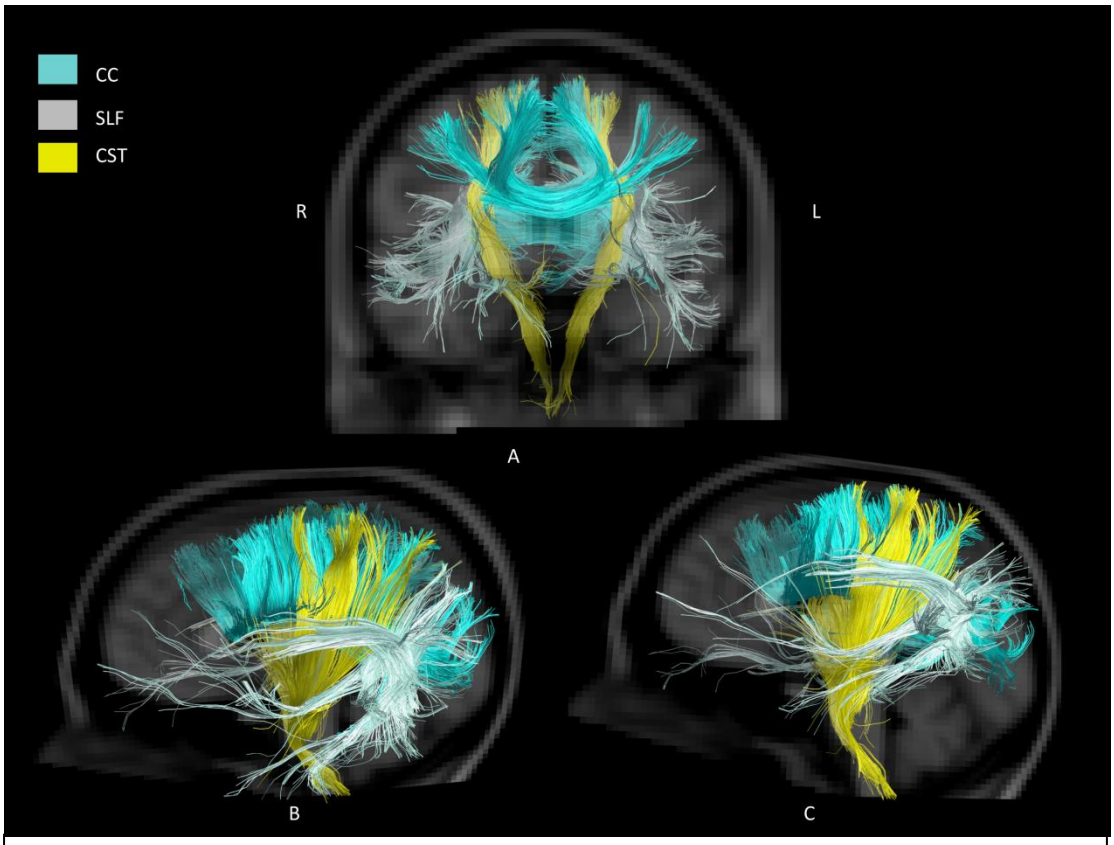

Figure 4.4: Visual representation of WM tracts that are found to be significant and shared in both ALS and FTD studies.

Discussion

DTI is a promising method to assess upper motor neuron dysfunction (Wang, Melhem et al. 2011, Turner, Agosta et al. 2012) and changes in frontal lobe function (Naik, Lundervold et al. 2010). We have systematically reviewed the abnormalities that are reported in ALS and in FTD using DTI. We report data obtained from 1674 patients across 64 studies. All studies have reported the results of FA; however, the other DTI measures were not consistently analyzed across all studies. Because there is an overlap of clinical features between ALS and FTD, we expected that there would be overlap of the white matter tracts involved. There have been previous meta-analyses of DTI abnormality in ALS (Foerster, Dwamena et al. 2012, Li, Pan et al. 2012). There are no previous systematic reviews of DTI in FTD. We were not able to obtain the raw data for most of these studies, and the studies varied in the use of sequences and in the techniques for analysis of the data, so we have made a comparison of the abnormalities that were reported.

DTI measures were variously obtained using ROI, TBSS, tractography and other methods. ROI is susceptible to inclusion of cerebrospinal fluid (CSF) or gray matter (GM) (Snook, Plewes et al. 2007). TBSS is considered to be the standard approach for voxel-based analysis (VBA) of DTI data (Smith, Jenkinson et al. 2006), but has drawbacks as described previously such as misalignment and alteration in the thickness of the skeleton (Edden and Jones 2011, Zalesky 2011, Keihaninejad, Ryan et al. 2012, Bach, Laun et al. 2014). Tractography is unable to determine the precise origin and termination of tracts, but can guide the ROI analysis of WM (Gong, Jiang et al. 2005, Yushkevich, Zhang et al. 2008, Goodlett, Fletcher et al. 2009, Jbabdi, Behrens et al. 2010). Because of the limited number of studies, we have combined the results of all the studies despite their different technical approaches, as outlined in the extracted data table in the appendix. However, we noted that some older studies were limited in resolution, power and field strength. This could account for some of the discrepancies. The tracts that showed significant changes are displayed in Figures 4.2 to 4.4.

ALS studies

The most common finding in ALS is the reduction in FA in the CST. Classical pathological studies of ALS show loss of upper and lower motor neurons and sclerosis of the CST in the spinal cord (Rafałowska and Dziewulska 1995). Other post mortem studies found gliosis affecting the posterior third of the internal capsule (Abe, Fujimura et al. 1997). Animal studies found similar results in cerebral motor cortex, where the CST originates (Martin 1999). Thus, abnormality of the CST in DTI of ALS studies is an expected finding. DTI studies also showed extensive abnormality of the CC, the cingulum and thalamus, confirming the findings of a previous review (Li, Pan et al. 2012).

The abnormalities found in ALS with DTI are consistent with the results of the imaging studies using other techniques. For example, structural MRI studies in ALS indicated that hyperintensities in CST and hypointensity in motor cortex are consistent features (Hecht, Fellner et al. 2001, Zhang, Uluğ et al. 2003). Analysis of regional atrophy in VBM studies in ALS patients suggests that the GM loss occurs in the motor cortex and frontal, temporal and limbic areas (Chang, Lomen-Hoerth et al. 2005, Kassubek, Unrath et al. 2005, Mezzapesa, Ceccarelli et al. 2007). Susceptibility weighted imaging (SWI) has also found abnormalities of these pathways in ALS (Prell, Hartung et al. 2015). Functional MRI (fMRI) in ALS patients has revealed that impaired letter fluency correlated with impaired activation in the frontal and cingulate gyri (Abrahams, Goldstein et al. 2004). Positron emission tomography (PET) also found reduced regional perfusion in the sensorimotor cortex, and also extra-motor areas such as prefrontal, cingulate, putamen and parietal cortices (Hatazawa, Brooks et al. 1988, Habert, Lacomblez et al. 2007) and that reduction is accompanied by mild neuropsychological deficit (Ludolph, Langen et al. 1992).

In ALS, many of the DTI studies show positive correlation between FA and performance on some cognitive examinations such as Trail-making, Stroop test scores and letter fluency. These changes are mainly in the projection fibers, commissural tract and association fibers. However, negative correlation was found with emotional recognition in the association fibers. Verbal learning, memory test and the apathy scores on FsRBe correlated with FA in the limbic tracts.

Our results found that the clinical measures positively correlated with the disease duration and disease progression but negatively with the UMN scores. ALSFRS-R had weaker correlation with FA in the commissural and association fibers but stronger negative correlation with MD in the CST and thalamic radiation. This is in support of the theory that ALS involves more than just motor dysfunction. Previous meta-analysis in ALS using DTI measures is in support of the findings in this review (Li, Pan et al. 2012).

FTD studies

The tracts that were abnormal in FTD included the SLF, ILF and IFOF as well as the corpus callosum and uncinated fasciculus. There also was abnormality of the CST. FTD is associated with neurodegeneration and atrophy of the frontal, parietal and temporal lobes (Whitwell, Jack et al. 2007). In a previous post-mortem DTI study, histopathologic correlation revealed changes and frontal degeneration with mild frontotemporal gliosis (Larsson, Englund et al. 2004). FTD subtype studies reported the involvement of association fibers in the disease prognosis particularly ILF, IFOF and SLF, and the callosal radiation in the left hemisphere in tvFTD.

Other MRI studies of FTD patients have shown patterns of GM loss primarily in the frontal, orbitofrontal (Harlow 1868, Hornberger, Geng et al. 2011) and anterior temporal lobes (Baron, Chetelat et al. 2001, Diehl, Grimmer et al. 2004, Grimmer, Diehl et al. 2004, Ishii, Sasaki et al. 2005, Jeong, Cho et al. 2005). Functional studies have shown the changes to be prominent in the parietal lobe and the cingulate gyrus (Bartenstein, Minoshima et al. 1997, Johnson, Jahng et al. 2006). Although several researchers have discovered a contribution by the association tracts in disease progression of FTD, less is known about the tract-specific contribution in cognition and this has not been fully explored (Cairns, Bigio et al. 2007).

Overlap between ALS and FTD

The pathways for which there is strong evidence of abnormality in both ALS and FTD are shown in Figures 4.2–4.4. It is thought that ALS and FTD lie on a spectrum and that subjects with the combination of ALS and FTD have worse survival than subjects with ALS without FTD (Mathuranath, Nestor et al. 2000). This review identified extensive evidence of CST abnormalities in ALS and also some evidence in FTD. The DTI studies show extra-motor involvement in ALS and FTD in the cingulum and the CC, especially the forceps minor which reflects the neuropathological findings of degeneration in the CC (Brownell, Oppenheimer et al. 1970) and corresponds to the severe atrophy of the most anterior CC that has been related to cognitive impairment (Yamauchi, Fukuyama et al. 1995). Earlier studies showed that a network of orbitofrontal, anterior temporal and mesial frontal brain regions and their connecting white matter tracts are involved in ALS and FTD (Hornberger, Geng et al. 2011, Mahoney, Beck et al. 2012). The integrity of WM tracts connecting these regions, namely uncF, forceps minor and callosal radiation appears critical. The atrophy of frontal regions is important as it is involved in Theory of Mind processing (Stone, Baron-Cohen et al. 1998, Ferstl and von Cramon 2002) and cognitive control (Miller 2000, Ridderinkhof, Ullsperger et al. 2004). Involvement of the cingulate region in cognition is well established (Bush, Luu et al. 2000). Moreover, association fibers such as uncF (Agosta, Pagani et al. 2010) which is involved in naming and language function, (Papagno 2011) and SLF (Abrahams, Goldstein et al. 2005) were involved in ALS. Several of the studies have reported increased AD of the uncF in ALS and suggested that this may relate to the behavioral disturbances of ALS patients.

Association tracts are also involved in cognition. In Alzheimer disease there is loss of integrity of the association fibers (Rose, Chen et al. 2000), and WM destruction and altered regional morphology of CC (Black, Moffat et al. 2000, Meng, Guo et al. 2012). Efficient organization of association fibers is essential for optimal cognitive performance (Schmithorst, Wilke et al. 2002, Frye, Hasan et al. 2010). There is evidence that emotional deficit follows selective damage in the ILF and IFOF (Philippi, Mehta et al. 2009). The role of the corticostriatal pathways in neurodegeneration has recently been emphasized (Shepherd 2013), so the involvement of subcortical area may underlie the cognitive and behavioral changes in ALS (Tsermentseli, Leigh et al. 2012).

The heterogeneity of impairment in ALS is well-studied and includes cognitive impairments encompassing language and memory impairment specifically the verbal fluency (Raaphorst, De Visser et al. 2010, Mackenzie, Ansorge et al. 2011). One complicating factor in most of the included studies is that not all subjects with ALS had cognitive testing. It is possible that the regions of abnormality that we have found to overlap between ALS and FTD are due to these abnormalities being present only in ALS subjects with cognitive impairment.

DTI metrics demonstrate the significant differences between controls and patients and may be used to predict disease progression. Further investigations are needed to identify the overlap of ALS and FTD in cognitive processes to address the progression of the disease. Although DTI findings are important to the scientific findings, to date, they are not readily transferrable to clinical practice. Standardization of DTI analysis is necessary to allow for clinical studies, which is still a challenge due to the rapid advances in DTI techniques.

Chapter 5: Baseline scan evaluating whole brain gray and white matter in ALS subgroups based on cognitive testing

Preamble

The systematic review (Chapter 4) highlighted the main shared WM tracts in ALS and FTD. To investigate aim two of this thesis "To identify brain regions involved in ALS patients with and without cognitive impairment compared to controls", I performed gray matter analysis of structural MRI and white matter analysis of diffusion MRI using voxel-based approaches. This chapter consists of a manuscript titled "A combined tract based spatial statistics and voxel based morphometry study of the first MRI scan after diagnosis of Amyotrophic lateral sclerosis" that has been submitted for publication to Journal of Neuroradiology.

Gray and white matter alterations in amyotrophic lateral sclerosis (ALS) patients.

Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, characterized by the loss of upper motor neurons of the primary motor cortex and lower motor neurons of brain stem (BS) and spinal cord (Kiernan, Vucic et al. 2011, Turner, Hardiman et al. 2013). In ALS, there is heterogeneity in the site of onset, disease progression and pattern of progression (Chiò, Calvo et al. 2011).

The diagnosis of ALS is based on clinical and electrophysiological features, with widely accepted diagnostic criteria (Brooks, Miller et al. 2000, de Carvalho, Dengler et al. 2008). There is a need for validated imaging or body fluid biomarkers for monitoring of ALS subjects (Turner, Kiernan et al. 2009), but none has yet reached general acceptance (Foerster, Welsh et al. 2013, Chiò and Traynor 2014).

Some ALS patients suffer from severe cognitive and behavioral dysfunction, and can be categorized as having frontotemporal dementia (FTD), usually of the behavioral variant (Achi and Rudnicki 2012). Other patients with ALS have mild cognitive dysfunction mainly in the domain of memory and language (Strong, Grace et al. 1996) and executive function (Raaphorst, De Visser et al. 2010, Phukan, Elamin et al. 2012, Elamin, Bede et al. 2013). The estimated frequency of cognitive dysfunction in ALS ranges from 10% – 75% (Frank, Haas et al. 1997, Barson, Kinsella et al. 2000, Ringholz, Appel et al. 2005, Rippon, Scarmeas et al. 2006, Phukan, Elamin et al. 2012). Consensus criteria have been developed to categorize patients as having ALS with cognitive impairment (ALScog) and ALS with behavioral impairment (Strong, Grace et al. 2009, Consonni, Iannaccone et al. 2013), but these are difficult to apply in routine clinical practice.

Pathological and genetic studies have demonstrated that there is an overlap between FTD and ALS. Several genetic mutations including TDP-*43* (Arai, Hasegawa et al.

2006, Seelaar, Kamphorst et al. 2008), c9orf72 (Irwin, Lippa et al. 2007, Benajiba, Le Ber et al. 2009, Serpente, Fenoglio et al. 2015), *TARDBP and FUS* (Mackenzie and Rademakers 2008, Vance, Rogelj et al. 2009) and progranulin gene (Mase, Ros et al. 2001, Baker, Mackenzie et al. 2006, Seelaar, Kamphorst et al. 2008), have been found in some patients with ALS and some patients with FTD.

Magnetic resonance imaging (MRI) allows non-invasive measurements of subtle changes in human brain structure and is increasingly being used to study ALS (Turner and Modo 2010). MRI can explore the anatomical configuration and shape of the brain and study the microscopic composition of brain tissue. Voxel based morphometry (VBM) is a fully automated method that is used to study changes in gray matter volume based on high-resolution structural MRI (Shah, Ebmeier et al. 1998, Vargha-Khadem, Watkins et al. 1998, Ashburner and Friston 2000). VBM whole brain normalization procedure ensures the analysis is not biased to one region (Chen, Ma et al. 2010, Tsujimoto, Senda et al. 2011). VBM has been used to demonstrate reduction in cortical volume in both frontal and temporal regions in the ALS population (Chang, Lomen-Hoerth et al. 2005). Diffusion tensor imaging (DTI) on the other hand allows mapping of tissue microstructural characteristics and is widely used to infer white-matter structural connectivity of the human brain. DTI has been used to assess the integrity of white matter tracts in ALS (Prudlo, Bissbort et al. 2012).

A number of quantitative metrics can be obtained from DTI. Fractional anisotropy (FA) is the most commonly used DTI measure and it has been shown to reflect myelination in white matter (WM) and fiber density (Pierpaoli, Jezzard et al. 1996, Le Bihan 2003). Mean diffusivity (MD) measures the total diffusion eigenvalues within a voxel, and is used clinically to identify damage in normal appearing white matter (Werring, Clark et al. 1999, Bammer, Augustin et al. 2000). Parallel diffusivity along the axonal fibers is referred to as axial diffusivity (AD). Radial diffusivity derived from DTI describes the averaged water movement perpendicular to the axonal fibers. There is little information about the value of these different measures in the study of ALS. Previous studies showed that ALS patients with cognitive impairment (ALScog) have greater frontal lobe structural changes in both gray and white matter (Lillo, Mioshi et al. 2012, Kasper, Schuster et al. 2014) than those with pure ALS with no cognitive impairment (ALSnon-

cog). However, little is known about differences in DTI parameters between these groups. The question remains whether there are imaging differences that can distinguish patients with pure ALS (ALSnon-cog) from their counterparts who have cognitive involvement (ALScog).

In this study, we used MRI to study patients with ALS with and without cognitive impairment, to determine characteristic changes in brain structures and DTI metrics that distinguish ALScog subjects from ALSnon-cog subjects.

Materials and methods

Subjects and recruitment

From May 2012 to December 2014, 30 patients (median age 62, range 28-80 years) with probable or definite ALS were recruited from the Royal Brisbane and Women's Hospital motor neuron disease (MND) clinic. The clinical diagnosis of ALS was made according to El Escorial criteria (Brooks, Miller et al. 2000). Patients were excluded if they presented progressive muscular atrophy or primary lateral sclerosis; were younger than 18 years; had other neurological or pathological disease; were unable to lie flat in the MRI scanner; had other contraindications for MRI; or had severe weakness at the time of recruitment or respiratory failure defined by clinical signs. None of the ALS patients had percutaneous endoscopic gastrostomy or respiratory symptoms at the time of scanning.

Nineteen healthy control participants (median age 58 range 39-74 years) with no history of neurological or psychiatric disorder were recruited from the community. Details of all participants are reported in Table 5.1.

Ethical approval was obtained from the Human Research Ethic Committee (HREC) of the Royal Brisbane and Women's Hospital (RBWH), (HREC 2008/98). Written informed consent was obtained for each participant.

Table 5.1: Details of participants.

	ALSnon-cog N=17	ALScog N=13	Controls N=19	P value
Age (median, range)	60.7 (28 – 74)	62.7 (41-81)	58 (39-74)	-
Gender (Male: female)	11:6	8:5	8:11	
Handedness (rt : lt)	13:4	12:1		-
Familial: sporadic	3:14	3:10		-
Limb/Bulbar/both onset	15/2/0	11/2/0		-
Disease duration	44.83 (SD=57)	19.20 (SD=12)		0.105
ALSFRS-R	40 (SD=5)	36 (SD=4)		0.051
ACE-III	94 (SD=3)	83 (SD=7)		0.0001*
Fab	16 (SD=1)	15 (SD=3)		0.074
ALS-FTD-Q *	10 (SD=6) (n=7)	23 (SD=17) (n=7)		0.188
Disease progression rate	0.62 (SD=1)	0.47(SD=1.6)		0.136

* only 14 subjects were tested with ALS-FTD-Q

Clinical Evaluation

Motor function

Motor function was assessed with the revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) (Cedarbaum, Stambler et al. 1999). The ALSFRS-R is a 12-item scale evaluating motor skills of ALS patients, including speech, breathing, swallowing, and walking (Cedarbaum, Stambler et al. 1999). Each item is scored from 0 to 4 (worst to best respectively) resulting in a maximum total score of 48. The disease progression rate was calculated using the following formula, adapted from Ellis et al (Ellis, Simmons et al. 1999):

Disease progression rate = (48 – ALSFRS score)/disease duration

Cognitive and behavioral testing

ALS patients received neuropsychological screening and behavioral evaluation on the same day as their MRI scans. Cognitive performance was assessed using the Addenbrooke's Cognitive Examination (ACE-III) (Hsieh, Schubert et al. 2013), the Frontal Assessment Battery (FAB) (Dubois, Slachevsky et al. 2000), and the ALS-FTD-Questionnaire (ALS-FTD-Q) (Raaphorst, Beeldman et al. 2012), which have been introduced in Chapter 1.

MRI acquisition

All MRI scans were performed at the Royal Brisbane and Women Hospital (RBWH) using a 3T Siemens Tim Trio (Siemens, Erlangen, Germany) equipped with a 12-channel parallel head coil. In addition to a standard series of clinical sequences, Diffusion-weighted images (DWIs) were acquired along 64 non-collinear directions at b = 3000 s/mm^2, with one non-diffusion weighted image. Acquisition parameters were: 60 axial slices, FOV 30x30 cm, flip angel 90, slice thickness 2.5 mm, matrix 128x128, TR/TE 3200/91 ms, iPAT factor 2. A field map was acquired using two 2D gradient-recalled echo images with TE1/TE2=4.92/7.3 and TR= 488 ms to assist in the correction of geometric distortions. The acquisition time for the diffusion dataset was 9:40 min. These parameters have been used in our previous DTI studies of MND (Rose, Pannek et al. 2012, Rose, Rowland et al. 2012). The b value was chosen to allow detection of crossing fibres. In addition, a high-resolution structural image was acquired using 0.44 mm^3 isotropic with interpolation 3D T_1 MPRAGE (slice thickness 0.9, FOV 23 × 25.6 × 17.6 cm, TR/TE/TI 1900/2.24/900 ms, flip angle 9) with total scan time 9:14 min.

Diffusion processing

Diffusion MRI data were preprocessed as described previously (Pannek, Boyd et al. 2014). Processing included correction for head movement with rotation of the b-matrix, detection and removal of signal intensity outliers, and correction for geometric distortions and intensity inhomogeneity. Maps of FA, MD, axial diffusivity (AD) and radial diffusivity (RD) were calculated.

Tract based spatial statistics (TBSS) analysis

Tract-based spatial statistics analysis was performed with the FSL package version 4.1 (www.fmrib.ox.ac.uk/fsl/tbss) which is a fully automated whole brain analysis technique that uses voxel-wise statistics on FA data while simultaneously minimizing the effects of misalignment (Smith, Jenkinson et al. 2006). Briefly, the main steps were a) non-linear alignment of FA images to 1x1x1 mm MNI152 standard space, b) creation of the mean FA image and its white matter "skeleton" representing the tracts that are common to all subjects, c) projection of individual FA maps onto the image skeleton. Non-FA metrics (MD, AD, and RD) were also projected onto the skeleton. The mean FA skeleton threshold was 0.2. We then performed voxel-wise statistical analysis on the skeleton, with statistical tests as described below. To correct for multiple comparisons, we employed permutation testing (5000 permutations) and threshold-free cluster enhancement (TFCE;(Smith and Nichols 2009)). We consider results to be significant at fully corrected $p < 0.05$.

Voxel-based Morphometry Analysis (VBM)

Structural three-dimensional T1-weighted images were analyzed using FSL-VBM toolbox in FMRIB software library package (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLVBM) (Douaud, Smith et al. 2007). FSL-VBM uses an optimized VBM protocol (Good, Johnsrude et al. 2002) carried out with FSL tools (Smith, Jenkinson et al. 2004) in which GM maps are co-registered into a standard space, allowing comparisons of GM density between groups. All data volumes were resampled at 1 mm^3 voxel size prior to FSL VBM processing. Structural images were brain-extracted and gray matter-segmented before registering to the MNI152 standard space using non-linear registration (Andersson, Jenkinson et al. 2007). The resulting images were averaged and flipped along the x-axis to create a left-right symmetric, study-specific gray matter template. All native GM images were then non-linearly registered to this study-specific template and "modulated" to correct for local expansion (or contraction) due to the non-linear component of the spatial transformation. The modulated gray matter images were then smoothed with an isotropic Gaussian kernel with a sigma of 4 mm. Finally, voxel-wise analysis was

performed using permutation-based non-parametric testing, correcting for multiple comparisons across space using TFCE (Smith and Nichols 2009).

Statistical tests

Age, neuropsychological (ACE-III) and behavioral (FAB and ALS-FTD-Q) tests, ALSFRS-R, disease duration and disease progression were compared across groups (ALScog, ALSnon-cog, and controls) using a two-tailed T-test.

To identify group differences in diffusion metrics (TBSS) and gray matter density (VBM), we used the "randomise" tool of FSL, with 5000 permutations. Correction for multiple comparisons was performed using threshold-free cluster enhancement (TFCE, (Smith and Nichols 2009)). Results were considered significant at $p<0.05$, corrected for multiple comparisons.

Results

Patient groups

Patients were grouped into those with normal cognition (pure ALS [ALSnon-cog]) and those with impaired cognition [ALScog], on the basis of performance on the ACE-III with a cut-off point of 88. There were 14 ALScog subjects with median age 62.7 (range 41-81 years) and 16 ALSnon-cog subjects with median age 60.74 (range 28 – 74 years). The ALScog group was slightly older than the ALSnon-cog group and had shorter disease duration. The details of participants, neurophysiological and behavioral tests and disease progression rate are shown in Table 5.1. ALScog group had significantly lower scores in all five cognitive domains compared to the ALSnon-cog group, Table 5.2.

Behavioral assessment using ALS-FTD-Q revealed greater abnormalities in the ALScog group than in the ALSnon-cog group. FAB showed a trend towards significant difference between the patient groups ($p=0.041$).

DTI studies of white matter (TBSS)

We tested for differences in DTI measures (1) between all ALS subjects and controls, (2) between males and females with ALS, (3) between ALS subjects with normal cognition (ALSnon-cog) and controls, (4) between cognitively impaired ALS subjects (ALScog) and controls, and (5) between ALS subjects with (ALScog) and without impaired cognition (ALSnon-cog).

TBSS revealed differences in FA, MD and AD between ALSnon-cog and controls, and between ALScog and controls, but not between ALSnon-cog and ALScog ($p<0.05$) (Figure 5.1). However, some trends of higher MD and RD in ALScog compared to ALSnon-cog patients ($p<0.1$) were detected (Figure 5.2). Table 5.3 demonstrates the significant measure of DTI metrics.

Table 5.2: ACE-III results for all domain comparing ALSnon-cog and ALScog.

	Attention	Memory	Fluency	Language	Visuspatial
ALSnon-cog	17.06 (SD=1.6)	24.13 (SD=1.26)	12.25 (SD=1)	25 (SD=1)	16 (SD=0.34)
ALScog	15.38 (SD=2)	21.85 (SD=2.7)	10 (SD=3)	22.23 (SD=4)	14.62 (SD=1.3)
Significance (p-value)	0.017*	0.013*	0.033*	0.027*	0.005*

Table 5.3: DTI and MRI changes in ALS patients. ALS (n=30) and controls (n=19).

	Presence of significant differences		
Method	ALSnon-cog vs HC	ALScog vs HC	ALSnon-cog vs ALScog
FA	Yes ($p<0.05$)	Yes ($p<0.01$)	No
MD	Yes ($p<0.05$)	Yes ($p<0.01$)	Yes ($p<0.1$)
RD	No	No	Yes ($p<0.1$)
AD	No	Yes ($p<0.01$)	No
VBM	Yes ($p<0.05$, one region)	Yes ($p<0.05$, widespread)	Yes ($p<0.05$, caudate nucleus)

Mean Diffusivity (MD)

TBSS showed significant differences between the whole group of ALS subjects and controls in MD which was increased in widespread brain regions including CST, Cg, anterior thalamic radiation (aTR) and CC. In the association fibers, the IFO and uncF showed increased MD at $p<0.05$ (Figure 5.1).

In Figure 5.1, comparing ALSnon-cog patients and healthy controls, MD was found to be significantly increased ($p<0.05$) bilaterally in patients in the uncinate fasciculus (unc.F), the inferior fronto-occipital fasciculus (IFOF), and the right temporal part of the superior longitudinal fasciculus (SLF). Increased MD in the body of the corpus callosum (bCC) showed changes that did not reach significance ($p<0.1$) especially in the forceps minor (FM) bilateral when comparing ALS subgroups (Figure 5.2).

When comparing ALScog patients with healthy controls, there was an increase in MD diffusely spread over the WM (Figure 5.1), with highly significant differences ($p<0.01$)

noted in most of the association fibers including the SLF, IFOF, IFL, unc.F, along the corticospinal tract (CST) including anterior corona radiate (CR) and anterior limb of internal capsule (ALIC), posterior orbital gyrus WM, tracts in the precentral gyrus WM, supplementary motor cortex WM, inferior frontal gyrus WM and middle and inferior temporal gyrus WM. Less significant increase in diffusivity ($p<0.04$) was seen in the CC forceps major left cingulum.

With MD, there was changes that did not reach significance ($p<0.1$) towards differences between ALSnon-cog and ALScog subjects in both the right frontal and temporal lobes, right CST at the level of internal capsule (IC) and the left anterior corpus callosum (CC) and bilaterally in the left CC (Figure 5.2).

Fractional anisotropy (FA)

FA was decreased in all ALS subjects compared to controls in the corticospinal tract (CST), the callosal radiation and the body of corpus callosum (CC), cingulum (Cg) at $p<0.01$, and in the association fibers in the superior longitudinal fasciculus (SLF) and the inferior longitudinal fasciculus (ILF) at $p<0.05$ (Figure 5.1).

There was a significant reduction in FA in ALSnon-cog patients compared to healthy controls ($p<0.05$, corrected for multiple comparison) (Figure 5.1). Widespread WM changes were detected along most of brain tracts, particularly in the projection fibers (along the CST), commissural fibers (bilateral forceps minor, splenium and genu of CC, left forceps major), and association fibers (including the temporal part of SLF and bilateral IFOF). FA showed more significant differences ($p<0.05$) in the frontal lobe tracts and in the association fibers (IFOF, left SFOF, bilateral SLF and left Unc.F) including the bCC.

In the ALScog group compared to controls, there was widespread reduction in FA in patients, and this was more prominent towards the right side of the brain. There was reduced FA bilaterally in the tracts that extend from frontal and central CC to the primary and premotor cortex including parts of the CST; superior CR and posterior limb of IC and rostral cerebral peduncle ($p<0.01$). Additional changes were observed

in the association fibers; IFO and ILF ($p \leq 0.02$) and SLF ($p \leq 0.05$). There were weaker regional differences in the precuneus, superior occipital gyrus WM, thalamus and tracts in the superior frontal gyrus ($p \leq 0.03$). Fornix and stria terminalis also showed FA reduction ($p \leq 0.05$) (Figure 5.1).

There were no differences in FA between ALSnon-cog subjects and ALScog at $p<0.05$ and $p<0.1$.

Axial diffusivity (AD)

Although the differences between the whole group of ALS subjects and controls in WM were less significant in AD (Figure 5.1), the changes seen in the association fibers were more widespread than with FA, and included changes in the SLF, ILF, IFOF and uncF. Changes in aTR and CST at the level of corona radiata were seen in AD.

ALScog patients have lower AD than healthy controls. These changes can be clearly observed in the right hemisphere WM, including tracts in the frontal orbital WM. The right SLF, ILF and IFOF showed lower AD in ALScog group compared to controls. A similar significant result was also seen in the genu of CC and forceps minor (FM) and forceps major (Fmj) (Figure 5.1). There were no differences between the ALSnon-cog group and healthy controls (Figure 5.1), and between ALSnon-cog and ALScog groups.

Radial diffusivity (RD)

The differences between the whole group of ALS subjects and controls in WM were less significant in RD compared to other DTI measures. However, changes were mainly seen in the association fibers which were more widespread than with FA, and involving the SLF, ILF, IFOF and uncF (Figure 5.1).

There was no significant change in RD in whole brain TBSS for any of the comparisons (ALSnon-cog vs healthy controls, and ALScog vs healthy controls); however when subcomparing ALSnon-cog with ALScog groups, some changes that did not reach significance in ALSnon-cog vs ALScog groups, and were observed in the right hemisphere WM in the corpus callosum, forceps minor and forceps major, the CST at the level of the corona radiata and the anterior limb of the internal capsule, the thalamic

radiation and the WM tracts in the temporal lobe and pre- and postcentral gyrus ($p<0.1$, Figure 5.2).

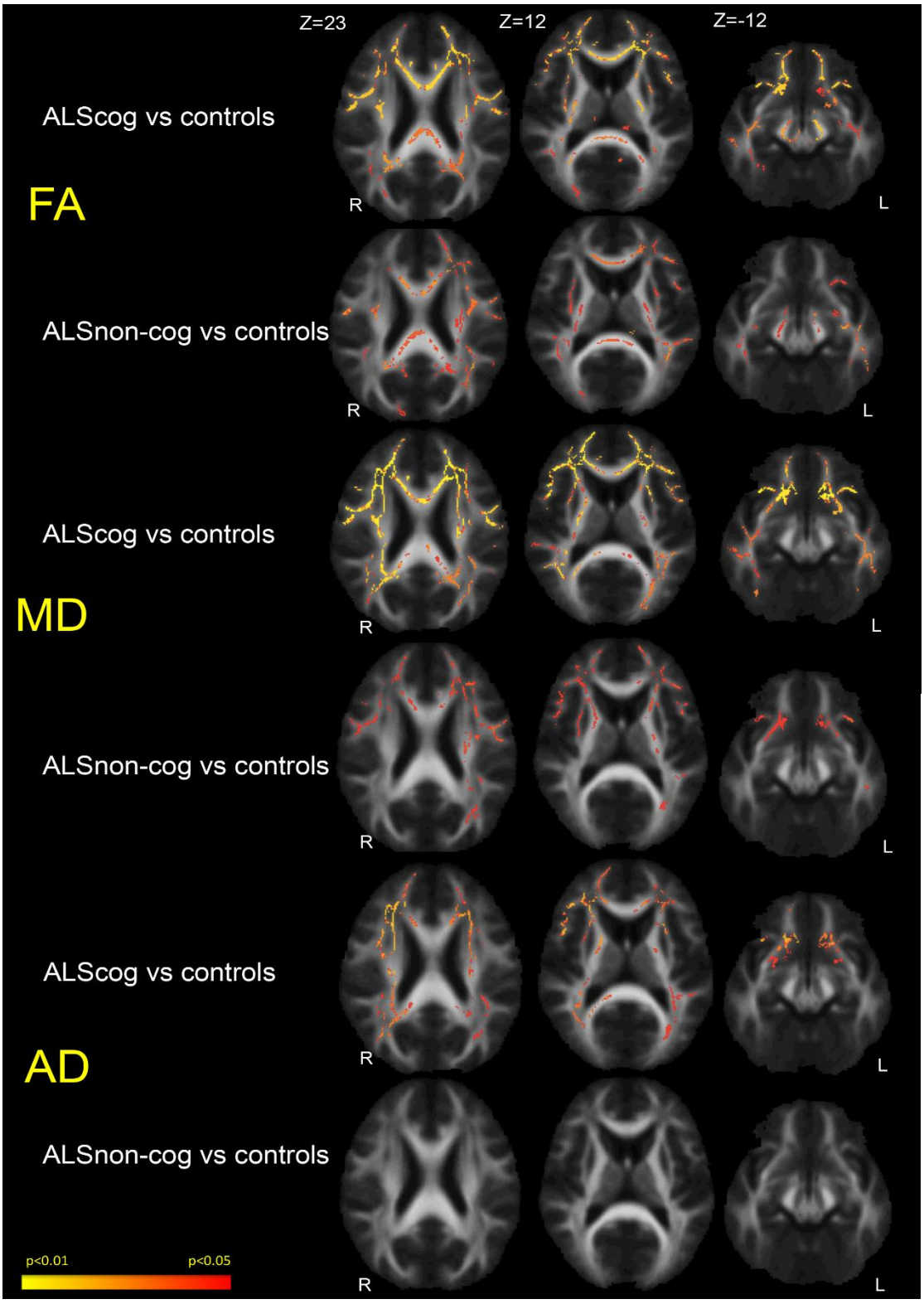

Figure 5.1: WM TBSS analyses of DTI metrics highlighting differences between ALScog and ALSnon-cog compared to healthy controls. ALScog exhibit widespread changes in the frontal, parietal and temporal lobes in FA, MD and AD. ($p<0.01$). Compared to controls, ALSnon-cog subjects have no AD changes but significant FA and MD changes in the CST, CC and temporal part of SLF and bilateral IFOF ($p<0.05$).

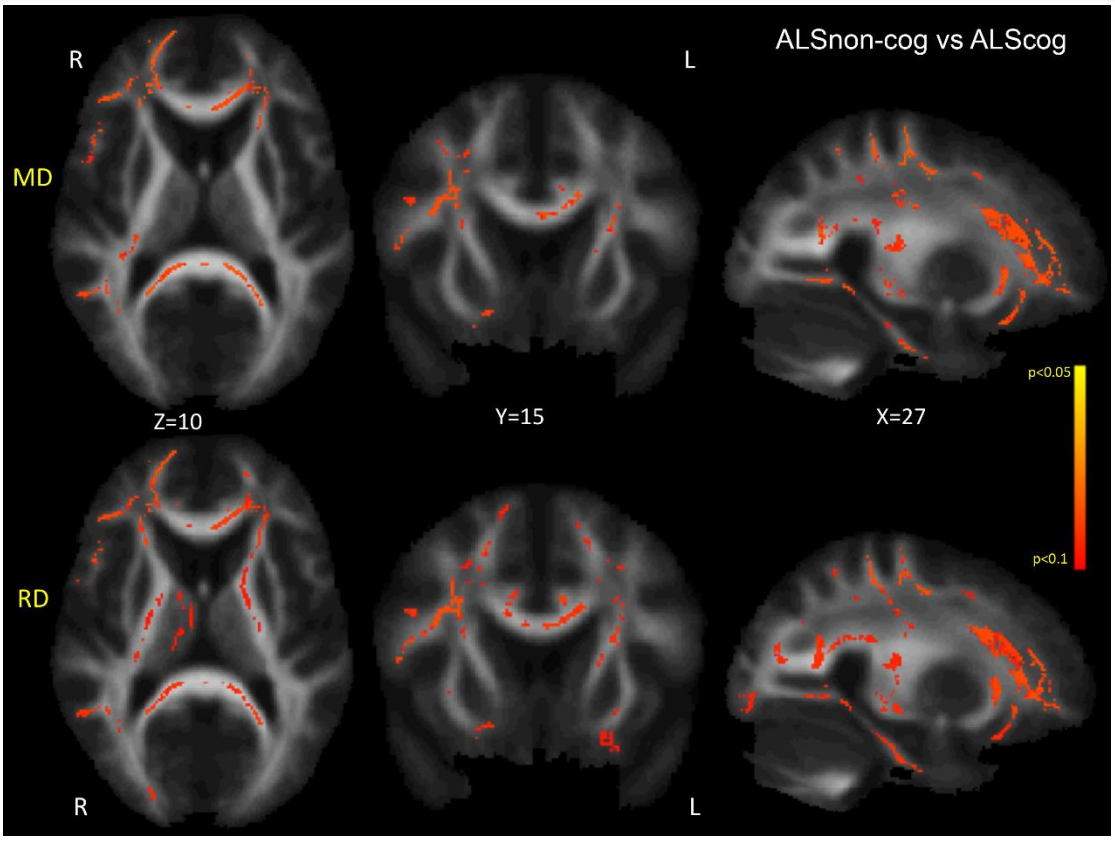

Figure 5.2: TBSS comparison of ALSnon-cog and ALScog. Trends of higher MD and RD in subjects with cognition involved were detected in the right frontal and parietal lobes including CST at internal capsule (p<0.1, corrected for multiple comparisons).

There were no differences between male (n=18) and female (n=12) subjects with ALS in any of the DTI measures.

VBM

When comparing all ALS subjects with controls, the right post- and pre-central gyrus showed significant differences in GM density at $p< 0.02$, and small regions in the left middle frontal gyrus and right central opercular cortex, right superior temporal gyrus and right cingulate gyrus showed a trend towards significant changes at $p< 0.06$ (Figure 5.3). These changes were confined to the right hemisphere except a small region in the left middle frontal gyrus.

Figure 5.4 depicts the regional pattern of significant differences in GM volumes compared between the patient and control groups. Comparing ALSnon-cog patients and controls showed no differences at $p<0.05$ but there was a some evidence ($p<0.1$) towards a reduction in GM density in patients in two small regions in the right pre- and postcentral gyrus in the parietal lobe.

Compared to controls, ALScog patients had widespread and substantial reduction ($p<0.01$) of GM volume in bilateral frontal and temporal poles, middle temporal gyri, paracingulate gyrus, left middle frontal gyrus and cortex, left temporo-fusiform cortex, left frontal operculum, right central opercular cortex and the right pre-central gyrus, (Figure 5.4).

In the limbic system, VBM revealed significant GM loss in the amygdala, bilateral para-hippocampal gyrus and bilateral anterior parietal lobe, middle frontal gyrus, putamen, and posterior thalamus in ALScog compared to controls ($p<0.05$), Figure 5.4.

ALScog patients had distinct bilateral loss of GM volume in the caudate nucleus (Cd) compared to ALSnon-cog at $p<0.05$ (Figure 5.4).

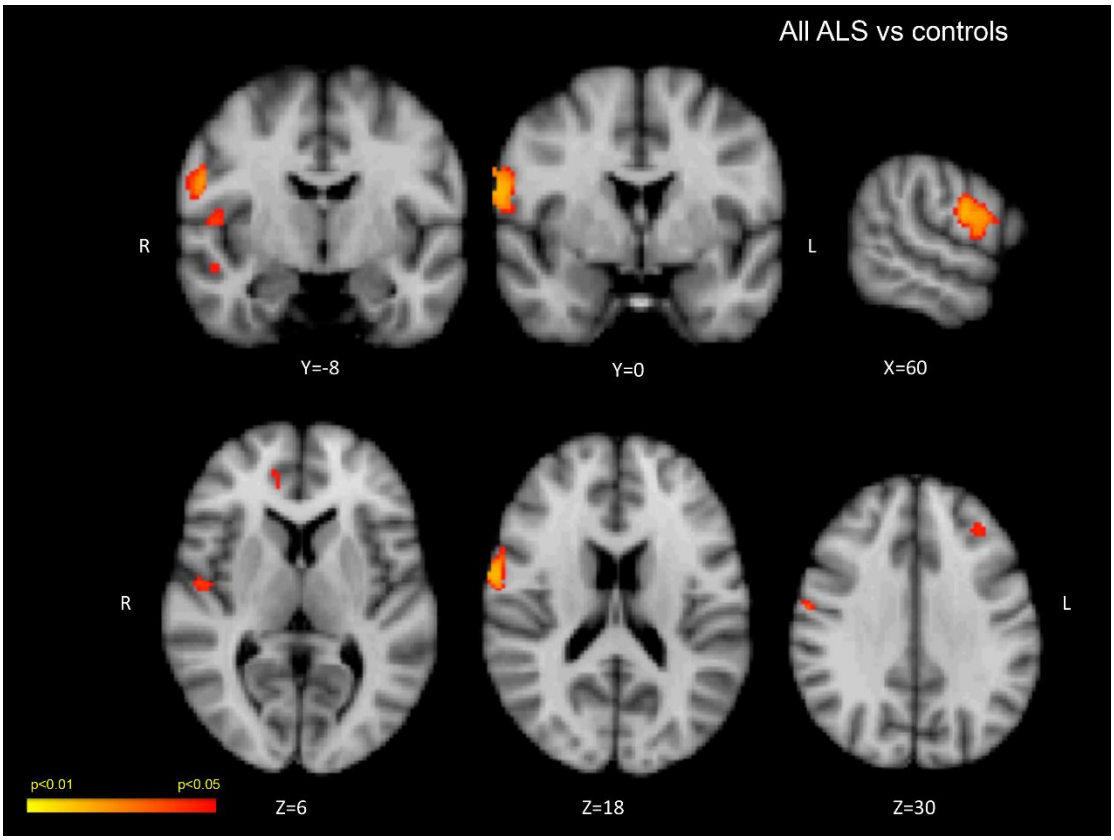

Figure 5.3: Clusters of significant GM density differences in VBM analysis when comparing all ALS subjects and controls. This shows reduction in the GM volume at p<0.02. The regions are pre- and post-central gyrus, opecular cortex, superior temporal gyrus and cingulate gyrus.

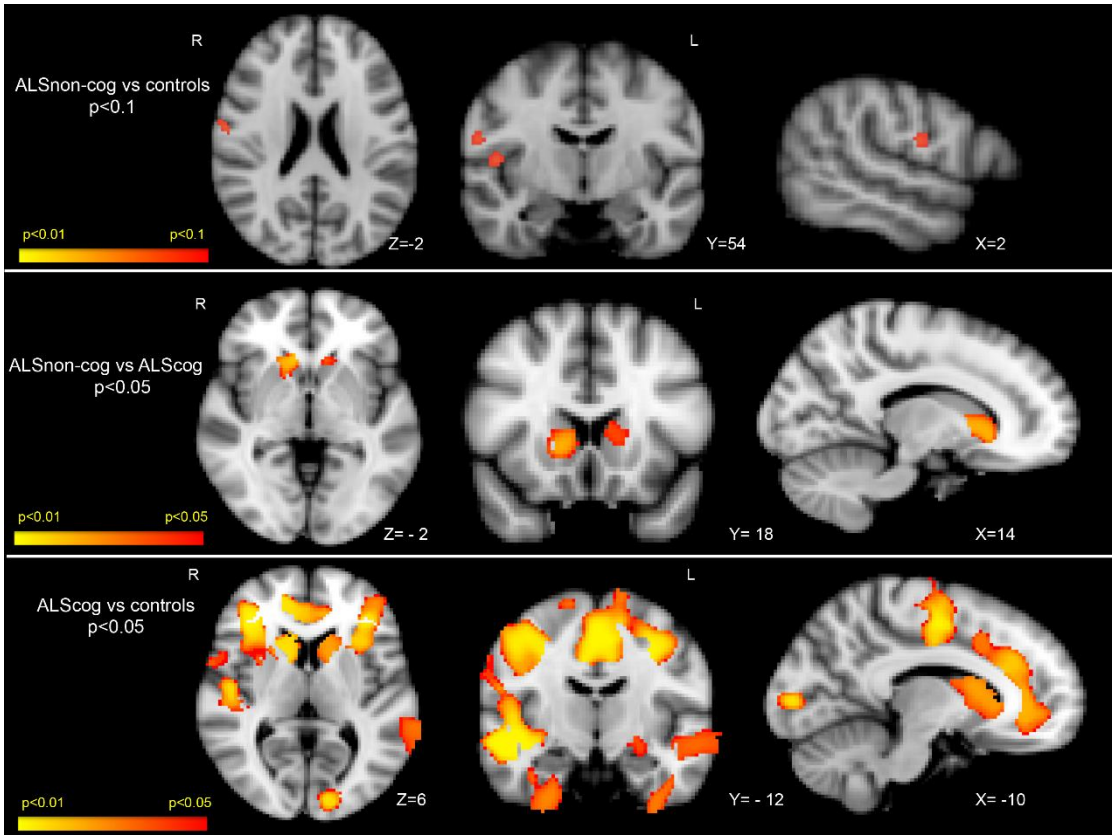

Figure 5.4: Clusters of significant GM signal differences in VBM analysis when comparing ALS subgroups with healthy controls. Results are overlaid on the axial sections of a T1-weighted template in the MNI standard space in neurological convention (TFCE p<0.05 corrected). ALScog have distinct reduction in the volume of caudate compared to ALSnon-cog subjects (middle row). Compared to controls, ALScog subjects (bottom row) have more widespread structural T_1 changes than ALSnon-cog subjects (top row).

Discussion

To study the underlying basis of cognitive impairment in ALS, this study investigated gray and white matter disruption in ALSnon-cog and ALScog patients, and compared the changes observed with DTI measures. We have demonstrated that ALS is associated with changes in both GM density and WM diffusivity and that the location of changes differs between ALSnon-cog and ALScog.

DTI changes were seen in the CST, and also in extra-motor areas. Changes in the CST are consistent with classical features of ALS and with previous MRI studies (Lillo, Mioshi et al. 2012). We defined ALScog patients by their performance on the ACE-III. This categorization is not as stringent as the definition proposed by Strong et al. for ALScog (Strong, Grace et al. 2009), but is the assessment that can be used in routine clinical practice. It does not test executive function, and in the future is likley to be replaced by other measures such as the ECASS (Abrahams, Newton et al. 2014) that were not available when this study was commenced on 2012.
Our ALScog patients did not have significant behavioural changes when measured by FAB but mild to moderate behavioural disturbances were detected by ALS-FTD-Q. The changes seen with MRI were more severe in the ALScog group than the ALSnon-cog group, but this cannot be explained by disease duration, because the ALScog group had shorter disease duration than the ALSnon-cog group ($p=0.1$). There was a majority of male subjects in both patient groups, consistent with the findings that ALS is more common in males than females (McCombe and Henderson 2010). The majority of patients in both groups were right handed. It is important to control for handedness because this can influence the spread of disease (Devine, Pannek et al. 2015). Others have found evidence of a significant correlation between DTI measures with disease severity measures in ALS (Ellis, Simmons et al. 1999). However, in the current study such correlation was not feasible due to the small sample size and variable disease presentation.

In patients with ALScog there were marked changes in DTI and VBM in the frontal and prefrontal cortex. The prefrontal cortex constitutes nearly one-third of the neocortex (Shallice and Evans 1978) and is a store of short-term memory (Miller and Cohen 2001). Abnormality of frontal regions in ALS with dementia has been previously observed in Japanese ALS patients

with high signal intensities on T_2-weighted images in frontotemporal WM and precentral gyri (Van Zandijcke and Casselman 1995).

In ALScog, DTI showed abnormality in most of the projection fibers (CST at the level of corona radiata and internal capsule and tracts in the pre- and post-central gyri) and the association tracts (SLF, IFOF, ILF). DTI changes were most prominent in the frontal connections. This may represent a reduction in functional connectivity with cognitive impairment. In ALScog these changes were observed in the SLF and were particularly severe in the anterior portion of the tract. The observation of predominantly anterior involvement is compatible with earlier studies (Kiernan and Hudson 1994). SLF DTI abnormality in ALScog patients is remarkable as this structure plays an important role in the language function (Catani and Mesulam 2008).

The CC has been implicated in human behavior and cognition (Witelson 1989). A previous study had identified atrophy in the anterior half CC in ALS subjects with cognitive decline (Yamauchi, Fukuyama et al. 1995), similar to our findings in ALScog. An autopsy study on patients with ALS has shown pathologic changes in the isthmus and posterior mid-body of CC (Sugiyama, Takao et al. 2013). We found a significant decrease of FA in the splenium of CC, which has also been described in other studies (Agosta, Pagani et al. 2007, Bartels, Mertens et al. 2008, Rose, Pannek et al. 2012).

DTI detected smaller difference in ALSnon-cog group vs controls compared to ALScog group vs controls. Between the ALSnon-cog and the ALScog group there were no significant differences at $p<0.05$, but at $p<0.1$, there were some evidence of changes in the motor cortex; pre- and postcentral gyrus, the commissural fibers in the fronto-parietal lobe; forceps minor and splenium of the corpu callosum, association fibers; inferior fronto-occipital and superior longitudinal fasciculus, and inferior longitudinal fasciculus in the temporal lobe. These changes were more prominent on the right hemisphere (Figure 5.2).

Using VBM, when comparing all ALS subjects with healthy controls, we found a reduction in the gray matter volume in the fronto-temporal regions and the pre- and post-central gyri. These regions connected with WM tracts that were found to show significant changes on TBSS. These changes reflect the physiological relationship between GM and WM, similar to the results

found in previously published studies (Bede, Bokde et al. 2013). ALSnon-cog patients showed asymmetric regional decrease of density in the grey matter of the right but not the left of primary motor cortex when compared to the control group. Previous studies have shown decreased signal intensity in the precentral gyrus on T_2 and proton density weighted imaging (Oba, Araki et al. 1993, Cheung, Gawel et al. 1995, Bowen, Pattany et al. 2000). Rooney et al also found that NAA/ (Cho+Cr) spectroscopy correlated with the UMN disease severity in the motor cortex (Rooney, Miller et al. 1998). In a post-mortem study, Smith found that a large number of degenerating fibers pass to or from the precentral gyrus in ALS patient (Smith 1960). Our finding of reduced GM on the right side is consistent with the findings of a meta-analysis that found preferential atrophy in the right precentral gyrus (Chen and Ma 2010). However, using different techniques, previous work from our group has shown that there is preferential involvement of the dominant motor cortex in ALS (Devine, Kiernan et al. 2014). The relationship of handedness to disease therefore requires further clarification.

Although disease duration was shorter in ALScog group, we found heterogeneous widespread volume reduction involving both anterior and posterior cerebral GM compared to controls. These early diffuse changes in the GM and WM in ALScog group may be an indicator of a later diagnosis of cognitive impairment in ALS patients. ALS patients with FTD were found to have shorter survival than those without (Olney, Murphy et al. 2005).

The ALScog group showed loss of GM in the caudate nucleus, compared with the ALSnon-cog group. There is mounting evidence that lesions in the caudate nucleus can cause cognitive deficits (Mendez, Adams et al. 1989). Previous post mortem studies have consistently found pathological changes in the frontotemporal cortex and subcortical structures such as caudate nucleus and putamen in ALS patients with dementia (Mackenzie and Feldman 2005). A previous quantitative imaging study revealed that involvement of subcortical GM structures including the caudate nucleus may form a key feature of ALS (Bede, Elamin et al. 2013) and other work found that these additional changes in ALS patients with cognitive impairment are correlated with neuropsychological and behavioral profiling (Kasper, Schuster et al. 2014).

Our findings of loss of volume of the caudate nucleus in ALScog is consistent with the finding of reduced FA and increased MD in several clusters of the right anterior parts CC. This suggests that a more prominent disruption of temporo-frontal connection in ALScog patients may

represent a hallmark for the presence of cognitive impairment. The diffusivity changes that we identified in many regions of WM were in tracts that connected atrophic GM tissue such as gCC and ant. SLF. This is a novel finding and suggests a mechanism of disease progression in relation to cognition. The medial temporal lobes are implicated in memory storage while the frontal lobe mainly serves in executive function and language.

It is of note that in ALScog compared to ALSnon-cog, we found GM loss in bilateral caudate nuclei but more in the right caudate nucleus. The subjects were mostly right handed. As with the possible role of handedness in motor cortex atrophy, the role of handedness and limb dominance in cognitive impairment requires further study. We speculate that memory and language deficits observed in ALScog may be due to selective impairment in the motor control and memory circuit as a consequence of the changes observed in caudate nucleus. Some studies have shown that the caudate nucleus receives input from frontal and temporo-parietal cortical areas and forms fronto-caudate anatomical loops (Kemp and Powell 1971). We also hypothesize that microstructural alteration of fronto-temporal structures might have led to cognitive and behavioral changes in ALS patients. Clinicopathological studies have confirmed the clinical overlap between ALS and FTD in multiple domains of cognition (Lomen-Hoerth, Anderson et al. 2002, Lillo, Mioshi et al. 2012).

DTI can generate eigenvector maps that provide visual comparison between trajectories of degenerated and intact tracts (Werring, Toosy et al. 2000). Although DTI studies typically report the findings with FA, more recently published work has reported other DTI metrics such as RD and AD and has linked the increase in both to loss of myelination (Song, Sun et al. 2002) and axonal degeneration (Budde, Xie et al. 2009), respectively. The need to gain insight into the white matter in ALS has raised prospects for the role of diffusion imaging. This insight has been achieved in our study through using four DTI metrics to assess cerebral tracts. Large MD increases were seen in all patients compared with controls and within subgroup comparison. Our findings highlight the potential advantages of DTI to detect the structural changes in white matter and associated degeneration of fiber pathways. Future longitudinal DTI studies may provide a better understanding of the pattern of WM changes in ALS.

One limitation of this study is the small sample size. There was also a slight variation in age between patient groups and healthy controls. Another limitation is that we used clinical

screening tools for assessment of cognitive function and did not use formal neuropsychological testing. These were used because they are readily available in the clinic.

In summary, diffusion tensor imaging has shown greater frontal lobe involvement in subjects with ALScog than ALSnon-cog, and showed the involvement of the caudate nucleus in ALScog. Ongoing longitudinal studies should provide opportunity to assess the possible early feature of caudate nucleus as early predictor for cognitive impairment.

Chapter 6: Two scans evaluation of motor and extra-motor pathways

Preamble

As shown in Chapter 5, DTI in patients with ALS found significant changes in the motor and extra-motor pathways compared to controls, with extra-motor involvement being more prominent in subjects with cognitive impairment. This chapter explores the changes in WM of motor and extra-motor pathways over time. A subset of patients studied in Chapter 4 (n=23) had two MRI scans, 6 months apart. Changes were explored using region-of-interest (ROI) analysis in 21 regions.

A 6–month evaluation using diffusion tensor imaging.

Introduction

Amyotrophic lateral sclerosis (ALS) is a disease that is defined by the loss of upper and lower motor neurons. However, extra–motor features can be found in ALS, with cognitive impairment being prominent (Beeldman, Raaphorst et al. 2016). MRI studies of ALS have reported pathological changes in white (WM) and gray matter (GM) (Agosta, Pagani et al. 2007, Canu, Agosta et al. 2011).

Diffusion tensor imaging (DTI), which measures movement of water molecules, can be used to assess the integrity of white matter (WM). DTI provides a variety of measures to characterize the diffusion of water, which is hindered and restricted by cellular microstructure. Currently, the most widely used DTI measure is fractional anisotropy (FA), which is a measure of the degree of anisotropy of diffusion (Koay, Chang et al. 2006). FA is believed to reflect myelination of white matter (WM) and fiber density (Pierpaoli, Jezzard et al. 1996, Le Bihan 2003). Mean diffusivity (MD) is another DTI metric, which measures the overall speed of diffusion within a voxel. MD is used clinically to identify damage in white matter that appears normal on structural imaging (Werring, Clark et al. 1999, Bammer, Augustin et al. 2000). Most DTI studies of ALS have been cross–sectional studies at a single time–point and have focused on abnormalities in the white matter (WM) of the motor system (Carrara, Carapelli et al. 2012, Kwan, Meoded et al. 2013, Müller, Turner et al. 2016). However, some studies have reported the involvement of association tracts and subcortical structures (Pettit, Bastin et al. 2012, Sharma, Sheriff et al. 2012).

Serial MRI studies could provide a measure for the rate of progression in neurological diseases and may also demonstrate the spread of pathology to different brain regions (Hu, Jin et al. 2016). However, the few longitudinal DTI studies of ALS have small numbers of subjects and inconsistent findings (Nickerson, Koski et al. 2009, Zhang, Schuff et al. 2011, Keil, Prell et al. 2012). Some have reported the progression of ALS by demonstrating decreasing FA over time along the corticospinal tract (CST) (Sage, Peeters et al. 2007, Nickerson, Koski et al. 2009). However, one study found that FA does not change on later scans (Blain, Williams et al. 2007) while others found negative results when using DTI and ^{1}H MRSI (Mitsumoto, Ulug et al.

2007). We hypothesize that there would be greater white matter damage in ALS in later timepoints and that white matter damage would correlate with disease severity.

Methods

Participants

Patients with ALS were recruited from the multidisciplinary Motor Neuron Disease clinic at the Royal Brisbane Women's Hospital (RBWH). Twenty-three patients (n=16 males, n= 7 females; age 32–84 years old, mean age=59±11 years) were included. All patients fulfilled the criteria for definite ALS according to revised El Escorial criteria (Brooks, Miller et al. 2000). This study was approved by the RBWH ethics committee (HREC 2008/98), and all patients provided written informed consent.

Clinical assessment

The revised ALS functional rating scale (ALSFRS-R) was administered at each clinical visit every three months (Cedarbaum, Stambler et al. 1999). On the same day as their MRI scans, ALS subjects also received cognitive and behavioral testing, using the Addenbrooke's cognitive examination ACE-III (Hsieh, Schubert et al. 2013) and Frontal assessment battery (FAB) (Dubois, Slachevsky et al. 2000).

Image acquisition

All subjects had two MRI scans, 6 months apart. MRI scans were performed at RBWH using a 3T Siemens Tim Trio (Siemens, Erlangen, Germany) equipped with a 12–channel parallel head coil. In addition to a standard series of clinical sequences, diffusion-weighted images (DWIs) were acquired along 64 non-collinear directions at b = 3000 s/mm^2, with one non-diffusion weighted image. Acquisition parameters were: in-plane resolution 2.34x2.34, 60 axial slices, FOV 30x30 cm, slice thickness 2.5 mm, matrix 128x128, TR/TE 9200/112 ms, iPAT factor 2. A field map was acquired using two 2D gradient-recalled echo images with TE=4.92 and TR= 488 ms to assist in the correction of geometric distortions. The acquisition time for the diffusion dataset was 9:40 min.

Diffusion processing

Diffusion MRI data were preprocessed as described previously (Pannek, Boyd et al. 2014). Preprocessing methods included correction for head movement with rotation of the b-matrix, detection and replacement of signal intensity outliers, and correction for geometric distortions and intensity inhomogeneity. Maps of FA and MD were calculated using MRtrix 0.2.9.

A custom FA template, generated using scripts provided with ANTs (http://picsl.upenn.edu/ANTS/) (Avants, Tustison et al. 2009), was derived from all subjects. We used ANTs symmetric diffeomeophic registrations using symmetric image normalization (Greedy SyN). The JHU 1mm FA was used for the initial rigid body registration to generate the template.

The JHU atlas (Wakana, Jiang et al. 2004) was normalized to this study template using symmetric diffeomorphic registration. ROIs of the JHU atlas were subsequently transformed to the individual datasets in native space and mean values of FA and MD were calculated for each ROI.

Region of interest (ROI) analysis

We performed an ROI analysis of the diffusion tensor data in 21 regions; non-midline structures were measured on both sides separately. The regions that were studied are listed in Table 6.1.

Using ITK-Snap software, the automated placement of ROIs was confirmed by one rater (A.R.A). Mean FA and MD values were extracted for each region Figure 6.1.

Table 6.1: List of regions-of-interest investigated in this study.

ROIs
The corticospinal tracts (CST)
Corona radiata (CR) (right and left)
Medial lemniscus (ML) (right and left)
Pons
Posterior internal capsule (PLIC) (right and left)
Callosal tracts
Minor forceps (FMi) (left and right)
Genu corpus callosum (gCC)
Body corpus callosum (bCC)
Splenium corpus callosum (sCC)
Association fibers
The superior longitudinal fasciculus (SLF) (right and left)
The inferior longitudinal fasciculus (ILF) (right and left)
Other extramotor tracts
Cingulum (Cg).
Hippocampus (Hpc) (right and left)
Anterior limb of internal capsule (ALIC) (right and left)

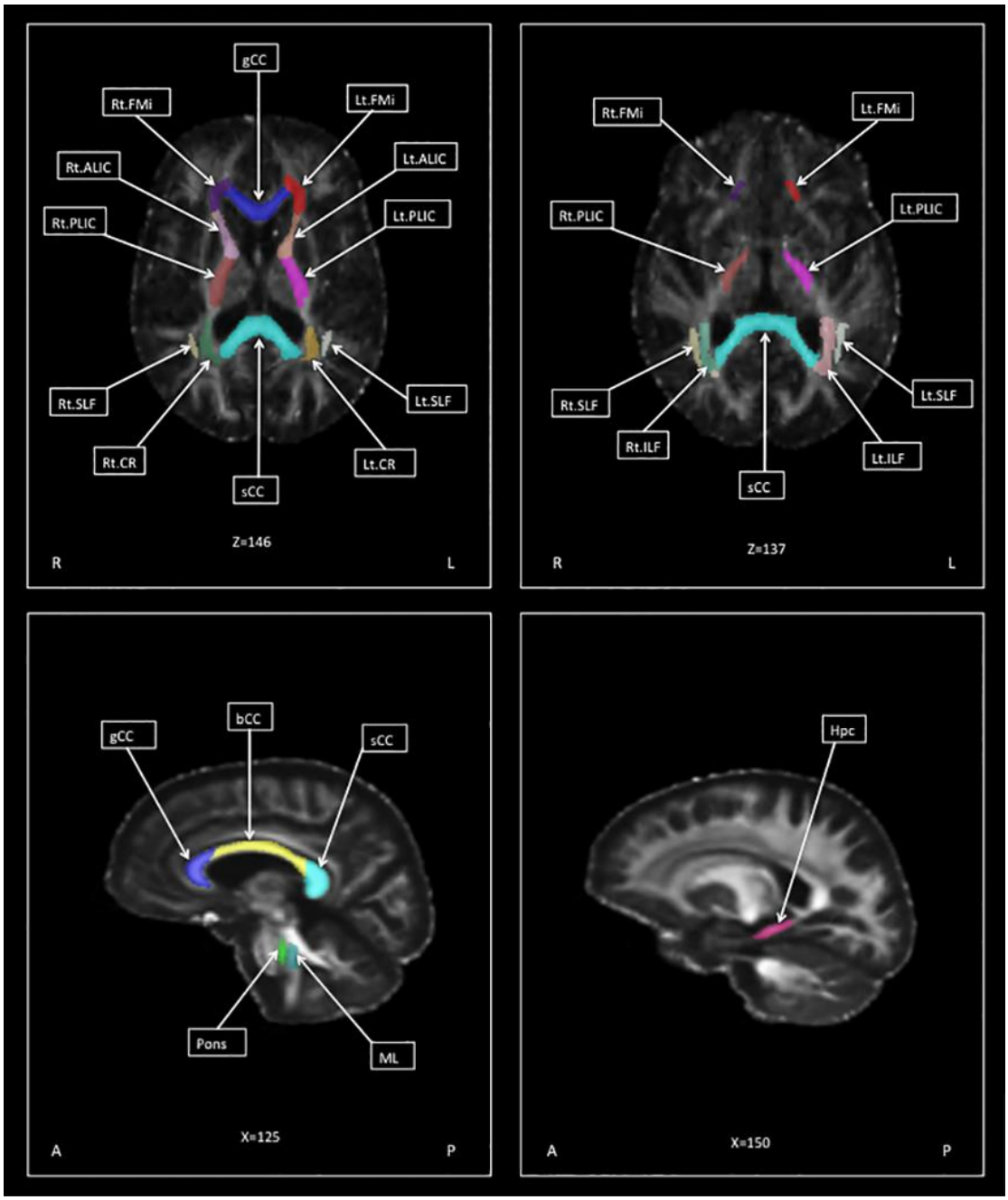

Figure 6.1: Regions of interest (ROIs) used in the analysis, overlaid on study specific template.

Tract-based spatial statistics (TBSS)

Tract-based spatial statistics analysis was performed with FSL (version 5.0) (www.fmrib.ox.ac.uk/fsl/tbss). TBSS is a fully automated whole brain analysis technique that uses voxel-wise statistics on FA data while simultaneously minimizing the effects of misalignment (Smith, Jenkinson et al. 2006). Briefly, the main steps were a) non-linear alignment of FA images to 1x1x1 mm MNI152 standard space, b) creation of the mean FA image and its white matter "skeleton" representing the tracts that are common to all subjects, c) projection of individual FA maps onto the image skeleton. The mean FA skeleton threshold was 0.2. We performed voxel-wise statistical analysis on the skeleton, with statistical tests as described below.

Statistical analysis

Statistical analysis for ROI measurements were performed using SPSS for Mac (ver. 23.0, SPSS Inc., Chicago, IL, USA). Mean and standard deviation values were calculated for each variable. All data were tested for normality using Shapiro-Wilk test. For data that were normally distributed, we used the paired-sample t-test, with significance taken to be $p<0.05$. For data that were not normally distributed, group differences were analyzed by Wilcoxon rank t-test, with a threshold for significance of $p<0.05$. To confirm our results, MANOVA was performed on the significant results correcting for age and gender as covariant.
The ROI measures were used to explore the relationship of FA and MD with disease severity (using ALSRFRS-R) and disease duration for each patient over time using a Pearson correlation.

TBSS analysis used a Two-Sample Paired T-test design to detect changes between two time-points from the same group of patients. To correct for multiple comparisons across space, we employed permutation testing (5000 permutations) and threshold-free cluster enhancement (TFCE; (Smith and Nichols 2009)). We consider results to be significant at a fully corrected $p < 0.05$.

Results

Subjects

The clinical features of the individual subjects are shown in Table 6.2. Table 6.3 summarizes the clinical data. There were more males than females (16:7). The timing of the first scan ranged from 3 to 112 months from date of onset (mean 27.6 months). Three patients had a longer disease duration (patient 4: 65 months, patient 7: 68 months; and patient 16: 112 months). In the interval between the first and second scans there was a significant decline in the ALSFRS-R score (p= 0.03) and also in ACE-III score (p=0.02) showing clinical progression of the disease over the 6-month interval.

Table 6.2: Clinical features of ALS patients.

Subjects	Age	Handedness	El Escorial category	Site of Onset*	Riluzole	Disease duration (months)**
1	57	Right	Definite	RLL	Y	15
2	68	Right	Definite	LUL	N	27
3	74	Right	Definite	RUL	N	48
4	63	Left	Definite	LLL	–	65
5	41	Right	Definite	RUL	Y	22
6	65	Right	Definite	LUL	N	17
7	51	Right	Definite	LL (Bilateral)	N	68
8	47	Mixed	Definite	LLL	Y	30
9	57	Right	Definite	LUL	Y	28
10	81	Right	Definite	RLL	Y	19
11	28	Right	Definite	LUL	Y	13
12	54	Right	Definite	LUL	N	2.8
13	66	Right	Definite	RLL	Y	6
14	63	Right	Definite	RUL	N	8
15	60	Right	Definite	LLL	–	31
16	71	Right	Definite	RUL	N	112
17	52	Right	Definite	LUL	Y	11
18	68	Right	Definite	LLL	N	14
19	60	Right	Definite	Bulbar	N	26
20	53	Right	Definite	LL	Y	27
21	59	Left	Definite	RLL	N	11
22	60	Left	Definite	LLL	N	24
23	71	Right	Definite	Bulbar	N	16

*RUL: **right upper limb**, RLL: **right lower limb**, LUL: **left upper limb**, LLL: **left lower limb**, LL: **lower limb**

Disease duration **at the time of the first scan

Table 6.3: Summary of clinical data.

	At first scan	At second scan	p value
Number of subjects	23	23	–
Mean age in years (Mean±SD) (range)	59±11 (29–80)	–	–
Gender (male:female)	16:7	–	–
Disease duration in months (Mean±SD)	27.6±24	33.0±24	–
ACE–III (Mean ±SD)	92±4	89±6	0.02
FAB (Mean ±SD)	16±2	15±2	0.24
ALSFRS–R (Mean ±SD)	39±5	38±4	0.03

TBSS

When whole brain analysis was performed using TBSS, there was no significant difference between the first and second scans.

FA and MD changes in motor pathways

The results for the individual patients for each ROI for FA are shown in Figure 6.2. It can be seen that there was only a small change in FA between the two scans. In some patients there was a decrease, and in other patients there was an increase in FA.

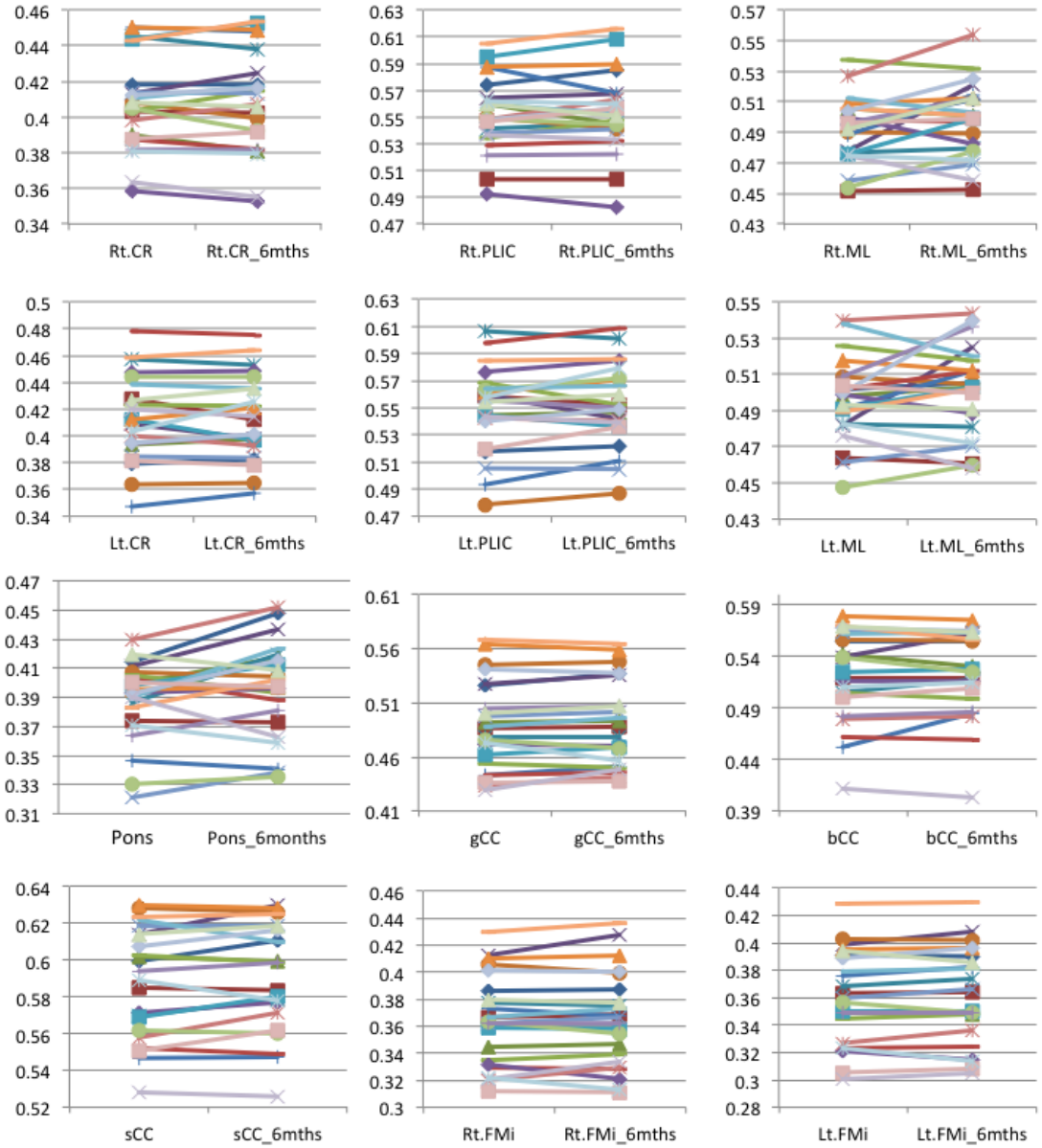

Figure 6.2: Individual FA plot at two time-points in the motor pathways and callosal tracts. In the motor pathways: corona radiata (CR), posterior limb of internal capsule (PLIC), medial leminescus (ML), pons, and the callosal tracts: corpus callosum (CC) at the genu (gCC), body (bCC), and splenium (sCC) and forceps minor (FM).

We also calculated, for each ROI, the mean of the differences between the two scans

$$\Delta FA = FA_1 - FA_2$$

$$\Delta MD = MD_1 - MD_2$$

where the subscript 1 or 2 indicates the first or second scan, respectively. The mean differences are shown in Table 6.4. The absolute values were small.

Table 6.4: Mean of FA and MD differences between two scans.

ROI	Mean (SD) ΔFA	Mean (SD) ΔMD
Rt.CR	0.0000 (0.007)	0.0006 (0.056)
Lt.CR	–0.0005 (0.008)	0.0006 (0.076)
Rt.PLIC	–0.0008 (0.009)	0.0173 (0.083)
Lt.PLIC	–0.0027(0.010)	0.0000 (0.114)
Rt.ML	–0.0076 (0.015)	0.0395 (0.180)
Lt.ML	–0.0049 (0.016)	0.0288 (0.182)
Pons	–0.0077 (0.017)	0.0709 (0.245)
Rt.FMi	–0.0008 (0.006)	–0.0079 (0.068)
Lt.FMi	–0.0013 (0.005)	–0.0208 (0.071)
gCC	–0.0021 (0.007)	–0.0190 (0.104)
bCC	–0.0009 (0.001)	0.0171 (0.152)
sCC	–0.0025 (0.007)	0.0006 (0.092)
Rt.Cg	–0.0004 (0.009)	–0.0165 (0.101)
Lt.Cg	–0.0021 (0.007)	0.0143 (0.115)
Rt.ILF	0.0022 (0.010)	–0.0045 (0.086)
Lt.ILF	–0.0003 (0.010)	–0.0014 (0.096)
Rt.SLF	0.0010 (0.007)	–0.0094 (0.282)
Lt.SLF	–0.0002 (0.009)	0.0048 (0.252)
Rt.ALIC	–0.0043 (0.009)	0.0078 (0.162)
Lt.ALIC	–0.0037 (0.012)	–0.0036 (0.095)
Rt.Hpc	–0.0063 (0.013)	0.0610 (0.167)
Lt.Hpc	–0.0037 (0.014)	–0.0226 (0.174)

Table 6.5 shows the mean FA for the first and second scans. These were compared with a paired t test. There was little difference between the mean values. There was a significant increase in the FA along the CST at the right medial lemniscus (ML) ($p=0.029$) and pons ($p=0.032$). After correcting for age and gender, right ML showed significant changes but pons did not. However, after correcting for multiple comparisons these differences would not be significant. Using MD, there were no significant changes over time at any level of the motor pathways (data not shown).

Table 6.5: Comparison of FA at first and second scans.

ROI	FA Mean (SD)		
	First scan	Second scan	p-value
Callosal Tracts			
Right FMi	0.3639 (0.03)	0.3647 (0.03)	0.586
Left FMi	0.3605 (0.03)	0.3618 (0.03)	0.266
gCC	0.4887 (0.04)	0.4908 (0.04)	0.190
bCC	0.5221 (0.04)	0.5230 (0.04)	0.689
sCC	0.5884 (0.03)	0.5909 (0.03)	0.124
Cortico–spinal Tracts			
Right CR	0.4076 (0.03)	0.4076 (0.03)	0.988
Left CR	0.4129 (0.03)	0.4133 (0.03)	0.801
Right PLIC	0.5517 (0.03)	0.5525 (0.03)	0.700
Left PLIC	0.5493 (0.03)	0.5520 (0.03)	0.220
Right ML	0.4912 (0.02)	0.4988 (0.02)	0.029*
Left ML	0.4958 (0.02)	0.5007 (0.03)	0.163
Pons	0.3879 (0.03)	0.3956 (0.03)	0.032*
Association Fibers			
Right ILF	0.4893 (0.03)	0.4895 (0.03)	0.306
Left ILF	0.5053 (0.03)	0.5031 (0.04)	0.907
Right SLF	0.4293 (0.02)	0.4283 (0.03)	0.506
Left SLF	0.4216 (0.03)	0.4218 (0.03)	0.898
Extra–motor Tracts			
Right ALIC	0.4803 (0.03)	0.4846 (0.03)	0.040*
Left ALIC	0.4732 (0.03)	0.4769 (0.03)	0.169
Right Hpc	0.3339 (0.02)	0.3402 (0.03)	0.038*
Left Hpc	0.3364 (0.03)	0.3401 (0.02)	0.243
Right Cg	0.4042 (0.03)	0.4046 (0.03)	0.832
Left Cg	0.4171 (0.03)	0.4192 (0.03)	0.177

***** Significant at $p<0.05$

FA and MD changes in extra-motor pathways

The values of the FA in individual patients are shown in Figure 6.3 and Figure 6.4. The mean differences between the two scan are shown in Table 4. Table 5 shows the results of a paired t-test comparison of the FA at first and second scans for association tracts and subcortical structures. There was a significant increase in FA in the tracts of the right hippocampus (p=0.038) and the right anterior limb of internal capsule (ALIC) (p=0.04). However, after correction for multiple comparisons these would not be significant. After correcting for age and gender, right ALIC showed significant changes but hippocampus did not.

We observed no changes in MD across 6–months interval between measurements (data not shown).

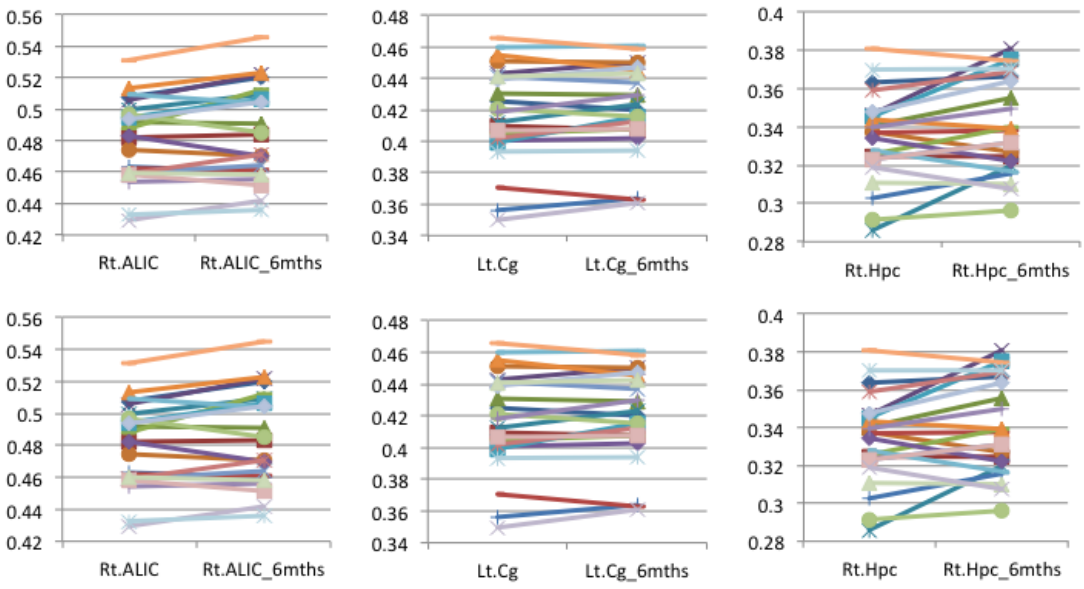

Figure 6.3: The individual FA plot at two time-points in the extra-motor gateways: anterior limb of internal capsule (ALIC), cingulum (Cg) and hippocampal gatways (Hpc).

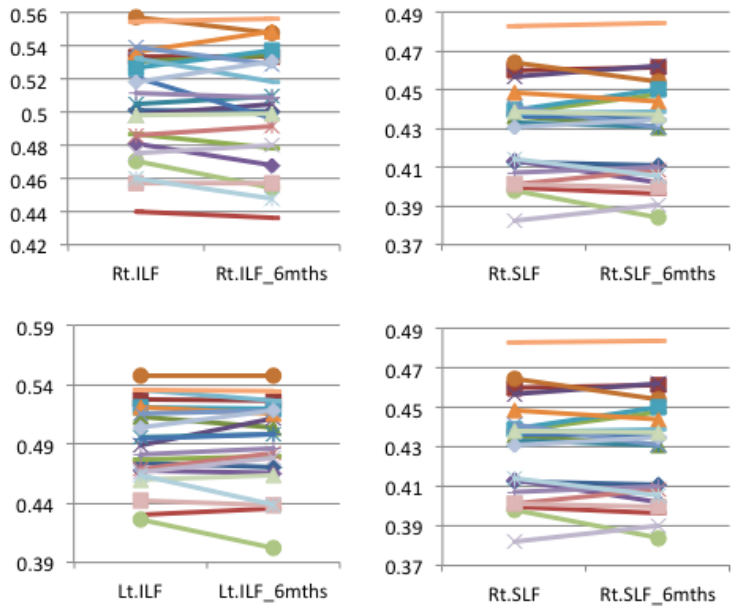

Figure 6.4: The individual FA plot at two time-points in the in the association tracts: superior longitudinal fasciculus (SLF) and inferior longitudinal fasciculus (ILF).

Correlation between DTI and ALSFRS-R

We investigated the correlation of DTI measures with ALSFRS-R and disease duration at both time-points. At the first scan, there was an inverse relationship between FA and ALSFRS-R scores in all ROIs except in the ML and right hippocampus, where the relationship was positive. Table 6.6 shows that significant correlations with ALSFRS-R at baseline were seen in the genu of CC, bilateral forceps minor and bilateral ILF at $p<0.05$. At the time of the second scan, there were no significant correlations (see Table 6.6).

At the first scan, there was a positive correlation between MD and ALSFRS-R scores in all ROIs (Table 6.7). There were significant correlations between MD and ALSFRS-R scores in the left hippocampus and bilateral ALIC. The association fibers showed strong correlations with ALSFRS-R in both ILF and SLF bilaterally. The cingulum on both sides, the forceps minor and the genu of CC also showed significant correlation at $p<0.05$. In the motor pathways, MD at baseline correlated with ALSFRS-R only in the corona radiata ($p<0.05$).

Table 6.6: The correlation of FA with clinical measures.

ROI	ALSFRS-R (Baseline)		ALSFRS-R (6–month)		Disease duration (Baseline)		Disease duration (6– month)	
	Pearson Correlation	p-value (2–tailed)	Pearson Correlation	p-value (2–tailed)	Pearson Correlation	p-value (2–tailed)	Pearson Correlation	p-value (2–tailed)
Rt.CR	–0.270	0.250	0.287	0.282	–0.362	0.090	–0.317	0.141
Lt.CR	–0.236	0.317	0.308	0.246	–0.249	0.253	–0.195	0.372
Rt.ML	0.103	0.667	0.142	0.600	–0.120	0.586	0.047	0.830
Lt.ML	0.008	0.972	0.206	0.444	–0.118	0.592	–0.069	0.755
Rt.PLIC	–0.178	0.453	–0.048	0.860	0.069	0.753	–0.179	0.414
Lt.PLIC	–0.127	0.595	–0.141	0.601	–0.041	0.852	–0.255	0.239
Pons	–0.134	0.574	–0.183	0.497	–0.064	0.772	–0.298	0.168
Rt.FMi	–0.649	0.002*	–0.173	0.521	–0.354	0.098	–0.378	0.076
Lt.FMi	–0.560	0.010*	–0.051	0.851	–0.342	0.110	–0.396	0.061
gCC	–0.542	0.014*	0.185	0.493	–0.203	0.352	–0.180	0.410
bCC	–0.232	0.325	–0.012	0.966	–0.169	0.442	–0.207	0.344
sCC	–0.372	0.106	–0.272	0.308	–0.138	0.530	–0.271	0.211
Rt.ILF	–0.594	0.006*	–0.266	0.319	–0.404	0.056	–0.431	0.040*
Lt.ILF	–0.597	0.005*	–0.088	0.745	–0.333	0.120	–0.435	0.038*
Rt.SLF	–0.399	0.081	0.123	0.650	–0.325	0.130	–0.333	0.120
Lt.SLF	–0.389	0.090	0.271	0.311	–0.245	0.261	–0.315	0.143
Rt.Cg	–0.333	0.152	0.091	0.736	–0.236	0.278	–0.310	0.150
Lt.Cg	–0.373	0.105	–0.063	0.815	–0.196	0.370	–0.178	0.417
Rt.Hpc	0.123	0.605	–0.103	0.704	0.132	0.547	–0.007	0.976
Lt.Hpc	–0.223	0.346	–0.217	0.419	–0.056	0.799	–0.276	0.202
Rt.ALIC	–0.349	0.132	0.130	0.632	–0.596	0.003*	–0.535	0.009*
Lt.ALIC	–0.381	0.098	0.115	0.671	–0.441	0.035*	–0.399	0.059

Table 6.7: Correlations of MD with clinical measures

ROI	ALSFRS-R (Baseline)		ALSFRS-R (6–month)		Disease duration (Baseline)		Disease duration (6– month)	
	Pearson Correlation	p-value (2–tailed)	Pearson Correlation	p-value (2–tailed)	Pearson Correlation	p-value (2–tailed)	Pearson Correlation	p-value (2–tailed)
Rt.CR	0.468	0.037*	0.088	0.745	0.477	0.021*	0.455	0.029*
Lt.CR	0.493	0.027*	–0.008	0.977	0.416	0.048*	0.390	0.066
Rt.ML	0.144	0.544	0.241	0.368	0.035	0.873	0.094	0.669
Lt.ML	0.224	0.342	0.202	0.453	0.174	0.428	0.281	0.195
Rt.PLIC	-.301	.198	-.195		-.277	.201	-.407	.054
Lt.PLIC	-.133	.575	-.299	.837	-.252	.247	-.240	.270
pons	0.206	0.384	0.236	0.379	–0.115	0.602	0.130	0.555
Rt.FMi	0.530	0.016*	0.212	0.430	0.351	0.101	0.344	0.109
Lt.FMi	0.651	0.0028*	0.257	0.337	0.474	0.022*	0.474	0.022*
gCC	0.508	0.022*	0.313	0.238	0.266	0.219	0.285	0.188
bCC	0.313	0.179	–0.034	0.901	0.193	0.378	0.308	0.153
sCC	0.362	0.117	0.276	0.301	0.089	0.686	0.214	0.328
Rt.ILF	0.652	0.002*	0.358	0.173	0.445	0.033*	0.520	0.011*
Lt.ILF	0.621	0.003*	0.256	0.338	0.353	0.098	0.438	0.037*
Rt.SLF	0.516	0.020*	0.223	0.406	0.445	0.033*	0.446	0.033*
Lt.SLF	0.623	0.003*	0.059	0.829	0.497	0.016*	0.530	0.009*
Rt.Cg	0.469	0.037*	0.189	0.482	0.295	0.171	0.307	0.154
Lt.Cg	0.582	0.007*	0.232	0.388	0.307	0.154	0.195	0.372
Rt.Hpc	0.310	0.183	0.417	0.108	–0.069	0.754	0.069	0.754
Lt.Hpc	0.478	0.033*	0.553	0.026*	–0.146	0.507	–0.001	0.995
Rt.ALIC	0.551	0.012*	0.310	0.242	0.647	0.001*	0.449	0.031*
Lt.ALIC	0.702	0.001*	0.311	0.241	0.459	0.027*	0.602	0.002*

At the second scan, in motor pathways, ALSFRS-R had positive correlation with MD in all ROIs other than the body of CC and left corona radiata. In the extra-motor pathways, significant correlations were seen only with the right hippocampus ($p<0.05$), (Table 6.7).

DTI correlation with disease duration

At the first scan, FA showed negative correlations with disease duration in all ROIs except the right PLIC and right hippocampus. The only significant correlations were only in left and right ALIC at $p<0.05$ (see Table 6.6). At the second scan, FA had a negative correlation with disease duration in all ROIs except the right PLIC (Table 6.7). Table 6.6 shows the significant correlations in the regions right ALIC and bilateral ILF. A negative correlation means that as disease duration increases there is a decrease in FA.

At the first scan, MD had a positive relationship with disease duration in all ROIs except the hippocampus and pons where it showed a negative correlation (Table 6.7). However, the significant correlations were in the ALIC, corona radiata and SLF and right ILF and left forceps minor. At the second scan, MD had positive correlation except in the right hippocampus. ALIC, ILF and SLF had a significant correlation with disease duration. Left forceps minor and right corona radiate showed a positive correlation with disease duration (Table 6.7). A positive correlation means that as disease duration increases there is an increase in MD.

Correlation between change in FA and change in ALSFRS-R

There was a negative correlation between the change in FA and the change in ALSFRS-R in the splenium of the corpus callosum ($p=0.029$) and the right cingulum ($p=0.009$), Table 6.8. In all but two ROIs (left CR and left Cg) the sign of the Pearson correlation coefficient was negative, indicating an inverse relationship between the change in FA and the change in ALSFRS-R. However, this relationship was not statistically significant.

Table 6.8: Correlation between change in FA and change in ALSFRS-R

ROI	Pearson correlation	p-value (2 tailed)
Rt.sCR	–0.009	0.969
Lt.sCR	0.066	0.764
Rt.PLIC	–0.140	0.523
Lt.PLIC	–0.016	0.942
Rt.ML	–0.362	0.090
Lt.ML	–0.186	0.395
Pons	–0.257	0.237
Rt.FMi	–0.150	0.496
Lt.FMi	–0.330	0.124
gCC	–0.095	0.665
bCC	–0.317	0.140
sCC	–0.454	0.029*
Rt.ILF	–0.188	0.391
Lt.ILF	–0.364	0.088
Rt.SLF	–0.045	0.840
Lt.SLF	–0.072	0.744
Rt.Cg	–0.534	0.009**
Lt.Cg	0.329	0.125
Rt.Hpc	–0.392	0.064
Lt.Hpc	–0.376	0.077
Rt.ALIC	–0.156	0.476
Lt.ALIC	–0.287	0.185

Discussion

There is a need to measure the progression of ALS, for use in prognosis and in clinical trials and to understand disease pathogenesis (Bowser, Turner et al. 2011). There has been interest in the role of ROI studies in DTI of fiber tracts to evaluate progression of ALS (Kassubek, Müller et al. 2014) but the results have been variable. This study was performed to determine the usefulness of DTI of WM tracts in ALS, as a measure of disease progression over a 6–month interval.

We used DTI to study WM changes on MRI scans performed 6–month apart. Over this time period there was some evidence of clinical progression as seen by decline in clinical scores of motor function and cognition. Over this time interval there was little difference in the mean values for FA and MD. ROI analysis of FA revealed marginally significant changes in the motor pathways. However, it should be noted that these changes would not be significant after correcting for multiple comparisons.

If we do not correct for multiple comparison, then there was evidence of abnormality in the distal portion of the intracranial CST in both ALIC, ML and pons. Microstructural degeneration of the caudal WM tracts confirms previous DTI studies (van der Graaff, de Jong et al. 2009, Prudlo, Bissbort et al. 2012, Sheelakumari, Madhusoodanan et al. 2015). Previous DTI studies using an ROI method showed a bilateral reduction in FA along the CST (Jacob, Finsterbusch et al. 2003) while other studies found changes in CST to be confined to the right hemisphere (Zhang, Schuff et al. 2011). The changes in motor pathways over time are consistent with the known atrophy in ALS (Jacob, Finsterbusch et al. 2003, Keil, Prell et al. 2012).

We found some evidence of progressive changes in the hippocampus. No previous study has identified DTI changes over time in the hippocampus, but there is known to be atrophy of the hippocampus in ALS (Abdulla, Machts et al. 2014). Hippocampal abnormality was found previously in one MRI study of ALS at advanced stages of the disease (Stoppel, Vielhaber et al. 2014). We found no significant changes in FA or MD in whole brain analysis using TBSS.

Therefore, our study therefore shows little change in DTI measures over 6 months in ALS patients, when the results are corrected for multiple comparisons. There have been inconsistent findings in other serial studies (Table 6.9), with some studies finding no change and others finding change in different directions. This study agrees with those who found little change over time. We scanned our patients in a higher field strength (3T) than some previous studies (Table 6). The lack of significant change between the ROIs or with TBSS could be due to the relatively short 6-month interval between scans, as other studies have reported that longer intervals have shown significant changes from baseline (Nickerson, Koski et al. 2009) or to the lack of sufficient progression in our patients over this time period. We note that some of our patients had long survival and so had slowly progressive disease.

Significant change in FA and MD using TBSS again could be due to the relatively short 6–month interval between scans, as other studies have reported that 9 months between scans may benefit to observe significant changes from baseline (Nickerson, Koski et al. 2009).

Table 6.9: Our findings of significant ROIs compared to previous studies.

Results of DTI changes over time in ALS in ROIs									
ROI	Our study (n=23)	Kwan et al (Kwan, Meoded et al. 2013) (n=9)	Steinbach (Steinbach, Loewe et al. 2015) (n=16)	Cardenas–Blanco (Cardenas-Blanco, Machts et al. 2016) (n=34)	Zhang (Zhang, Schuff et al. 2011) (n=17)	Keil (Keil, Prell et al. 2012) (n=15)	Nickerson (Nickerson, Koski et al. 2009) (n=2)	Mitsumoto (Mitsumoto, Ulug et al. 2007) (n=30)	Menke (Menke, Körner et al. 2014) (n = 27)
CR	–	No (FA, MD)	–	–	Yes (↓FA, no MD)	–	–	–	–
ALIC	Yes	–	–	–	Yes (↓FA, no MD)	↑ADC in IC, ExC	–	–	–
ML	Yes	–	–	–	–	–	–	–	–
Pons	Yes	No (FA, MD)	–	–	–	Yes (↓FA)	–	–	–
FMi	–	–	–	–	–	–	–	–	–
gCC	–	–	–	–	–	–	–	–	–
bCC	–	–	–	–	–	–	–	–	–
sCC	–	–	–	–	–	–	–	–	–
ILF	–	–	–	–	–	–	–	–	–
SLF	–	–	–	–	–	–	–	–	–
Hpc	Yes	–	Yes (CI)	–	–	–	–	–	–
Cg	–	–	–	–	–	–	–	–	–
CST	–	No (FA, MD)	No (CI)	Yes, in ALS vs HC (in ↓FA, ↑RD, no MD) No within ALS group		Yes (↓FA)	Yes (↓FA)	No–PLIC and PreCG.	Yes (↑L1)
Notes	–	N=23 but 9 had longitudinal scans.	• Used connectivity index (CI) • Results more in right side	–	Right superior CST	mesencephalic level CST	↓ intensity PLIC=↓FA	–	↑L1 in the left CST

Yes: **significant results reported**, No: **studied but no significant was found.**

A further possibility is that degeneration of upper motor neurons is an early event in ALS, and that by the time we have performed the scans, degeneration is complete in the tracts linked to the upper motor neurons. In this case, no further change would be observable by brain DTI. The evidence for early damage to upper motor neurons comes from studies showing early changes in cortical excitability (Menon, Kiernan et al. 2015) and our study that showed that upper motor neuron signs appear before lower motor neuron signs as the disease spreads (Devine, Kiernan et al. 2014). This was the explanation favored by Menke et al in their study (Menke, Körner et al. 2014), which found little change in DTI over time.

Another reason for the lack of significant change could be the small sample size. If we did not correct for multiple comparisons, then there were some suggestive results. The necessity for correction for multiple comparison is argued (Rothman 1990). Possibly with increased numbers these would be more significant.

We found changes only in the right hemisphere, which is consistent with previous work by Steinbach et al (Steinbach, Loewe et al. 2015). The role of handedness in ALS is poorly understood. It has been proposed that ALS with upper limb onset is more likely to be concordant with handedness than ALS with lower limb onset with footedness (Turner, Wicks et al. 2011). A recent study from our group has found that handedness influences both the site of onset and the spread of pathology (Devine, Kiernan et al. 2014). This asymmetry in brain pathology has also been found in other diseases such as FTD.

In the present study, we found correlation between change in some DTI measures and disease progression measured by change in the ALSFRS–R in the CC and Cg. This is similar to findings of a previous study (Abhinav, Yeh et al. 2014). A relationship between DTI metrics in motor pathways and disease duration has been documented previously (Steinbach, Loewe et al. 2015). However, the significance of our findings is unclear because some of the correlations were with increasing FA, which is contrary to what is expected, and further work on DTI correlations is required.

We also found that the change in FA over 6–months was negatively correlated with the change in ALSFRS–R over 6–months in all but two regions and was statistically significant in the corpus callosum. Our findings support a recent MRI study that highlighted the degeneration of the corpus callosum in ALS (Bartels, Mertens et al. 2008). The lack of any correlations between FA and disease severity measure (Mitsumoto, Ulug et al. 2007, Iwata, Aoki et al. 2008) or disease duration after correcting for multiple comparisons is in agreement with other studies (Trojsi, Corbo et al. 2013, Grapperon, Verschueren et al. 2014). Previous studies of the consistency and reporducibiuity are given in Chapter 7.

In summary, this study shows little change with DTI over 6 months, but the change that we did observe were in the motor pathways and there was some correlation with clinical progression. Given the heterogeneity of ALS, in terms of the clinical features and progression, it is likely that larger studies are needed to explore this further.

Chapter 7: 12–month follow-up DTI evaluating whole brain and corticospinal tract using TBSS and ROI approaches

Preamble

Results from the conducted systematic review (chapter 4), highlighted the consistent finding of the involvement of corticospinal tract. In the previous chapter (chapter 6), 21 regions of interest were examined from both motor and extra- motor pathways. Based on the results from both studies, in this chapter I will address aim four of this thesis "to investigate the DTI changes of CST over 12 months to determine if there are significant changes over time and whether the rate of change of DTI over 12 months correlates with clinical progression". This will be submitted for publication as a manuscript entitled "Serial DTI studies over twelve months in patients with amyotrophic lateral sclerosis".

Serial DTI studies over 12 months

Introduction

Amyotrophic lateral sclerosis (ALS) is a disease that is characterized by the progressive loss of lower motor neurons (LMN) in the spinal cord and brain stem and of upper motor neurons (UMN) in the motor and premotor cortex (Rafałowska and Dziewulska 1995). The pathology of ALS is accumulation of aggregates of proteins within cells, with the majority of patients having accumulation of the protein TDP-43 (Neumann, Sampathu et al. 2006, Kwong, Neumann et al. 2007). Pathological studies suggest that this protein accumulation gradually spreads to other regions as disease progresses (Brettschneider, Del Tredici et al. 2013). Eventually there is loss of the corticospinal tract and gliosis, which leads to the naming of the disease for the loss of muscle bulk (amyotrophy) and gliosis of the corticospinal tracts (lateral sclerosis) (Schiffer, Cordera et al. 1996).

MRI studies in ALS have shown abnormalities in voxel–based morphometry (VBM) and diffusion weighted imaging (DWI) (Graham, Papadakis et al. 2004, Hong, Lee et al. 2004, Chang, Lomen-Hoerth et al. 2005, Kassubek, Unrath et al. 2005, Bede and Hardiman 2014). Diffusion-weighted imaging is sensitive to water motion; thus, any alterations in the degree of diffusion can reflect alterations in the microscopic environment of the water molecule. The restriction of water diffusion and higher diffusion anisotropy are associated with WM maturation and myelination. Measurements of diffusion can be obtained using diffusion tensor imaging (DTI). The measurement that is most widely used in clinical studies is fractional anisotropy (FA).

DTI changes in can be evaluated using Tract-Based Spatial Statistics (TBSS), first described by Smith et al (Smith, Jenkinson et al. 2006). This is a voxel-wise method which increases the sensitivity and the interpretability of DTI images and aims to solve the drawbacks of simple voxel-wise methods by projecting measurements onto a white matter skeleton.

DTI changes can also be evaluated using region of interest (ROI) studies. This analysis groups anatomically related voxels, which increases the statistical power. ROI analysis can be performed by manual or semi-automated methods (Aribisala, Cox et al. 2013). Manually drawn

ROIs have been considered to have problems with reproducibility, because the technique is operator dependent. In the manually drawn ROI, selected regions typically contain fewer voxels than automated methods. However, manual ROI can easily be used in clinical laboratories.

Cross-sectional DTI studies have consistently shown reduction in FA in the corticospinal tract (CST) in ALS patients compared to controls using either whole brain or regional analyses (Abe, Yamada et al. 2004, Karlsborg, Rosenbaum et al. 2004, Iwata 2007, Sarro, Agosta et al. 2011). In contrast to cross-sectional studies, longitudinal DTI studies of ALS have shown conflicting findings (Blain, Williams et al. 2007, Nickerson, Koski et al. 2009, Ajroud-Driss, Mansour et al. 2012, Keil, Prell et al. 2012, Verstraete, Walhout et al. 2012). Thus, it is yet unclear whether DTI is sensitive to changes in CST over time.

Here we have performed a serial study of patients with ALS, with the aim of determining whether there are changes over time in the white matter tracts, using TBSS, and whether there are changes over time in the CST, using an ROI approach. The second aim was to compare the semi-automated ROI with manual ROI. The final aim was to examine the correlation of change in FA with survival.

Methods

Subjects:

Fifteen ALS patients (mean age 60±13, 10 males and 5 females) were enrolled in this study. They were scanned three times at 6 monthly intervals. All patients had a diagnosis of ALS by the revised El Escorial criteria (Brooks, Miller et al. 2000). Three of the patients had slowly progressive course (survival of more than 60 months from the date of onset). Disease severity was measured using ALSFRS-R, which is used for assessing functional and respiratory decline in ALS patients (Cedarbaum, Stambler et al. 1999). Thirteen healthy controls (mean age 50±16, 6 males and 7 females) were used as control group. This project was approved by the RBWH ethics committee HREC (2008/98). All subjects provided written informed consent. Table 7.1 shows the details of patients and controls.

Patients were grouped into those with normal cognition ALSnon-cog and those with impaired cognition (ALScog), on the basis of performance on the ACE-III with a cut-off point of 88. There were 4 ALScog subjects with median age 63 (range 51-80 years) and 11 ALSnon-cog subjects with median age 59 (range 28 – 73 years).

Table 7.1: Subject details and clinical measures.

		ALS N=15	Controls N=13
	6-months	12-months	
Age (years) Mean (SD)	62±11	60±13*	50±16*
Gender (Male:female)	16:7	10:5#	6:7#
ALSFRS-R Mean (SD)	38±4	38.7±4.5	NA
Disease duration Mean (SD) Median (months)	33.0±24	41.6±26 123-7	NA
Number of patients deceased at end of study		6	0
ACE-III Mean (SD)	89±6	88±7	NA
FAB Mean (SD)	15±2	15±3	NA
* p=0.005, # not significant			

MRI acquisition and pre–processing of HARDI

MRI scans were obtained and pre-processed as described in Chapters 5 and 6.

TBSS analysis

Tract-based spatial statistics (TBSS) was used to perform voxel-wise statistical testing on DTI variables. The alignment of the DT images to a reference space is required. This allows spatially overlapping voxels of different datasets to correspond to the same anatomical structures. In this study we used TBSS to perform comparisons of two or three groups of scans. For the comparison of two groups, DTI whole brain analysis was performed following the standard procedure of voxel–wise cross-subject analysis using TBSS. After correcting for motion artefacts, the resulting individual FA volumes were directly registered to the FMRIB58 template (Smith, Jenkinson et al. 2006, Smith, Johansen-Berg et al. 2007). The mean FA-image was created following registration and thinned to represent the mean FA skeleton using the

FSL TBSS manual. Individual FA volumes were then projected onto this common skeleton (Liu, Spulber et al. 2011). Following these steps, data were fed into voxel-wise cross-subject statistical analysis with different group comparisons, including a paired test. The statistical analyses were performed by employing the voxel-wise general linear model (GLM) and significant clusters were formed by employing the TFCE method to correct for multiple comparisons (Smith and Nichols 2009), implemented in FSL randomise. P-values were determined using 5000 random permutations (Nichols and Holmes 2002). The results are considered significant at $p<0.05$, corrected for multiple comparisons using TFCE.

To analyze differences among three groups, we combined the scans from all three time-points of patients in one group and ran the TBSS steps as above to create FA skeleton of whole group. Before the randomise run, we separated scans for each time-point from the FA skeleton. Next, two time-points were merged to one skeleton. Then, using randomise with multiple permutations and correcting for multiple comparisons using TFCE ($p <0.05$), we ran two different statistical approaches: (a) one sample t-test; by subtracting each time-point from the other one in six contrasts (1 minus 2, 1 minus 3, 2 minus 1, 2 minus 3, 3 minus 1, 3 minus 2) and the output is the difference between time-points; (b) paired sample t-test; comparing two time-points each time, separately, using the contrast as (scan 1 versus scan 2, scan 2 versus scan 3, scan 1 versus scan 3); c) triple t-test; comparing all three time-points; d) two-sample unpaired T-test to compared each time-point with controls. These statistical contrast were performed excluding longer disease duration and again with ALS patients sub-grouped based on their cognitive testing

The final results from TBSS comparisons of ALS patients at each time-point were superimposed using FSL (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/).

Automated ROI analysis methods

For the automated ROI, Advanced Normalization Tools (ANTs) was used on FA data using the methods described in Chapter 6. The datasets were analyzed by one rater (A, R A) who was unaware of the identity of the subject. There were no FA skeleton created using ROI method. We examined the CST at the level of pons, which is shown in Figure 7.1 and the posterior limb of the internal capsule (PLIC) as shown in Figure 7.2. These regions were chosen because

degeneration of the CST is a cardinal feature of ALS. We included the PLIC expecting the highest sensitivity where highest neuronal alignment and no crossing fibers present. The FA values were averaged for each region on both sides.

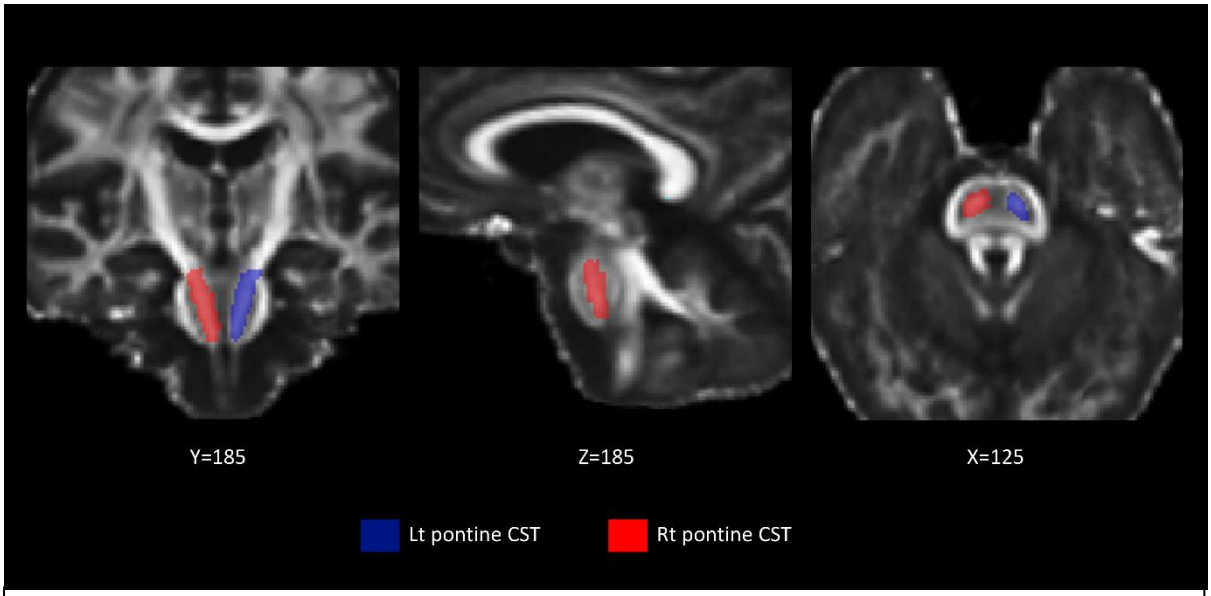

Figure 7.1: Atlas-based ROI of the CST at the pons. This figure illustrates, in 3 planes, the mask applied for the CST in the pons using the FA template with atlas-based ROI. (Left in blue and right in red). With this method, all the voxels from the entire structure are included in the ROI.

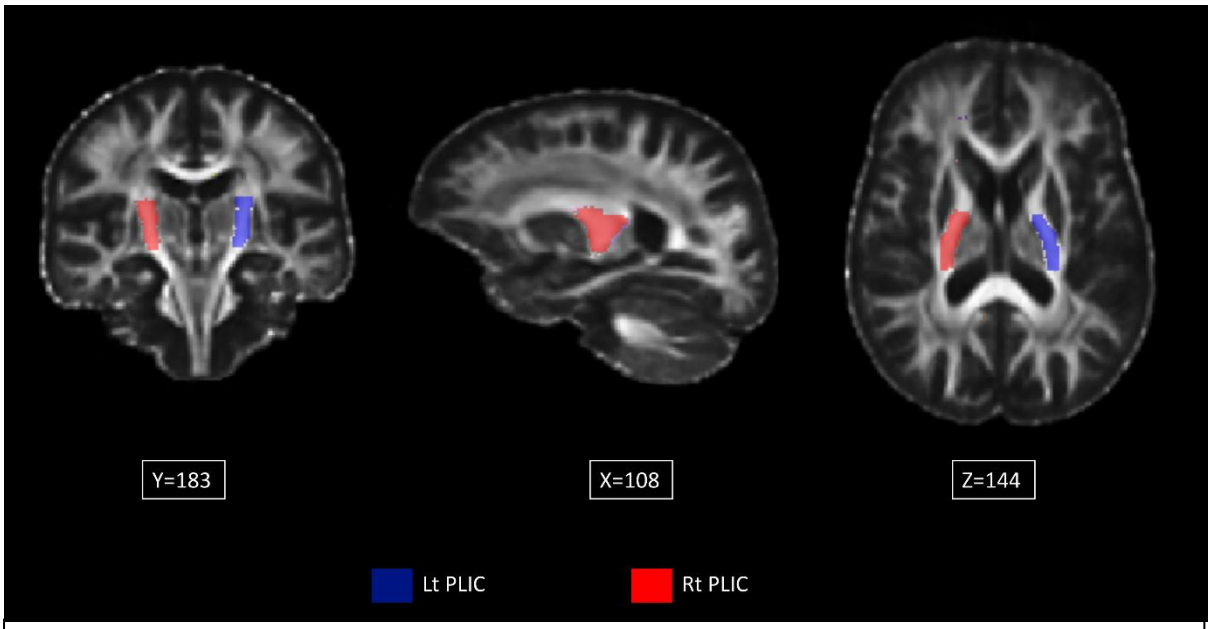

Figure 7.2: Atlas-based ROI of the PLIC. This Figure illustrates, in 3 planes, the mask applied for the PLIC, using the FA template with atlas-based ROI. (left in blue and right in red). With this method, all the voxels from the entire structure are included in the ROI.

Manual ROI methods

Manual ROIs were drawn on the colored FA maps based on anatomical knowledge. One axial slice was selected for each region. To delineate the CST in the pons, the ROI was drawn at the level of brain stem where the pontine crossing tracts (pct) are a straight red line, which is part of the middle cerebral peduncle (mcp). The pontine CST can be seen as two blue-purple regions anterior to the pct (Figure 7.3, left).

For the PLIC, the ROI was drawn on the last axial slice where the genu and splenium of corpus callosum and the fornix are seen in one plane. On colored FA maps, the PLIC appears as a blue-purple region; anterior to it is the green anterior limb of internal capsule (ALIC) and posterior to the PLIC is the retrolenticular part of internal capsule (rLIC), (Figure 7.3, right).

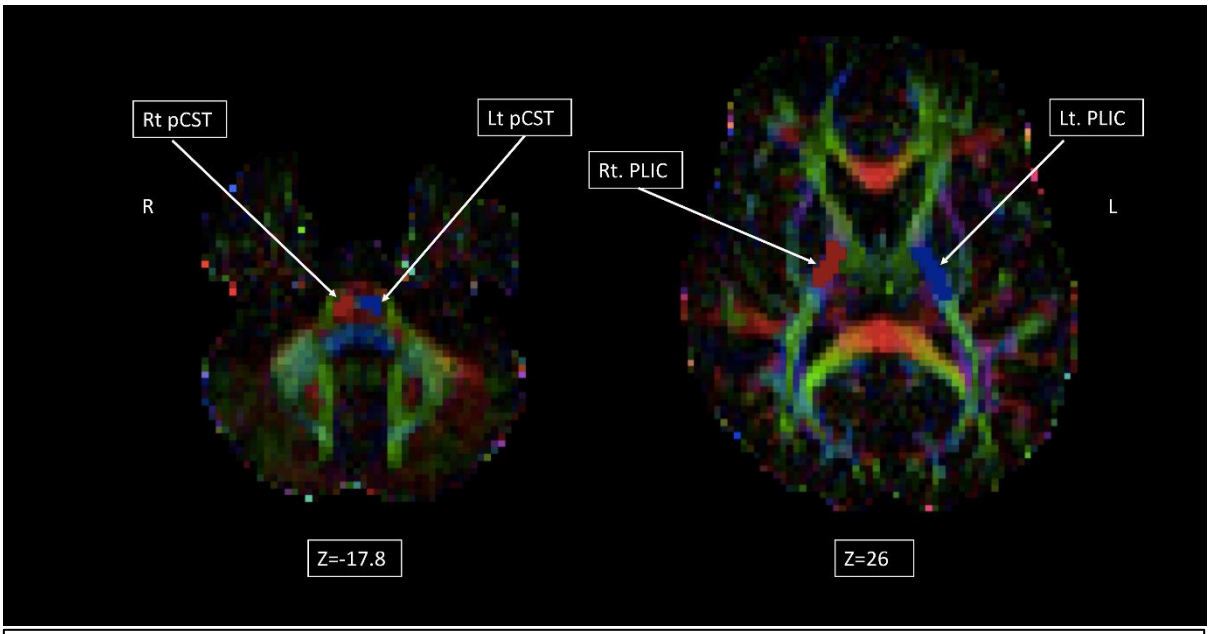

Figure 7.3: Manual ROI of the CST at the pons and PLIC. This Figure illustrates in two different axial planes for individual patient FA maps and the mask applied for the manual ROI for the pontine CST (left) and PLIC (right). With this method, only the voxels from one plane are included in the mask of the ROI.

Statistical analysis

Statistical analyses were performed using SPSS for Mac (Ver. 23.0, SPSS Inc., Chicago, IL, USA). Mean and standard deviation values were calculated for each variable. All data were tested for normality using Shapiro–Wilk test. MANOVA was performed when testing ROIs at three time-points for each region of interest.

We used TBSS to compare the values at three different time-points (Scan 1, Scan 2 and Scan 3). We also repeated this analysis after excluding subjects with long survival, who would be expected to show a lesser change over time. We also compared the scans from ALS subjects at each time-point to controls. Finally we compared the patients with cognitive impairment with those without cognitive impairment at each time-point, and made a comparison of the first scans with the third scans.

To examine whether the rate of loss of FA was related to clinical feature, the FA slope was calculated from raw FA data at three different time-points, for each subject, using MatLab

(R2014b, The MathWorks Inc., Natick, MA). We then used Pearson correlation test implemented in MatLab to determine whether there was a correlation between the FA slope and survival from the date of onset to death or censoring. The correlation was performed to determine if the rate of change of FA is linked to disease progression. The Spearman's correlation and the Pearson correlation were used to examine nonlinear and the linear relationship, respectively. We then pooled all the FA data from all subjects from all time-points into one regression analysis to examine the overall trend of the relationship between both ROI methods for each region. We examined the relationship between manual ROI and atlas ROI using Spearman's correlation.

The accuracy and reproducibility of ROIs is an important aspect of the quality control. It depends on the operator's ability to draw the same region accurately and consistently. Here, five random patients were selected to repeat the manual ROI in one-month difference from the first measure.

Results

TBSS results

The results of TBSS analysis are shown in Table 7.2. There was no significant difference among the scans of ALS subjects at three time-points when comparing whole ALS group or when excluding patients with longer disease duration.

Table 7.2: Comparisons that were examined with TBSS.

Group1	Group2	Group3	Significance
Comparison of 3 groups			
Time-point 1 (N=15)	Time-point 2 (N=15)	Time-point 3(N=15)	NS
Time-point 1 exclude longer DD (N=12)	Time-point 2 excluding longer DD (N=12)	Time-point 3 excluding longer DD (N=12)	NS
Comparison of 2 groups			
Time-point 1 (N=15)	Controls (N=13)		$p<0.05$
Time-point 2 (N=15)	Controls (N=13)		$p<0.05$
Time-point 3 (N=15)	Controls (N=13)		$p<0.05$
Time-point 1 ALS non-cog (N=11)	Time-point 1 ALS-cog (N=4)		NS
Time-point 2 ALS non-cog (N=10)	Time-point 2 ALS-cog (N=5)		NS
Time-point 3 ALS non-cog (N=10)	Time-point 3 ALS-cog (N=5)		NS
Time-point 1 (N=15)	Time-point 3 (N=15)		NS

However, at each of the three time-points, the overall FA in the ALS scans was reduced compared to that of controls. Figure 7.4 shows the TBSS results of three scans superimposed on the mean subject FA template ($p<0.05$; corrected TFCE). Figure 7.4 shows that the changes in the CST and CC were more widespread at the third scans (shown in green) than in the earlier time-points (shown in red and blue).

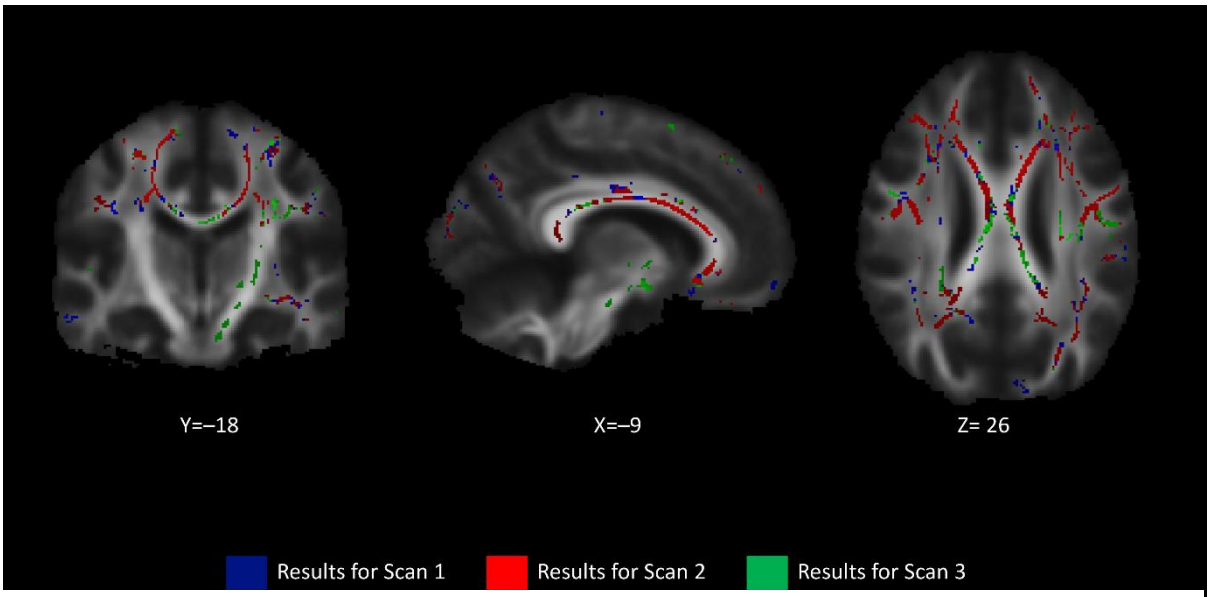

Figure 7.4: TBSS results of three time-points for ALS subjects compared to controls. The differences between ALS and controls are more extensive in the third scan in the CST and corpus callosum.

When subgrouping patients based on their cognitive scores (ACE-II), there were no significant difference between ALS without cognitive impairment and those with cognitive impairment at, at any of the three time-points.

ROI results

FA results at each time-point

Atlas-based ROI

The results of the atlas-based ROI analysis of FA for individual patients at each time-point are shown in Figure 7.5. There was considerable variation among individuals, with some showing increase in FA and some showing a decrease over time.

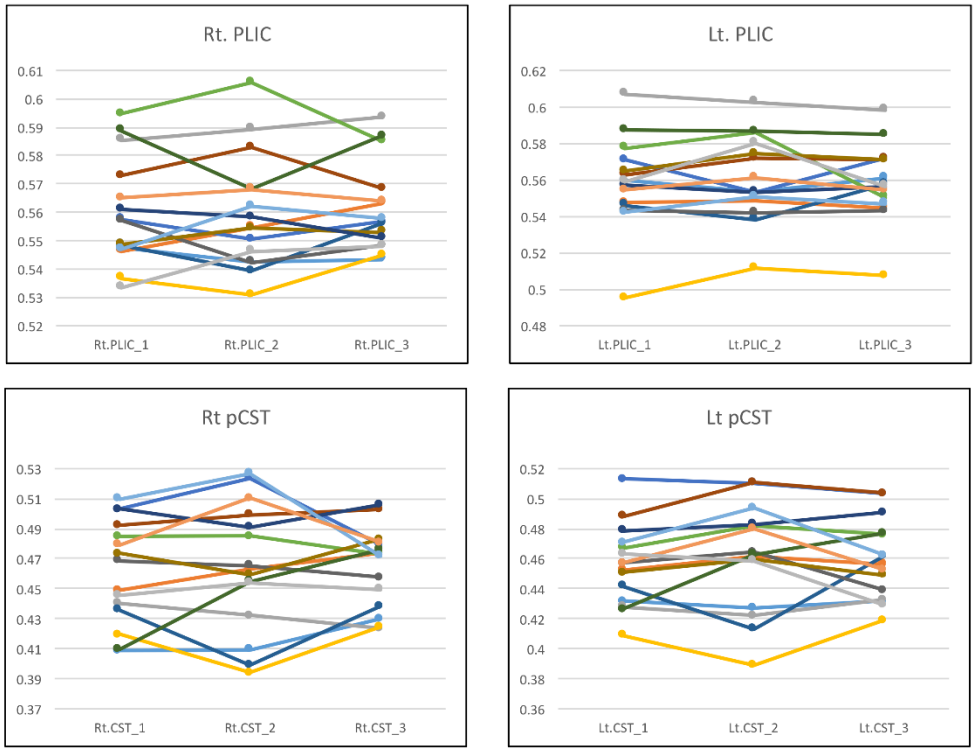

Figure 7.5: Atlas-based ROI FA measurement for individual patients at three time- points in the PLIC and CST in the pons. Using MANOVA there were no significant difference among the mean values at each time-points.

Manual ROI

The results of the manual ROI analysis of FA for individual patients at each Time-point are shown in Figure 7.6. There was considerable variation among individuals.

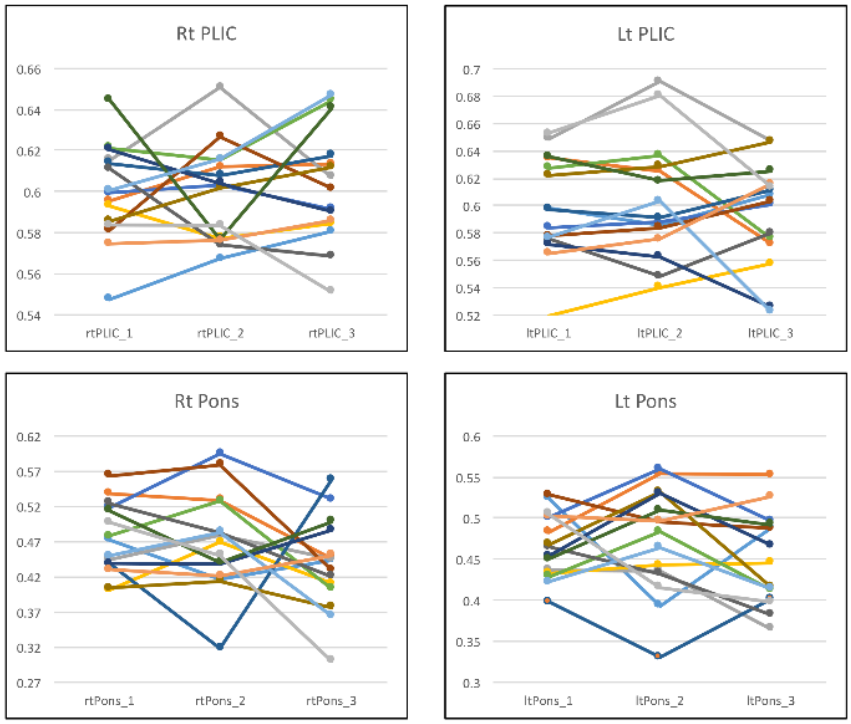

Figure 7.6: Manual ROI FA measurement for individual patients at three time-points for the CST at the pons and PLIC. Using MANOVA there were no significant difference among the mean values at each time-points.

Average FA for each atlas based and manual ROI methods

We performed MANOVA to determine whether the average FA values were significantly different over time (Table 7.3). There was no significant difference among the three time-points for either method. Therefore, we acquired the estimated marginal means for each time-point using both methods to test whether both methods are comparable, Figure 7.7.

Table 7.3: Means (SD) for FA at three Time-points using atlas-based ROI and manual ROI.

Atlas based ROI				
Region	FA1 mean (SD)	FA2 mean (SD)	FA3 mean (SD)	P value (MANOVA)
rt CST	0.462 (0.026)	0.465 (0.042)	0.465 (0.034)	0.832
lt CST	0.456 (0.027)	0.461 (0.035)	0.459 (0.027)	0.475
rt PLIC	0.559 (0.016)	0.560 (0.020_	0.561 (0.019)	0.690
lt PLIC	0.558 (0.021)	0.561 (0.023)	0.558 (0.025)	0.644
Manual ROI				
rt CST	0.474 (0.065)	0.469 (0.070)	0.437 (0.050)	0.226
lt CST	0.466 (0.056)	0.472 (0.064)	0.450 (0.040)	0.517
rt PLIC	0.599 (0.028	0.599 (0.023)	0.602 (0.024)	0.928
lt PLIC	0.599 (0.038)	0.604 (0.044)	0.594 (0.037)	0.782

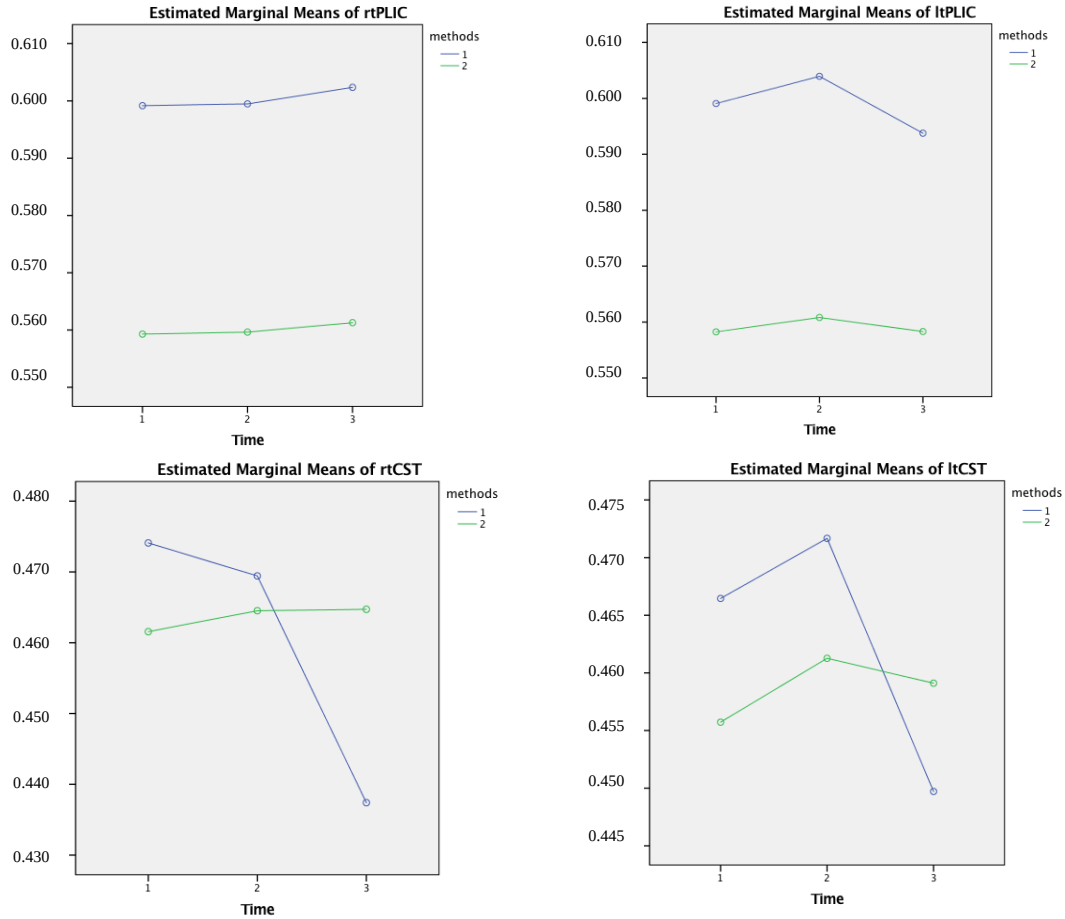

Figure 7.7: Estimated marginal means for every ROI in both methods (mROI in blue (1) and aROI in green (2)). There was better agreement for the PLIC (upper panels) than for the CST in the pons (lower panels).

A summary of the FA at the three time-points for each region, using both methods, can be seen in Figure 7.8. In general, the values for the manual ROI are greater than the atlas based values, and show greater spread.

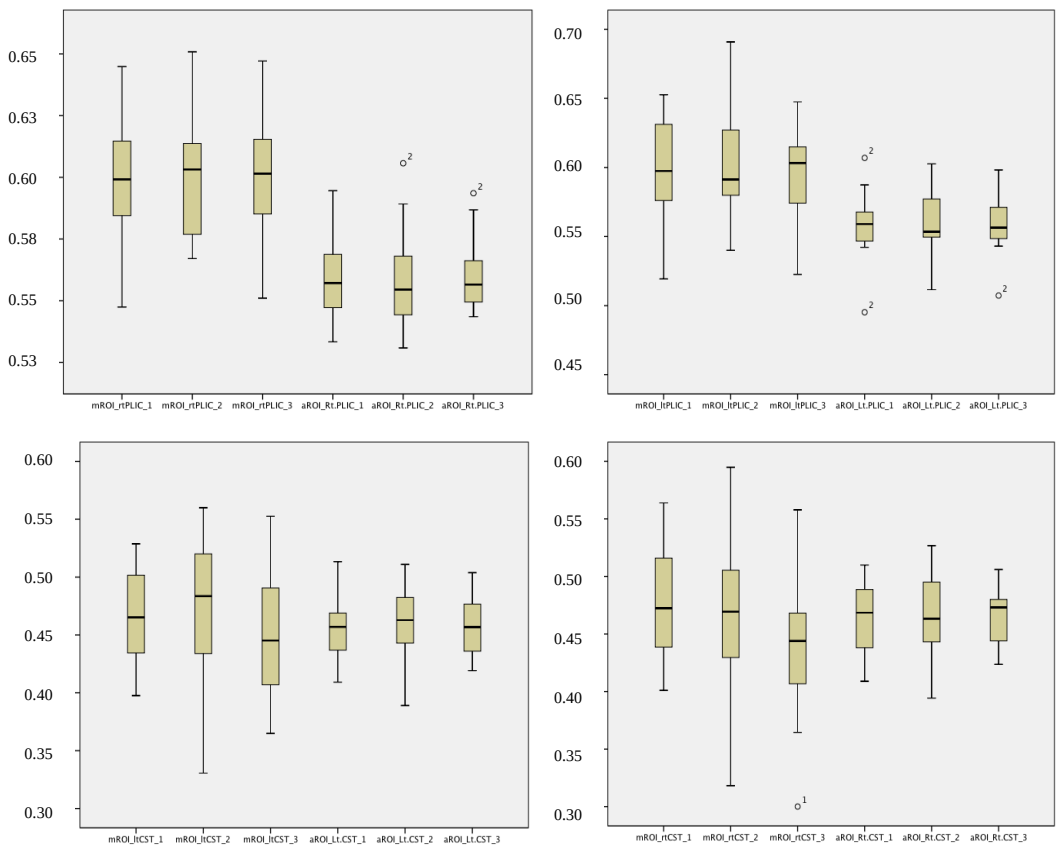

Figure 7.8: The mean and median differences for each ROI in every time-point using both methods.

To compare the FA value between the PLIC and pontine CST, we have combined the 90 FA values for both sides from all time-points and calculated the mean for two methods (Table 7.4). The average FA from the PLIC is significantly greater than that of the CST in the pons, using both methods.

Table 7.4: FA averaged mean of ROIs in both methods.

ROI	Mean FA	SD
mPLIC	0.60	0.001
aPLIC	0.56	0.003
mCST	0.46	0.001
aCST	0.46	0.003

Correlation of mROI with aROI

We performed a linear regression between atlas-based and manual-based measurements for each ROI using all the observations from the three time-points (Figure 7.9). There was better agreement for the values from the PLIC than the values from the pons.

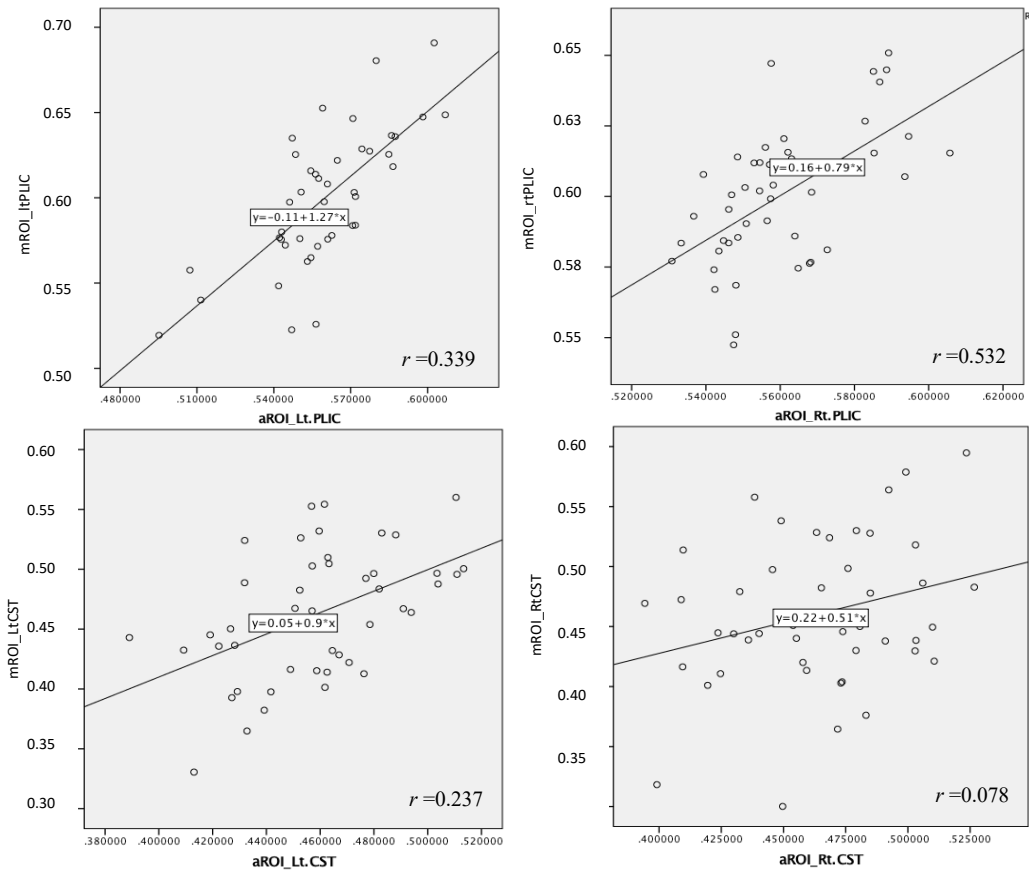

Figure 7.9: Regression results for FA values for all scans in every ROI comparing mROI and aROI.

We also performed a Spearman's correlation of ROI measurements to compare the manual versus atlas-based ROI methods testing if both methods are comparable (Table 7.5). The correlation in the left PLIC was significant (p=0.01, r=0.6)

Table 7.5: Correlation of FA in both methods.

ROI	p value	R value
Rt PLIC	0.069	0.482
Lt PLIC	0.011	0.636
rt CST	0.713	0.104
lt CST	0.296	0.289

Slope of the curve of FA at each time-point

Linear regression was performed on three scans for each subject, to determine the slope of the curve using data from all three time-points. Results are shown in Tables 7.6 and 7.7. A negative value represents a decline in FA over time.

Table 7.6: Slope the FA curve for the atlas-based ROI.

	Slope of the FA curve for the aROI (*10^{-3} per month)			
Subject	rt.CST	Lt.CST	Rt.PLIC	lt.PLIC
1	0.05719	0.00005	-0.01061	0.00361
2	0.06433	0.01122	0.04388	-0.00717
3	-0.03519	0.01126	0.01758	-0.01854
4	0.01601	0.02797	0.02182	0.03035
5	-0.06136	-0.02240	-0.00024	0.00748
6	-0.02899	0.02037	-0.02489	-0.06796
7	0.00915	0.05567	0.02065	0.03082
8	0.02798	0.04801	-0.00460	0.02487
9	-0.02847	-0.04821	-0.02336	0.00051
10	0.02664	-0.00628	0.00911	0.01233
11	0.00754	0.03414	-0.02787	-0.00180
12	0.18502	0.14051	-0.00446	-0.00695
13	-0.11025	-0.03194	0.02233	0.00925
14	-0.00255	-0.01735	-0.00325	-0.00163
15	0.00829	-0.07877	0.03232	-0.00936

Table 7.7: Slope of the FA curve in manually drawn ROI.

	Slope of the FA curve for the mROI (*10^{-3} per month)			
Subject	Rt CST	Lt CST	Rt PLIC	Lt PLIC
1	-0.07528	-0.09136	0.08952	-0.00087
2	-0.24096	0.18076	0.04642	0.00523
3	-0.00532	-0.15835	-0.02525	0.00008
4	0.01916	0.03263	-0.02132	-0.00318
5	0.00681	-0.02550	-0.01938	-0.00121
6	-0.19115	-0.04689	0.05721	0.00395
7	0.32633	0.01437	0.00954	-0.00115
8	-0.30329	-0.11183	0.07035	-0.00211
9	-0.27625	-0.22039	-0.11235	-0.00037
10	-0.06941	-0.13876	0.05965	-0.00175
11	0.13134	0.03616	-0.08274	0.00416
12	-0.04049	0.11661	-0.00965	0.00094
13	-0.24392	-0.03962	0.11939	0.00450
14	0.05890	0.06808	0.03191	-0.00424
15	-0.45347	-0.23641	-0.07563	0.00277

Figure 7.10 illustrates a summary of the results of the slope of the curve for change in FA. There was greater variability of the result obtained using the manual ROI than these obtained with the atlas based ROI.

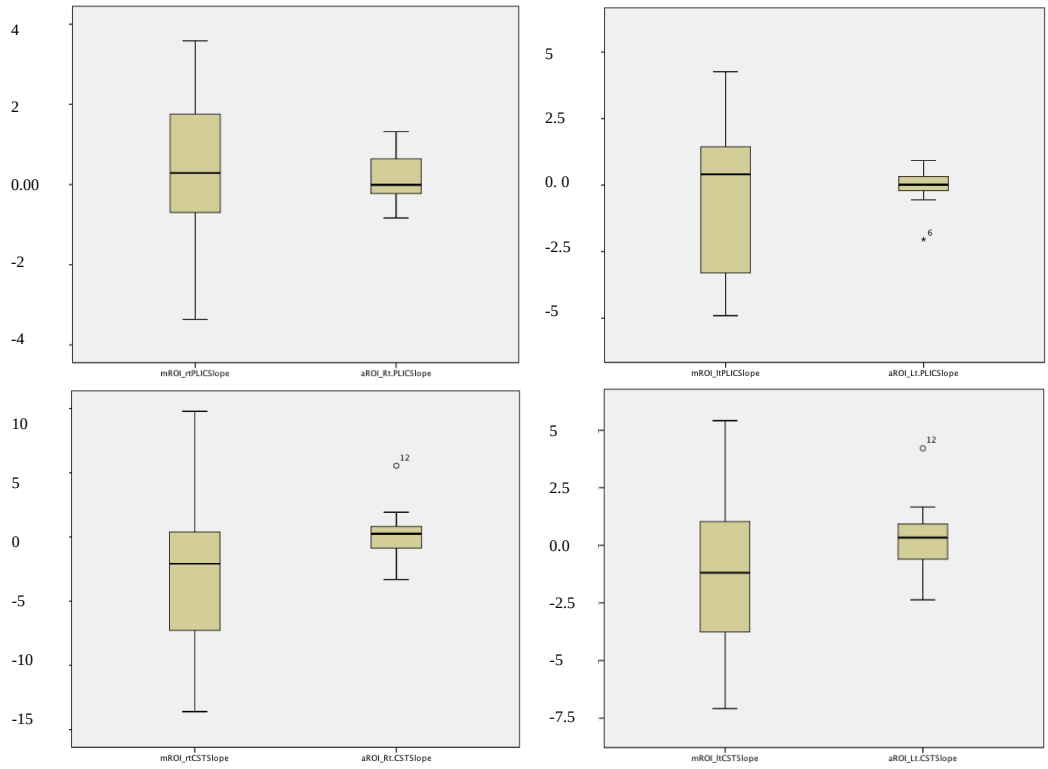

Figure 7.10: FA slope using both ROI analyses. There was a greater range of values for the manual than for the atlas based method.

Correlation of rate of loss of FA with clinical features

To determine if the rate of change of FA is linked to disease progression, the slope of the FA curve obtained with each method was correlated with survival from onset to death or censoring. This was done using both the Spearman's correlation to examine nonlinear relationship (Table 7.8) and the Pearson correlation; for the linear relationship (Table 7.9). A positive correlation indicates that as FA loss increases, survival is shorted and a negative correlation indicates that as loss of FA declines, survival is longer. Using Spearman's correlation, there was a significant negative correlation of survival with the FA of the CST in the pons, using manual ROI.

Table 7.8: FA slope correlation with survival from onset to death or censoring using Spearman's correlation.

	Survival	
	Spearman's correlation	p-value
mROI_rtCST	0.532	0.041*
aROI_rt.CST	0.089	0.752
mROI_ltCST	0.625	0.013*
aROI_Lt.CST	0.107	0.704
mROI_rtPLIC	0.096	0.732
aROI_Rt.PLIC	0.189	0.499
mROI_ltPLIC	-0.029	0.919
aROI_lt.PLIC	0.243	0.383

Table 7.9: FA slope correlation with survival from onset to death or censoring using Pearson correlation.

	Survival	
ROI	Pearson correlation	p-value
mROI_rtCST	0.396	0.144
aROI_rt.CST	0.052	0.854
mROI_ltCST	0.574	0.025*
aROI_Lt.CST	0.156	0.579
mROI_rtPLIC	-0.128	0.650
aROI_Rt.PLIC	0.020	0.945
mROI_ltPLIC	-0.215	0.441
aROI_lt.PLIC	0.181	0.517

Using the same methods, we also investigated whether the rate of loss of FA was predictive of survival from the time of the third scan to death or censoring, Table 7.10 and 7.11. There was a correlation of survival with the FA of the left but not the right CST, using manual ROI.

Table 7.10: FA slope correlation with survival from last scan to death or censoring.

ROI	Pearson Correlation	p-value
mROI_rtCST	0.207	0.460
aROI_rt.CST	0.224	0.423
mROI_ltCST	0.651	0.009**
aROI_Lt.CST	0.361	0.186
mROI_rtPLIC	0.352	0.199
aROI_Rt.PLIC	0.315	0.253
mROI_ltPLIC	0.201	0.473
aROI_lt.PLIC	0.494	0.061

Table 7.11: FA slope correlation with survival from last scan to death or censoring.

ROI	Spearman's rho	p-value
mROI_rtCST	0.201	0.474
aROI_rt.CST	0.376	0.167
mROI_ltCST	0.641	0.010*
aROI_Lt.CST	0.344	0.210
mROI_rtPLIC	0.367	0.178
aROI_Rt.PLIC	0.337	0.220
mROI_ltPLIC	0.240	0.389
aROI_lt.PLIC	0.555	0.032*

Reproducibility of manual ROI

Reproducibility of manual ROI was tested with one month difference between manula ROI placements. The within-subject coefficient of variation (CV) was 0.1–3.6% in the aROI and was 0.007–11% in the right PLIC in the mROI to be. In terms of reliability, the inter-rater and intra-rater variability can be assessed using the intra-class correlation coefficient (ICC) (Koch

2004). This have been reported to be between 17% – 37% (Bonekamp, Nagae-Poetscher et al. 2005). In our study, ICC was between 24% – 38% for the right pons while the left pons was 74% – 85%. In the right PLIC, the ICC was 37% – 54% and the left PLIC was 65% – 79%.

Discussion

ALS is a disease that shows progressive muscle weakness leading to death in 3–5 years. The underlying pathology is death of upper motor neurons, degeneration of the CST, and death of lower motor neurons. In the CST in the cord there is eventual gliosis. To measure these processes during life is difficult. DTI is a measure of fiber tracts and in this study we have examined changes in DTI measures, with the hypothesis that there would be degeneration over time and that this would be seen as loss of FA. We used TBSS for an overview of all the fiber tracts and ROI of the PLIC and CST to determine whether MRI can show changes in these motor pathways that are known to degenerate in ALS. We also investigated whether the rate of change of FA in the motor pathways was related to survival.

We have examined patients at three different time-points, 6 months apart. With TBSS there were no significant differences among the groups of scans at each time-point. Compared to controls, there were significant differences in the ALS patients at each time-point, and these were more extensive on the third scan than on the earlier scans. In the scans at the third time-point, the changes were more extensive in the CST and the commissural fibers. Thus, with TBSS there was some evidence of change over time in motor pathways.

We also used ROI analysis to test our hypothesis that there would be changes in the FA in the CST at pons and PLIC. The FA of different ROIs was calculated using atlas–based and manual methods. There were no significant differences in the average FA between time-point. However, there was considerable variation among patients, and some patients showed loss of FA overtime while others did not. This difference between TBSS and ROI has been noted previously, in a study that found that ROI methods showed less significant results than TBSS methods (Kasper, Schuster et al. 2014).

There are possible reasons why there would be little change in FA over time. Firstly, ALS patients are heterogeneous in their survival, with some patients showing prolonged survival

(Turner, Parton et al. 2003, Mateen, Carone et al. 2010, Pupillo, Messina et al. 2014). In our cohort, some of the patients had long survival and slowly progressive disease, and therefore would not be expected to show rapid change in fiber tracts.

Next, it is likely that after a fiber tract has progressively degenerated, there will be no further change in DTI. Some of our patients were later in the stage of the disease, and would be likely to have reached a state when the fiber tracts had been lost. If loss of fiber tracts leads to decline in FA, then the decline would be expected to cease at this point and therefore were incapable of further pathological change. After there has been loss of fibers, there is a subsequent phase of reactive gliosis, for which there is unlikely to be any further change in FA (Schiffer, Attanasio et al. 1994).

However, it is also possible that, even early in disease, the degeneration in the motor tracts is substantially complete. In a study by Menke, similar results were found with little change in DTI over time (Menke, Körner et al. 2014). These authors suggested that this indicates that upper motor neuron degeneration occurs early in the disease which is clinically evident. Our previous study of clinical signs in ALS show that UMN degeneration also occurs early (Devine, Kiernan et al. 2014), and neurophysiological studies of excitability also suggest that upper motor neuron degeneration occurs early (Vucic 2011). This could mean that changes in FA occur early in disease.

However, even though there was no significant change in DTI in the whole group, there was change in some patients and some correlation of rate of change in FA with survival (Vucic 2011). This could suggest that in those patients in whom change in FA is seen, this is a marker of progression.

In the current study we compared the value for atlas based and manual ROI. The manual methods gave a wider spread of values of FA. Our results would suggest that better results are obtained from the PLIC than the CST in the pons. This is in alignment with previous work that targeted FA measurement at the PLIC and suggested that PLIC may provide prognostic information (Menke, Abraham et al. 2012). Linear regression showed a significant relationship between the manual and atlas based methods for the PLIC but not the pons. This can be explained by the WM pathways crossing at this level which can lead to a substantial drop of

FA in the map which may affect the result reliability. Using Spearman's correlation, there was a significant correlation of the values for the left PLIC and the correlation approached significance for the right PLIC. Manual ROI has been criticized for being operator dependent and thus less reliable than atlas based methods (Prokscha, Guo et al. 2014). However, we found that both methods are still comparable which is in support of previous studies (Mori, Oishi et al. 2008, Oishi, Faria et al. 2009). Rigorous published work have compared different automated methods (Babalola, Patenaude et al. 2009) and test-retest reproducibility (Morey, Selgrade et al. 2010) in segmentation approaches and suggests that atlas-based methods are the best (Aribisala, Cox et al. 2013).

Limitations of our study are the small sample size, and the duration between scans during the study. We did not perform serial MRI in controls, so we cannot comment on whether the changes that we see on repeated testing differ from those of controls. Previous studies have reported that although inter-site DTI measurement variability was found to be quite high (Zhu, Hu et al. 2011), the intra-subject variation was less than 1% in different brain regions using DTI (Magnotta, Matsui et al. 2012). Our patients showed variation of more than 1%, therefore changes we observed are unlikely to be caused by measurement error alone. The ICC from the current study are similar to these of published studies except for the differences between the left and right side of the pons. The pons is well recognized to be a difficult site for DTI because of the crossing fibers (Bonekamp, Nagae-Poetscher et al. 2005). We have also noted that there is asymmetry of the changes in ALS.

In conclusion, we found little evidence of progression of ALS using DTI. We found that there were greater changes in TBSS with increasing time and some correlation of change in FA with disease progression, as well as reduced FA in the pons compared to the PLIC. However, the changes were small, and not significant in serial ROI studies. Larger studies, with patients with more rapidly progressive disease are needed to confirm this, but we find little evidence that serial DTI studies will be a good marker for progression of ALS.

Chapter 8: Discussion

This study was concerned with the role of MRI in assessing changes in the brain of patients with ALS. It focused particularly on the MRI abnormalities associated with cognitive change in patients with ALS, and the use of serial DTI to monitor disease progression. Up to three MRI scans were performed at 6–month intervals. Patients were screened for cognition and behavioral impairment using ACE-III and FAB.

The overall aim of the thesis was to investigate the use of DTI in studies of disease progression of ALS. The specific aims were;

1. To identify the tracts that are altered in both ALS and FTD by performing a thorough review of the literature on DTI in these diseases.
2. To identify brain regions involved in ALS patients with and without cognitive impairment compared to controls by using WM and GM analyses to investigate the DTI and structural changes.
3. To investigate the WM changes, either in motor or extra-motor pathways in ALS patients, in serial DTI over 6 months. Based on the results of my second aim, I used whole brain and region-of-interest analyses.
4. To explore the DTI changes of CST in ALS patients over 12 months to determine if there are significant changes over time and whether the rate of change of DTI over 12 month correlates with clinical progression. This was performed using whole brain and two ROI analyses.

Systematic Review of DTI in ALS and FTD

To establish the existing evidence of brain microstructural changes in ALS patients with or without cognitive impairment, we first performed a systematic review of the literature. All studies reporting on DTI in the brain in patients with ALS and FTD were included. We found that the most common abnormality shared between ALS and FTD was a reduction in FA in the CST, corpus callosum and SLF.

The corticospinal tract (CST) controls motor function of the limbs and trunk. From the pre- and postcentral cortices, the corticospinal and corticobulbar fibers descend in the internal capsule and the middle portion of the cerebral peduncle to the spinal cord. Pathological studies have identified the degeneration of motor pathways as a cardinal feature of ALS (Cosottini, Giannelli et al. 2010, Rajagopalan, Yue et al. 2012). The abnormality of motor pathways has been confirmed with MRI. There are also some reports of CST abnormality in FTD, based on MRI (Avants, Cook et al. 2010). Such motor abnormalities are not a classical feature of FTD, however some patients with FTD, particularly those with C9orf72 repeat expansion, can have co-existing ALS (Prado, Bicalho et al. 2015).

The CC is the largest fiber bundle that connects the brain's right and left hemispheres. The callosal WM fibers from part of the commissural fibers, playing a role in the motor, sensory and cognitive transfer between hemispheres. The CC has been implicated in human behavior and cognition (Witelson 1989). An autopsy study on patients with ALS has shown pathologic changes in the isthmus and posterior mid-body of CC (Sugiyama, Takao et al. 2013). Resting state fMRI of ALS patients suggested that significant decrease in interhemispheric functional connectivity can be a feature of early disease (Jelsone-Swain, Fling et al. 2010), which is consistent with structural callosal involvement observed with DTI (Filippini, Douaud et al. 2010). Efficient organization of association fibers is essential for optimal cognitive performance (Schmithorst, Wilke et al. 2002, Frye, Hasan et al. 2010).

SLF connects the tempro-parietal regions to the ipsilateral frontal areas (Madhavan, McQueeny et al. 2014)and plays an important role in the language function (Catani and Mesulam 2008). The medial temporal lobes are implicated in memory storage while the frontal lobes mainly serve in executive function and language (Karlsgodt, van Erp et al. 2008, McKinnon, Nica et al. 2008). In DTI studies, SLF have been found to be involved in behavioral changes in ALS (Trojsi, Corbo et al. 2013).

There have been reports of abnormalities of extra-motor pathways in ALS (Sato, Aoki et al. 2010, Barbagallo, Nicoletti et al. 2014, Kasper, Schuster et al. 2014, Menke, Körner et al. 2014, Sarica, Cerasa et al. 2014) and FTD (Avants, Cook et al. 2010,

Whitwell, Avula et al. 2010, Agosta, Scola et al. 2011). The majority of studies involving ALS subjects did not test cognition; it is therefore possible that the extra-motor abnormalities found on DTI in ALS are due to a subset of patients with clinical features of extra-motor disease such as cognitive impairment, which is found in some patients with ALS (Lillo, Savage et al. 2012). As well as cognitive impairment, some ALS patients have other extra-motor features such as sensory disturbance (Pugdahl, Fuglsang-Frederiksen et al. 2007), autonomic disturbance (Baltadzhieva, Gurevich et al. 2005) and cerebellar disturbance (Tan, Devenney et al. 2014). In some patients there is thought to be an ALS plus syndrome, with prominent extra-motor features. These patients are likely to have more widespread pathology than those with pure ALS (McCluskey, Vandriel et al. 2014).

There is also clinical heterogeneity in the site of onset, disease progression and pattern of progression of ALS and FTD which can cause difficulty when comparing studies. However, the consistent finding of the review is that the CST, SLF and callosal tracts show abnormalities in DTI metrics in both ALS and FTD (Rajagopalan, Allexandre et al. 2011, Lillo, Mioshi et al. 2012, Trojsi, Corbo et al. 2013, Cardenas-Blanco, Machts et al. 2014).

Differences between ALS patients with and without cognitive impairment

Our baseline MRIs, which had the largest number of subjects, showed some evidence of differences in brain microstructure and macrostructure between ALS patients with cognitive impairment and those without. These patients were defined on the basis of performance on the ACE-III R , which is a screening tool, rather than with detailed cognitive evaluation.

Using a direct comparison between the ALS patient subgroups in whole brain analysis, there was significance only at $p<0.1$. However, by comparing each group with healthy controls it could be seen that, for patients with cognitive impairment, the changes were more widespread and evident in the frontal lobe. This is consistent with the finding that some patients with ALS also have FTD (Chang, Lomen-Hoerth et al. 2005, Lillo,

Savage et al. 2012). Fronto-subcortical circuits are thought to be involved in determining behavioral impairment (Meier, Charleston et al. 2010). It is not fully understood why some ALS patients progress to FTD or have cognitive dysfunction. Some of the patients with cognitive dysfunction in ALS have C9orf 72 repeat expansions, or other genes that are linked to FTD, but others do not. The explanation for cognitive and extra-motor abnormalities needs further study, and further MRI studies of extra-motor white matter tracts may deepen our understanding of this co-morbidity.

In addition to the microstructural changes mentioned above, we also found microstructural changes in the caudate nucleus in patients with ALS with cognitive impairment compared to those without the cognitive impairment. This has been found by other studies (Mendez, Adams et al. 1989) and is consistent with the known role of the caudate nucleus in cognition (Elamin, Bede et al. 2013). The caudate nuclei are part of the fronto-striatal circuit, serve as the input for the basal ganglia, and receive cerebral cortical and the prefrontal cortex input (Karnath, Himmelbach et al. 2002). It is believed that caudate nucleus serves in executive cognitive functions (Chow and Cummings 1999), and in planning and problem solving (Voelbel, Bates et al. 2006). It has also been correlated with working memory and contributes to classification learning (Seger and Cincotta 2005). Earlier observations found the caudate nucleus to be associated with pathological behavior (Caplan, Schmahmann et al. 1990, Kumral, Evyapan et al. 1999). Abnormalities in the caudate nucleus might result in neurobehavioral deficits (Bhatia and Marsden 1994). This finding of basal ganglia involvement in ALS is new and not part of our usual understanding of ALS. However, there are some studies that report widespread pathology in ALS, including the basal ganglia and some ALS patients have clinical signs suggestive of Parkinsonism (Pupillo, Bianchi et al. 2015). Previous MRI studies have revealed that there is a callosal connection to the caudate nuclei (Huang, Zhang et al. 2005, Josephs, Whitwell et al. 2010, Murray, DeJesus-Hernandez et al. 2011). Understanding the role of this circuit in cognition may be relevant to the understanding of cognitive and behavioral impairment in ALS.

We also found that the WM regions that show changes in ALS are the ones that connect to the GM structures that showed structural changes. The pre- and post-central gyri

showed abnormality in our GM analysis along with the CST on the WM analysis. In addition, the caudate nuclei were involved on GM study while abnormalities of the frontal lobe WM tracts were statistically significant in our WM results. These structures are believed to share a neuronal network (Huang, Zhang et al. 2005, Murray, DeJesus-Hernandez et al. 2011). Studies that examine only WM are limited in that they do not provide the functional specificity of GM region-based analysis. Combining structural and functional methods can increase the anatomical data from the functional data (Kim, Kim et al. 2008).

Serial MRI studies

While all patients were invited to participate in the serial study, only 23 of 30 patients returned for followed up assessment at 6 months. Of these 15 went on to have a follow-up assessment at 12 months. The patients who were unable to return for 12–month follow–up had increasing weakness in muscle movement and swallowing, as it is expected in ALS.

In the group of patients who had baseline and 6–month MRI, we observed little change in brain microstructure over time. Our whole-brain analysis of two scans showed greater changes in the more distal CST in the later serial scans. We found some evidence for involvement of the hippocampus in ALS, as has been reported previously (Abdulla, Machts et al. 2014). In the 3 time-point serial scans, the whole brain voxel-wise analysis showed more CST and corpus callosum involved at later stages of the disease. Our ROI-based method allowed specific hypothesis testing for changes in CST at three time-points at the level of PLIC and pons. Both manual and atlas ROI methods at PLIC and pons found no difference over time. However, the changes with ROI were less significant than those of TBSS (Kasper, Schuster et al. 2014).

Most DTI studies of ALS have been cross-sectional studies at a single time-point, which have focused on abnormalities in the white matter (WM) of the motor system (Carrara, Carapelli et al. 2012, Kwan, Meoded et al. 2013, Müller, Turner et al. 2016). Advanced MR techniques such as DTI and spectroscopy have shown significant reduction in FA

(Sood, Gupta et al. 2010) and NAA/creatine (Govind, Sharma et al. 2012), along CST. The findings of greater changes more distal to CST is in support of previous work suggesting greater pathology in the distal parts of the CST (Toosy, Werring et al. 2003, Sheelakumari, Madhusoodanan et al. 2015). This would be consistent with a process of Wallerian degeneration. The pathology of ALS includes defects in neuronal energy metabolism and mitochondrial function. This is thought to lead to problems with axonal transport of proteins, and could explain the distal degeneration (Millecamps and Julien 2013).

Some other DTI studies have reported the involvement of association tracts and subcortical structures in ALS (Pettit, Bastin et al. 2012, Sharma, Sheriff et al. 2012). Other studies found that ubiquinated protein aggregates are present in the hippocampus and frontal cortex in ALS (Irwin, Lippa et al. 2007) with increased density in ALS with dementia (Wilson, Grace et al. 2001). There are several recent suggestions that hippocampus has its own contribution to the control of behavior (Nadel 1968, Fortin, Agster et al. 2002) and plays a role in the temporal sequencing of events (Bannerman, Rawlins et al. 2004). Abnormality of these functions of the hippocampus could contribute to cognitive changes in ALS.

Studies in structural MRI such as Fluid-attenuated inversion recovery (FLAIR) (Cheung, Gawel et al. 1995) proton-density weighted imaging (Hecht, Fellner et al. 2001), and magnetization transfer imaging (Kato, Matsumura et al. 1997) reported changes in the internal capsule of ALS patients. One study found that reduced FA in the distal CST from PLIC and pons were more evident in ALS subjects than in subjects with primary lateral sclerosis (PLS) (Iwata, Kwan et al. 2011). We know that CST is the signature for ALS pathology but these studies confirm the selectiveness of CST changes in MRI.

Although there was some evidence of change on CST over time, this was neither large nor significant when corrected for multiple comparisons. This is similar to the findings in a previous study (Menke, Körner et al. 2014). This lack of change could be due to small sample size, heterogeneity in ALS clinical presentation, or to the lack of clinical progression in some patients. The clinical heterogeneity could be due to individual

patients having involvement of different brain regions. This variability could lead to difficulty in studying changes in ALS when patients are grouped together. This can be overcome by subgrouping patients based on their cognitive performance.

There have been proponents of the dying back hypothesis, where distal degeneration of motor axons is the first abnormality in ALS (Karlsborg, Rosenbaum et al. 2004). However, modern evidence supports the hypothesis that upper motor neurons are the site of the first pathology in ALS (Ishikawa, Nagura et al. 1993, Vucic 2011, Devine, Pannek et al. 2015) which is supported by our findings in this project. Autopsy studies of ALS clearly show that there is loss of upper and lower motor neurons and degeneration of the corticospinal tracts. The sequence and timing of these events is unclear. Once the corticospinal tract has degenerated, there is a phase of gliosis (Troost, Sillevis Smitt et al. 1992, Schiffer, Cordera et al. 1996), which could explain the variations in FA in serial DTI measurement as further pathology evolves.

When performing serial studies, it is important to know the reproducibility of the test as this allows verifying the longitudinal results. In our longitudinal studies, results of within-subject coefficient of variation (CV), were 0.1–3.6% in the aROI in the right PLIC and 0.007–11% in the mROI. Unfortunately, healthy controls underwent only a single MRI, and we therefore cannot determine the reproducibility of our measures directly. Previous DTI studies of healthy volunteers have evaluated the reproducibility of different DTI metrics in human brain and reported a small variability; FA maps showed coefficient of variation below 2% (Sasaki, Yamada et al. 2008, Pagani, Hirsch et al. 2010, Vollmar, O'Muircheartaigh et al. 2010). However, it has been illustrated that the imaging processing pipeline is important for reproducibility (Vollmar, O'Muircheartaigh et al. 2010). The differences in DTI due to different numbers of diffusion gradients and acquisition parameters are small relative to the variability of FA measures between sessions, which is in accordance with previous systematic evaluation of the reproducibility of FA (Bisdas, Bohning et al. 2008, Vollmar, O'Muircheartaigh et al. 2010). Whole brain analysis has good reproducibility but cannot be sensitive to pathological changes in clinical studies because it is hard to estimate the pathological state of the tissue (Jansen, Kooi et al. 2007, Assaf and Pasternak 2008). The clinically more relevant ROI methods that do not require preprocessing of the data, can show

greater sensitivity to pathological changes, but have lower reproducibility as a result of operator variations from image processing (Vollmar, O'Muircheartaigh et al. 2010). However, in previous studies, DTI exhibited between-subject coefficient of variation (CV) ranging from 1–13% and within-subject CV less than 10% (Liu, Yang et al. 2014). In terms of reliability, the inter-rater and intra-rater variability can be assessed using the intra-class correlation coefficient (ICC) (Koch 2004). This have been reported to be between 17% – 37% (Bonekamp, Nagae-Poetscher et al. 2005). In our study, ICC was between 24% – 38% for the right pons while the left pons was 74% – 85%. The variability can be another issue in the relative lack of change that we observed. The difference in ICC between the right and left pons is difficult to explain, but as noted there are asymmetries in degeneration in ALS.

In addition to the manual ROI, an atlas-based method was used to seek converging evidence of abnormalities in the selected ROIs and to compare the results of both methods. We have explored the agreement between the manual ROI and the atlas-based ROI and found that both methods are comparable. The manual ROI method is still regarded as the gold standard in clinical practice (Babalola, Patenaude et al. 2009). Others have shown that atlas-based ROI is the most successful and time efficient method with less variability compared to other automated methods (Aribisala, Cox et al. 2013) if there were no major pathological or imaging distortion.

DTI provides information about the fiber orientation based on the diffusion of water molecule. WM that appears normal on conventional MRI can be abnormal on DTI (O'Sullivan, Morris et al. 2004). Any changes in FA are thought to be sensitive to WM microstructural changes but not to be specific to pathology (Assaf and Pasternak 2008). MD reflects the diffusion of water in the WM and is increased in cases such as edema (Filippi, Iannucci et al. 2000). In ALS it is known that there is degeneration of fibers, and the changes with DTI are thought to reflect this. However, the pathological basis for changes in FA, the most widely reported measure, are not certain. Some studies have suggested that longitudinal FA changes may be driven by the changes in demyelination rather than axonal damage (Farbota, Bendlin et al. 2012).

Furthermore, in ALS, after the stage of axonal loss there are other changes (Bruijn, Miller et al. 2004). Particularly, in ALS, gliosis occurs secondary to the loss of motor neurons in CST (Schiffer, Cordera et al. 1996). Some ALS cases have reactive astrocytes distribution (Nagy, Kato et al. 1994). However, these astrocytes can form a glial scarring around the cells as part of the healing process. This could result in a more anisotropic diffusion which could explain the increase in FA in some patient in the second scan, as with. When axonal loss occurs due to gliosis, diffusion is closer to being isotropic and thus FA drops.

Limitations and future directions

There are a number of limitations to this study. One limitation is that the number of patients was small. Because ALS is a progressive disease, it is difficult for patients to return for MRI studies as they become weaker. Indeed, there is difficulty in lying flat and still for many subjects with ALS, making an MRI scan a challenge. Of 30 ALS subjects, 8 are deceased and 7 withdrew due to relocation or because they were too advanced in the disease. While this study recruited 30 patients who participated baseline MRI, only 23 returned for the 6–month follow-up, and 15 for the 12–month follow-up.

Another limitation is that we used screening tools to detect cognitive impairment rather than detailed neuropsychological testing. With screening tools, we were not able to detect subtle differences in cognition. However, these simple screening tests have the benefit of being applicable in ALS clinics and enabled us to define two groups of ALS patients (with and without cognitive impairment) who differed on MRI. The screening tools used in this study were the ACE-III and the FAB. Other screening tools that have now been developed for ALS are the Edinburgh cognitive and behavioural screen (ECASS) (Abrahams, Newton et al. 2014) and the ALS cognitive behavioural screen (CBS)(Woolley, York et al. 2010).

For a serial study, 12 months of follow-up is short. However, due to the progressive nature of ALS, after 12 months more severely affected patients are unable to continue. Indeed, in a serial study, the patients who persist are likely to be those with slowly

progressive disease. Some patients have very long survival, but these are not typical of the majority of ALS patients. The other limitation is that some of the baseline scans were performed late after the date of onset or diagnosis and so are not representative of early ALS. Earlier and more frequent follow-up could improve the quality of the study.

A more fundamental limitation of DTI studies is the need for a clear understanding of the pathological and clinical correlates of MRI changes in ALS (Furtula, Johnsen et al. 2013), and the lack of serial pathological studies of changes in white matter tracts in ALS. There are limitations to DTI that are well-acknowledged. The sensitivity to any physiological motion could interfere with DTI results. Long scanning time that is required for DTI may also affect motion suppression, which is difficult to control in patients with ALS. In addition, tensor calculations require the measurements to be acquired along multiple directions which can reduce the amount of information from anatomical structures (Mori and Zhang 2006).

DTI studies can have heterogeneity in imaging acquisition, processing and analysis methods. However, neuroimaging modalities along with other forms of biomarkers show promise in helping to understand the etiology of the disease. Both sensitivity and specificity for imaging in ALS can be improved by combining different MRI techniques (Filippini, Douaud et al. 2010). Earlier, longer and more frequent, with 3-month interval, serial studies with standardized techniques are warranted to prove the validity of DTI in ALS. Choosing a homogenous sample based on a validated ALS staging system has several benefits and subgrouping patients based on cognitive testing may improve the validity of the research conclusions.

Conclusion

In conclusion, the present study results indicate that the motor and extra-motor tracts are both affected in patients with ALS. We found a reduction in the gray matter volume in the fronto-temporal regions and the pre- and post-central gyri. These regions connected with WM tracts that were found to show significant changes on WM analysis. This also confirms the subcortical structures involvement in ALS. Longitudinally, FA revealed marginally significant changes in the motor pathways and

hippocampus. We observe greater changes in whole brain analysis towards distal CST and CC with some correlation with survival. The pathological changes underlying WM abnormalities in ALS may include genetic (Siddique and Deng 1996, Al-Chalabi, Jones et al. 2012) and environmental (Juntas-Morales, Pageot et al. 2014) factors (Al-Chalabi and Hardiman 2013). Studying the presence of factors in combination with DTI is an important area for future research.

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Appendix

INCLUSION CRITERIA:

- ≥ 18 y.o.
- El Escorial &/or Neary criteria
- Adequate descriptions of sample (M/F, age, other neuro dx)
- DTI tractography or ROI for WM tracts (ALS or FTD vs. HC)
- Adequate description of imaging protocol (MR sequence, b-value, diffusion directions, threshold) & stats (mean, SD)
- Measured FA and MD values in specific WM tracts with defined coordinates

QUADAS CRITERIA:

Item		Yes	No	Unclear
1.	Was the spectrum of patients representative of the patients who will receive the test in practice?	( )	( )	( )
2.	Were selection criteria clearly described?	( )	( )	( )
3.	Is the reference standard likely to correctly classify the target condition?	( )	( )	( )
4.	Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?	( )	( )	( )
5.	Did the whole sample or a random selection of the sample, receive verification using a reference standard of diagnosis?	( )	( )	( )
6.	Did patients receive the same reference standard regardless of the index test result?	( )	( )	( )
7.	Was the reference standard independent of the index test (i.e. the index test did not form part of the reference standard)?	( )	( )	( )
8.	Was the execution of the index test described in sufficient detail to permit replication of the test?	( )	( )	( )
9.	Was the execution of the reference standard described in sufficient detail to permit its replication?	( )	( )	( )
10.	Were the index test results interpreted without knowledge of the results of the reference standard?	( )	( )	( )
11.	Were the reference standard results interpreted without knowledge of the results of the index test?	( )	( )	( )
12.	Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?	( )	( )	( )
13.	Were uninterpretable/ intermediate test results reported?	( )	( )	( )
14.	Were withdrawals from the study explained?	( )	( )	( )

Study	Subjects	T	DTI Measures	Sequences & Analysis	Smoothing	Directions	B value	FA Thresh	WM Regions	Extracted Data	QUADAS
Toosy et al (2003)	21 ALS 14 HC	1.5	Averages FA MD	Single-shot SE EPI DispImage (manual ROI) SAS 6.12 PROC MIXED used for stats	Not stated	7 non-collinear directions	Max 700 s/mm2	Not stated	CST at 4 levels (internal capsule, peduncles, pons, pyramids)	• Results all presented in graphs • ↓ trend in FA ○ From peduncles to pyramids (p = 0.0001), but no different in ALS and HC (p = 0.48) ○ Mean FA is lower in ALS compared to HC at IC (p=0.036), CP, pons and pyramids (p=0.038) • ↓ mean FA in ALS than HC at all levels (results given for midpoint 55 y.o.): ○ Lt. IC mean difference in FA = 0.049 (p = 0.032); rt. IC mean difference in FA = 0.058 (p = 0.011) ○ Lt below IC mean difference in FA = 0.052 (p = 0.001); rt. below IC mean difference in FA = 0.042 (p = 0.01) • ↑ trend in MD from IC to pyramids ○ Significant difference in MD between ALS and HC at Left IC only (p = 0.01)	1. Yes 2. No (no exclusion criteria) 3. Yes 4. Yes (duration included in formula) 5. Yes 6. Yes 7. Yes 8. Yes 9. Yes 10. No (El Escorial already known) 11. Yes 12. Yes 13. Unclear **14.** Unclear (no withdrawals)
Abe et al (2004)	7 ALS 11 HC	1.5	FA MD	Single-shot SE EPI dTV software (2-ROI methods) SPM99; Matlab 5.3;	8mm FWHM, then 12mm FWHM Gaussian kernel	6 non-collinear directions	1000 s/mm2	FA = 0.1	CST	• ↓ FA in ALS compared to HC ○ Rt. frontal subgyral WM ↓ FA (p < 0.007) ○ Lt. frontal preCG (p < 0.042) • Tractography corresponded to ↓ FA in average CST • No exact values for FA provided (visual only)	1. Unclear (small numbers) 2. Yes 3. Yes 4. Unclear 5. Yes 6. Yes 7. Yes 8. Yes 9. Yes 10. No (El Escorial already known) 11. Yes 12. Yes 13. Unclear 14. Unclear (no withdrawals)
Karlsborg et al (2004)	8 ALS 11 HC	1.5	FA ADC	SE-DWI-EPI sequence ROI (on T2-WI then aligned to DTI)	Not stated	6 directions	550 s/mm2	Not stated	CST in 3 regions (corona radiate (CR), internal	• Overall ADC of CST higher than in HC (p = 0.04) and overall FA lower than in HC (p = 0.01); (no raw values given) ○ CR ADC = 0.80 ± 0.03 (p = 0.66 vs HC)	1. Unclear (small numbers) 2. Yes 3. Yes 4. Yes (El Escorial applied at time of MRI) 5. Yes

				MRI software not stated; BMDP and Minitab used for stats					capsule (IC), pons)	○ IC ADC = 0.79 ± 0.02 (p = 0.02 vs HC) ○ Pons ADC = 0.79 ± 0.04 (p = 0.06 vs HC) ○ CR FA = 0.44 ± 0.06 (p = 0.48 vs HC) ○ IC FA = 0.59 ± 0.04 (p = 0.06 vs HC) ○ Pons FA = 0.57 ± 0.03 (p = 0.03 vs HC)	6. Yes 7. Yes 8. Unclear (software not stated) 9. Yes 10. No (El Escorial already known) 11. Yes 12. Yes 13. Unclear 14. Unclear (no withdrawals)
Sach et al (2004)	15 ALS 12 HC	1.5	FA	Single-shot stimulated echo acquisition mode (STEAM); not EPI; Diffusion weighting with Stejskal-Tanner spin echo prep SPM99; Matlab 5.3	6mm FWHM	6 directions	750 s/mm2	Not stated	WM pathways from motor (MC) & premotor cortex (preMC)to brainstem (BS)	• ↓ FA in ALS cf. HC: ○ rt PLIC (p < 0.00001) ○ lt PLIC (p = 0.012) ○ rt.CR under PMC (p = 0.03) ○ lt.CR under PMC (p = 0.006) ○ rt.CR under PreMC (p = 0.018) ○ lt.CR under PreMC (p = 0.001) ○ rt pyramidal tract in BS (p = 0.022) ○ lt pyramidal tract in BS (p = 0.042) ○ CC (p < 0.01) ○ rt.Th (p < 0.01)	1. Yes 2. Yes 3. Unclear (not all patients fulfilled El Escorial at the time of MRI, but all later fulfilled the El Escorial at follow-up) 4. Unclear (see #3) 5. Yes 6. Yes 7. Yes 8. Yes 9. Yes 10. No (El Escorial already known) 11. Unclear (6 patients had repeat El Escorial after the MRI scan) 12. Yes 13. Unclear 14. Unclear (no withdrawals)

Borroni et al (2007)	36 FTD (28 fvFTD, 8 tvFTD), 23 HC	1.5 T	FA	VBM , DTI DTI in single subject using (FACT in Brain Visa software)	10 mm	6	1000 s/mm^2	NA	SLF, ILF, IFOF	VBM analysis of GM and WM • fvFTD vs HC: GM atrophy ($p<0.05$) in: • Dorsolateral FC • ant Cg C • Insula • Superior temporal G • Bilateral Th • tvFTD vs HC GM atrophy ($p<0.05$) in: • Middle inferior TG • Temporal pole • Orbitofrontal G • Sup TG. • Superior FG. • DTI analysis of WM ○ fvFTD vs HC ($p<0.05$) • ↓ FA in rt SLF ○ tvFTD vs HC ($p<0.05$) • ↓ FA in bilateral ILF and IFOF • ↓ FA in lt CC, lt SLF Correlation analysis of FA and behavioral and neuropsychological examination • No correlation between FA and demographics • In SLF, -ve correlation between FA and FBI A, FBI B and FBI AB. • Personal neglect (eg, lack of personal hygiene, disorganization in planning and organizing complex activity, impulsivity or poor judgment and utilization behavior were related to ↓ FA in SLF. Multiple regression analysis	1. Yes. 2. Yes (Neary & McKhann refs) 3. Yes. 4. Unclear. 5. Yes. 6. Yes. 7. Yes. 8. Yes. 9. Yes. 10. No (El Escorial known) 11. Yes. 12. Yes. 13. Unclear. 14. Unclear

										• Trail-making Test B scoring have –ve correlation with FA in SLF. • tvFTD correlation were not investigated due to sample size. • The significantly associated FBI sub items independently related to ↓ FA.	

Mitsumoto et al (2007)	43 ALS 9 PMA 6 PLS 6 fALS 29 HC	1.5	Mean FA, Diffusion constant	SSEPI	NA	26	NA	NA	PLIC Pyramidal tract, preCG, preMC	• At Baseline FA and MD (n=64): o No sig in ALS at PLIC o Sig in fALS ($p<0.002$) • Correlation with clinical measures: o No sig with FA or MD with (DD, ALSFRS-R, MMT) • Analysis of changes overtime (n=30): o No sig. changes in imaging markers (from DTI and MRSI) Refer to the original paper for more details of other irrelevant results to current review.	1- Yes 2- Yes 3- Yes 4- Yes 5- Yes 6- Yes 7- Yes 8- No 9- Yes 10- Yes 11- Yes 12- Yes 13- Yes 14- Yes
Iwata et al (2008)	31 ALS 31 HC	1.5	FA	SSEPI ROI	NA	13	1000 s/mm3		CST (CR, IC, CP, basis pontis, medulla oblongata) Extramotor WM (gCC, sCC, superior CP, middle CP, inferior CP, cerebellar WM)	• ↓FA in ALS than HC: o Sig at CR, PLIC, CP, basis pontis and medulla oblongata ($p<0.0001$) o Not sig at the extramotor WM • Correlations with clinical measure: o Reduced FA with higher UMN scores at CR, IC, pyramids of medulla oblongata. o Not sig at extramotor WM o No sig with ALSFRS-R in any ROI • Correlation between FA and CMTCs: o Decreased FA along CST with delayed CMCTs and CTX-BS more detailed results on TMS can be found in the original paper.	1. Yes 2. Yes (only based on El Escorial criteria without exclusion criteria) 3. Yes 4. Unclear 5. Yes 6. Yes 7. Yes 8. No (no reference to the method or detailed description) 9. Yes 10. Yes 11. Yes 12. Yes 13. No 14. No
Matsuo et al (2008)	FTD=20 HC=17	1.5	FA, ADC	SS-EPI Tractography (PRIDE) ROI	NA	15	1000 s/mm2	NA	Pyramidal tract (CP), Unci.F,ILF, cc, Arc.F,	mFA measure in FTD and HC: o ↓ FA in association fibers (Arc.F, UnciF, ILF) in FTD than HC ($p<0.01$) o ↓ FA lower in FTD than HC in gCC ($p<0.01$) while slightly decreased in splenium ($p<0.05$).	1. Yes 2. Yes 3. Yes 4. Unclear 5. Yes 6. Yes

										○ No sig difference in pyramidal tracts. ○ No sig difference in between FTD subgroups (early vs advanced) except in lt.UnciF ($p<0.05$) Correlation between FA and neuropsychological scores Sig correlated in lt.UnciF ($p<0.05$)	7. Yes 8. Yes 9. Yes 10. Unclear (diagnosis of FTD already known) 11. Unclear 12. Yes 13. Unclear 14. Yes (no withdrawals)
Zhang et al (2009)	18 FTD 19 HC	4	FA, DR, DA	GRAPPA, Dual SE, EPI Volume-one and dTV software (TOI, Voxel-wise Analysis)	FWHM, 4 mm	6	800 s/mm2	FA = 0.18	CC, Cg, Unc.F Brain CST	FA: ○ Lt.d.Cg, lt.Unc, lt.a.Cg ($p<0.0001$), rt.Unc ($p=0.005$), rt.d.Cg ($p=0.003$), a.CC ($p=0.0002$), rt.a.Cg ($p=0.005$) RD: ○ lt.p.Cg ($p=0.008$), rt.p.Cg ($p=0.01$), lt. and rt.a.Cg ($p<0.0001$), a.CC ($p=0.0002$), p.CC and rt.CST ($p=0.01$), rt.d.Cg ($p=0.003$), L.d.Cg ($p=0.001$) AD: ○ a.CC ($p<0.0001$), rt.a.Cg ($p= 0.03$), L.d.Cg ($p= 0.008$), R.d.Cg ($p= 0.04$), L.Unc ($p= 0.001$), R.Unc ($p <0.0001$) Find results of VWA and TOI in the original paper.	1. Yes 2. Yes 3. Yes 4. No 5. Yes 6. Yes 7. Yes 8. Yes 9. Yes 10. Yes 11. Yes 12. Unclear 13. Unclear 14. Unclear

Chen et al (2009)	7 FTD 20 HC	1.5	FA, MD	SSPE-EPI ROI analysis (on b=0 then transferred to MD and FA images)	NA	25	1000 s/mm2	NA	Temporal WM, gCC, sCC, medial SC periventricular area (PV)	• FA o Lt.Temporal ($p<0.05$) , R.ant.PV ($p<0.05$), bilateral post.PV ($p<0.05$) . • MD: o Bilateral Temporal ($p<0.05$) , Rt.ant.SC ($p<0.05$), bilateral post.PV ($p<0.05$), gCC ($p<0.05$)	1. Yes 2. Yes 3. Yes 4. Unclear 5. Yes 6. Yes 7. Yes 8. Yes 9. Yes 10. Yes 11. Yes 12. Yes 13. Unclear 14. Unclear
Avants et al (2010)	25 FTD 24 AD 23 HC	3	FA	SSPE-EPI Sparse canonical correlation analysis (SCCA)	FWHM, 2 mm	30	1000 s/mm2	FA>0.2	CST, ILF, IFOF, Unc.F, CC, SLF	• WM significant correlated with cortical thickness: o Bilateral CST, ILF, IFOF, UncF, CC and Rmcmillan o SLF	1. Yes 2. Yes 3. Yes 4. Unclear 5. Yes 6. Yes 7. Yes 8. Yes 9. Yes 10. Unclear 11. Unclear 12. Unclear 13. Unclear 14. Unclear
Filippini et al (2010)	24 ALS 24 HC	3	FA, MD, AD, RD	Whole brain DWI SE TBSS VBM	NA	60	1000 s/mm^3	NA	CC, CST	FA between ALS vs HC ($p<0.05$ corrected): o Within CC o Bilateral WM tracts from central CC to PMC and preMC. o Rostral CST o Weaker difference in CST at BS ($p<0.05$ uncorrected) ↓ FA correlated clinical score in ALS: o ↑ **UMN score** Bilateral CST ($p<0.05$ corrected) but not in CC o ↓ **ALSFRS-R** weaker correlation ($p<0.05$ uncorrected) in CC.	1. Yes 2. No 3. Yes 4. Yes 5. Yes 6. Yes 7. Yes 8. Yes (provided online) 9. Yes 10. No (El Escorial already known) 11. Unclear

										○ ↑ **DD** Bilateral CST (p<0.05 corrected) ↑ RD correlated clinical score in ALS ○ In CC and bilateral WM connecting PMC and preMC (p<0.05 corrected) No significance found in mean or axial diffusivity. ↓ GM volumetric atrophy corresponding with FA and RD: ○ PMC, preMC, supplementary MC, Cg cortex, TL Post hoc combining measure: ○ FA correlated with GM differences p= 0.64, $p < 10^{-4}$ RD correlated with GM differences GM: p=0.61, $p < 10^{-4}$	12. Yes 13. Yes (uncorrected data reported in the result but not shown) 14. Yes (no withdrawals)
Whitwell et al (2010)	16 FTD 4 SMD 7 NFA 19 HC	3	FA, RD, AD	SSEP FLAIR MPRAGE SENSE (ROI-analysis DtiStudio-software)	FWHM, 8 mm	21	NA	0.05	CC, Cg, Unc.F, ILF, SLF, CST, AC	• DTI changes in WM in bvFTD (p<0.05 corrected): ○ ↓ FA and ↑RD bilateral Unc.F, AC. ant. SLF, ant. ILF with altered RD in gCC and post. ILF ○ ↑FA in CST Refer to original paper for details of other groups' results and detail FA and RD values.	1. Yes 2. No 3. Yes 4. Unclear 5. Yes 6. Yes 7. Yes 8. Yes 9. Yes 10. Yes 11. Yes 12. Yes 13. Unclear 14. Unclear
Agosta et al (2011)	33 FTLD (bvFTD= 13) HCbvFTD=25 PPA=20 (NFA=9, semantic	3	FA, MD, RD, AD	Pulsed-gradient SE-EPI. WM hyperintensities (WMHs) TBSS. VBM; GM ROI	8-mm fullwidth at half-maximum (FWHM)	32	1000 s/mm2	FA=0.2	WM tracts: CC, Cg, Unci.F, IFOF, ILF, paraHpc,	• **bvFTD vs HC (**p<0.05, corrected**)** ○ ↓ FA in CC, Cg, CR, External Capsule (ExC), IC, subcortical WM near frontal cortex (FC), parietal cortex (PC), temporal, occipital WM, Fx, cerebellar peduncle (CP).	1. Yes 2. Unclear (selection criteria clear for HC but not for FTLD) 3. Yes 4. Unclear 5. Yes 6. Yes

	=7, logopenic=4) HCPPA=27								SLF, CST, Fx GM regions: Cg C, FC, preMC, FG, PL, temporal gyri (TG), temporal pole, OC, OFC, white	○ ↑ MD in CC, Fx, bilateral EC, frontal and temporal WM. Bilateral superior and inferior parietal WM ○ ↑ RD similar tracts to the ↓ FA except cerebellum. ○ ↑ AD in CC, bilateral orbital and dorsolateral frontal WM, Fx, rtCR, ExC, ant.Temporal WM. • NFA vs HCPPA ○ ↓ FA in bilateral (CC, Cg, Fx) but was decreased only in the left hemisphere in (CR, ExC, inferior and dorsolateral frontal WM, temporal and occipital WM. ○ ↑ MD in CC, ltCg, ExC, ltCR, lt.orbitofrontal and temporal WM. ○ ↑ RD similar to the regions with ↓ FA except the occipital WM. ○ ↑ AD in lt.ant dorsolateral frontal WM and lt.inf parietal WM. • Semantic vs HCPPA ○ ↓ FA in antCC, lt.CR, ltExc, lt. orbitofrontal WM, lt dorsolateral frontal WM, bilateral ant. Temporal WM ○ ↑ AD, RD and MD were found in CC, Cg, CR, ExC and IC, bilateral orbitofrontal and temporal WM, BS and cerebellum • Logopenic vs HCPPA ($p<0.05$ uncorrected) ○ ↓ FA in bilateral (CC, Cg, Fx) but was decreased only in the left hemisphere in (CR, ExC, inferior and dorsolateral frontal WM, temporal and occipital WM. ○ ↑ MD in CC, ltExC, orbital dorsolateral frontal, inferior pareital and ant.temporal WM. ○ ↑ RD in lt. antCC, lt.Cg, lt. orbital and dorsolateral frontal WM, lt inferior parietal WM.	7. Yes 8. Yes 9. Yes 10. Unclear 11. Yes 12. Yes 13. Yes (in supplementary material) 14. Yes (no withdrawals)

										○ ↓ FA in lt.ant CC, lt. ant and post. Cg, ltCR, lt Exc, bilateral orbitofrontal and left inferior frontal WM, lt temporal and lt. inferior parietal WM. ○ ↑ AD in CC, lt.Cg, Fx, lt Temporal and inferior parietal WM	
Hornberger et al (2011)	14 bvFTD 15 AD 18 HC	3	FA,	TBSS	FWHM, 8 mm	32	1000 s/mm2	NA	Unc.F, CF, FMF, Cg, FM, CC	• **DTI ↓ FA** (p<0.001, uncorrected) **in:** ○ Unc.F ○ CF ○ FMF • VBM ↓GM in bvFTD vs HC ○ a.Cg ○ OF ○ Insula ○ a.TL • Correlation: ○ FA of UncF and Hayling task, -ve. ○ FA of FM and Cg ○ FA of UncF and Neuropsychiatric inventory disinhibition= -ve	1. Yes 2. Yes 3. Yes 4. Unclear 5. Yes 6. Yes 7. Yes 8. Yes 9. Yes 10. Yes 11. Unclear 12. Yes 13. Unclear 14. Unclear
Sarro et al (2011)	16 ALS 15 HC	1.5	FA, MD, RD, AD	FLAIR Pulsed-gradient EPI Tractography, Fmri	NA	12	1000 s/mm3	0.4	CC, CST, Cg, SLF, IFOF, ILF, UnciF, Fx	• FLAIR hyperintensities: ○ Bilateral CST; caudal PLIC, CR and ventral BS • DTI ALS vs HC: ○ ↑ MD of CC (p<0.04), CST (right, p=0.001, left, p=0.002), bilateral SLF (p=0.02), rt.Cg (p=0.2), bilateral Unci.F (p=0.02) ○ ↓ FA in CST (right, p=0.001, left, p=0.002) ○ ↑ RD in bilateral CST (p<0.001), bilateral SLF (p<0.03), Unci.F (p=0,3) ○ ↑ AD in rt.SLF (p=0.4), rt.Cg (p=0.3), bilateral Unci.F (right, p=0.03, left, p=0.002) • Regression analysis between DTI metrics and neuropsychological tests: ○ Trail-making test scores correlated with CC (MD and FA), rt. ILF (MD), CST,	1. YES 2. YES 3. YES 4. YES 5. YES 6. YES 7. YES 8. YES 9. YES 10. YES 11. YES 12. YES 13. Unclear (reported only significant data) 14. Yes (excluded 2 patients)

										Cg, IFOF, ILF all bilaterally (FA), rt. Unci.F (FA). o Stroop test scores associated with CC, rt.IFOF, ILF (FA) o Wisconsin Card sorting test correlated with bilateral IFOF and ILF (MD), rt.IFOF (FA). o Phonemic fluency test scores correlated with lt. Cg (FA) o Verbal learning and memory test scores correlated with Fx (FA and MD) o Visual-spatial correlated with Unci.F (FA)	
Tsujimoto et al (2011)	21 ALS 21 HC	3.0	FA MD	Stejskal-Tanner sequence with Single-shot SE EPI dTV-II.SR; Volume-One 1.72; SMP5	8mm isotropic Gaussian kernel	Not stated	1000 s/mm2	Cluster extent threshold = 20 voxels	CST; Frontal lobe areas	• ↓ FA in bilateral CST and frontal lobe gyri; ↑ MD in bilateral CST (all $p < 0.001$; visual no absolute values) • Correlations between FA and increased apathy score on FrSBe: o Rt. mFG, R sub-gyral FL areas, rt. middle FG, R para.Hpc G, rt.ant.CG, lt Th, lt.sup.FG (all $p \leq 0.002$) • Correlations between MD and increased apathy score in FrSBe: o Lt.sup FG, rt.sup.TG (all $p \leq 0.002$); see tables in paper	1. Yes 2. Yes 3. Yes 4. Yes 5. Yes 6. Yes 7. Yes 8. Yes 9. Yes 10. No (El Escorial already known) 11. Yes 12. Yes 13. Unclear 14. Unclear (exclusions not withdrawals)
Zhang et al (2011)	20 FTD 20 AD 21 HC	4	FA	dual spin-echo EPI, GRAPPA (dTV.II software)	FWHM, 8 mm	6	800 s/mm2	NA	FL, TL CC, Cg Unc, Th, Caudate, LPL.	• DTI ↓ FA in FTD vs HC ($p<0.001$, uncorrected): o frontal and temporal lobes o a.CC o a.Cg • VBM ↓GM in bvFTD vs HC o Frontal and temporal lobes o Rt. frontoinsular gyrus o Limbic lobes such as bilateral anterior cingulate gyrus o Uncus	1. Yes 2. Yes 3. Yes 4. Unclear 5. Yes 6. Yes 7. Yes 8. Yes 9. Yes 10. Unclear 11. Yes

										○ Subcortical nuclei including the bilateral caudate ○ The thalamus ○ The lateral parietal lobes.	12. Yes 13. Unclear 14. Unclear
McMillan (2012)	50 FTLD 42 AD 38 HC	3	FA	SSPE-EPI, MPRAGE (TSA) (whole-brain GM density analyses.)	FWHM, 4 mm	30	1000 s/mm2	NA	CC, CST, IFOF, ILF, SLF, UncF	• DTI ↓ FA in FTD vs HC ○ bilateral CST, IFO, ILF, SLF, and UncF, CC most prominent in anterior portions of these tracts. • DTI ↓ FA in FTLD vs AD ○ More in the ant.CC in FTLD ○ No sig areas in AD group Other irrelevant results on GM density are on the research paper.	1. Yes 2. Yes 3. Yes 4. Yes 5. Yes 6. Yes 7. No 8. Yes 9. Yes 10. No (El Escorial already known) 11. Yes 12. Yes 13. Yes (provided online) 14. Yes
Lillo et al (2012)	10 ALS, 10 ALS-FTD, 15 bvFTD, 18 HC	3.0	FA	FDT toolbox in FSL; TBSS	Not stated for DTI	32 gradients	1000 s/mm2	Unclear	Whole brain	• Comparisons: ($p < 0.05$ FWE corrected) ○ **bvFTD vs. HC:** WM degeneration in FM, ant. CC, ant. ILF, CST ○ **ALS-FTD vs. HC:** WM degeneration same as above, but less in FM and ant. CC (more in ant ILF and CST) ○ **ALS vs. HC:** WM degeneration in CST (only small changes in FM, ant. CC and ILF) ○ **bvFTD vs. [ALS-FTD and ALS]:** more WM degeneration in FM, ant. CC and ILF; less in CST and temporal poles ○ **ALS-FTD vs. ALS:** more WM degeneration in FM, ant. CC and ILF; less in CST	1. Yes 2. Yes (revised FTD Criteria in 2011 Brain paper) 3. Yes 4. Yes 5. Yes 6. Yes 7. Yes 8. Yes 9. Yes 10. No (ALD/FTD classification already known at time of MRI) 11. Yes 12. Yes 13. Unclear 14. Unclear (no withdrawals)

Pettit et al (2012)	30 ALS 30 HC	1.5	FA, MD	Whole brain Single shot SE EPI	NA	64	1000 s/mm3	0.2-0.4	Cg, Th, SFG, CR, CC, UnciF, CST, ILF, SLF, superior PL, temporal gyri, OG, optic radiation.	ROI analysis sig. gp difference in frontal regions: o Ant. Cg, ant.ThR (FA,MD) Unci.F (MD) In prefrontal regions: o Sup., inf., mid FG (FA, MD) o Ant.CR (MD) In temporal regions: o hippocampus and Cg (MD) o Temporal G, ILF, CR (FA) CST and CC (FA, MD) CC segmentation: o FA (P=0.02) o MD (P=0.02) No sig between DTI measures and optic radiation or OG No sig correlation with FVC, onset site or cognitive tests Neuropsychological data correlation with MRI Letter fluency: o Sup.FG (MD, p=0.012), o inf.FG (FA, p=0.017), o CST (FA, p=0.01), o CC (FA, p=0.049) Reverse digit span • hippocampus of Cg (MD, p=0.018)	1. Yes 2. Yes 3. Yes 4. Yes (disease duration presented and consistent) 5. Yes 6. Yes 7. Yes 8. Yes 9. Yes 10. No (El Escorial already known) 11. Yes 12. Yes 13. Yes (table containing all significant and non-significant results) 14. Unclear
Prell et al (2012)	17 ALS 17 HC	1.5	FA ADC	EPI SPM2; MATLAB 12	6mm FWHM	24 directions	1000 s/mm2	Unclear	Whole brain	• Multiple areas of significance (large tables and images in paper); no raw values given, but set at $p < 0.05$ corrected. • Comparisons: o ADC in ALS vs. HC o FA in ALS vs. HC o ADC in Bulbar ALS vs. HC o ADC in Limb ALS vs. HC o FA in Bulbar ALS vs. HC o FA in Limb ALS vs. HC o ADC in Bulbar ALS vs. Limb ALS (n.s.)	1. Yes 2. Yes (El Escorial , but no ref given) 3. Yes 4. Unclear (not stated which El Escorial category they were at the time of the scan) 5. Yes 6. Yes 7. Yes 8. Yes

										○ FA in Bulbar ALS vs. Limb ALS (n.s.)	9. Unclear (no ref provided) 10. No (El Escorial already known) 11. Yes 12. Yes 13. Unclear 14. Yes
Prudlo et al (2012)	15 ALS 7 LMN variant 21 HC	1.5	FA	EPI Whole-brain TBSS (using FSL 4.1); followed by ROI analysis of selected WM regions (DTIstudio version 2.4.01)	States TBSS does not need a smoothing kernel	30 directions	1000 s/mm2	Not stated	Whole brain	[Comparisons at $p < 0.05$ significance level] ↓ FA in All ALS (n=22) vs. HC (n=21): ○ CST, ATR, aLIC, pLIC, GCC, Fj, FM, UncF, SLF, ILF, IFOF, Cg, MCP ↓ FA in Classic ALS (n=7) vs. HC (n=7): ○ CST, ATR, aLIC, pLIC, bCC, Fj, FM, Unc.F, SLF, ILF, IFOF, Cg, MCP, ICP ↓ FA in LMN -ALS (n=7) vs. HC (n=7): ○ CST, ATR, aLIC, pLIC, GCC, Fj, FM, UF, SLF, ILF, IFOF, MCP, ICP ↓ FA in Classic ALS (7) vs. LMN variant (7): ○ No sig	1. Yes 2. Yes 3. Yes 4. Yes 5. Yes 6. Yes 7. Yes 8. Yes 9. Yes 10. No (El Escorial already known) 11. Yes 12. Yes 13. Unclear 14. Unclear (no withdrawals)
Bastin et al (2013)	30 ALS 30 HC	1.5	FA, MD,RD	Whole brain SE EPI TractoR (tract segmentation)		64	1000 s/mm3		Genu and splenium CC, CCG, CST, UnciF, ILF, Arcuate F	Tract integrity ALS vs HC ○ ↑ MD in all 12 tracts and significant in rt. CCG (p=0.03), lt.CST (p=0.02), rt.CST (p=0.001), ○ ↓ FA in lt. CST(p=0.002), rt. CST (P=6X10-5), rt. UnciF (p=0.07) Tract shape difference: ○ Sig. in lt.CST (p=0.04), rt. CST (p=0.02), rt. UnciF ($p<0.05$) Correlation with disease progression: ○ ⇓ correlation with FA along lt.CST (p=0.02), rt. CST (p=0.01) ○ ⇓ correlation with RD along lt.CST (p=0.07)	1. Yes 2. Yes 3. Yes 4. Yes 5. Yes 6. Yes 7. Yes 8. Yes (appears to have enough detail about MRI method) 9. Yes 10. No (El Escorial already known) 11. Unclear 12. Yes

											13. Yes (table with all significant and non-significant results) 14. Yes (same number in the result section)
Bede et al (2013)	39 ALS (C9pos= 9, C9neg=30) 44 HC	3	FA, MD, RD	SE-EPI TBSS, VBM, Cortical thickness.	NA	32	0,1,100 s/mm2	NA	CC, CST, cerebellar pathways, superior motor tracts	• DTI in C9pos vs C9neg ($p<0.05$, corrected): o FA, RD,MD changes in frontotemporal abnormalities in C9pos o More changes involved in motor WM. o Overlap WM pathology in both groups was significant in bCC and superior CST. o gCC, AC and bilateral Th related to genotype-specific gp. • Cortical thickness analysis o Atrophy GM in C9pos vs C9neg: lt.fusiform, lt. supramarginal, lt.sup TG, lt. OFC, lt. lateral OC, lt. posterior CG. o C9pos vs HC: bilateral prefrontal cotices, insular cortex, fusiform G, supramarginal C, lateral OC, precuneus, temporal poles, inferior FG. o No significant findings in C9neg and HC • VBM: o Orbitofrontal, opercular and temporal changes in the C9pos compared to HC and C9neg	1. Yes 2. Yes 3. Yes 4. Yes 5. Yes 6. Yes 7. Yes 8. Yes 9. Yes 10. Unclear 11. Unclear 12. Yes 13. Unclear 14. Yes (no withdrawals)
Bozzali et al (2013)	FTLD(+ GRN) =6 FTLD(- GRN) =17 HC=12	1.5	FA, MD	DW SE-EPI VBM Probabilistic tractography	10mm FWHM Gaussian kernel	12	1000 s/mm2	probabilistic threshold=0.1 + n 0.02	CC divided to 5 regions: 1.Most anterior - prefrontal cortex (PFC) 2.Rest of ant CC-PMC and	DTI between FTLD and HC: o Widespread pattern of ↓ FA and ↑ MD across all regions of CC. Similar finding when comparing each group against HC. DTI between FTLD(+GRN) and FTLD(-GRN) o ↓ FA and ↑ MD in the most antCC; bilaterally more prominent in the left side.	1. Yes 2. No (same subjects recruited for previous study) 3. Yes 4. Yes 5. Yes 6. Yes 7. Yes 8. Yes 9. Yes 10. Yes

									supplmentary MC. 3.mid CC-pre-rolandic C. 4.posterior CC-post-rolandic cotices. 5.most posterior CC-connecting post. Parietal, temporal, occipital cortices.	VBM between FTLD(+GRN) and FTLD(-GRN) o ↓ mGM near the most antCC Correlation analysis o VBM: mGM positively correlated with mean FA and negatively with mean MD for the most antCC in FTLD(+GRN) but not FTLD(-GRN) o Voxel wise analysis (VWA) confirmed the same findings as VBM between mGM and mean FA particularly in lt.CC.	11. Yes 12. Yes 13. No 14. Yes (no withdrawals)
Furtula et al (2013)	30 HC; 14 ALS	3T	FA	Double spin echo single shot EPI Deterministic tractography		26	1000 s/mm2		CST, PLIC	• No significant difference in mean FA between ALS and HC (CST, PLIC) • In ALS, no significant difference in FA between more affected and less affected side of CST • No correlation between FA (CST or PLIC) and ALSFRS-R or disease duration	1. Unclear (2 patients diagnosed with PMA) 2. Yes (ALS according to El Escorial + 2 extra PMA patient recruited) 3. Yes 4. Yes 5. Yes 6. Yes 7. Yes 8. Yes 9. Yes 10. No (El Escorial / PMA status already known) 11. Yes 12. Yes 13. Yes (all data completely reported for each individual patient) 14. Yes (withdrawal(s) explained)

Frizell Santillo et al (2013)	14 bvFTD 22HC	3 T	FA,MD, RD, AD	Track Vis Tractography QuTE analysis	NA	48	800 s/mm^2	0.2	Cg	DTI bvFTD vs HC: • ↓ FA in the ant Cg.but not the posterior part. • Lt hemisphere is slightly more affected more than rt. • ↓ FA ($p<0.001$) and ↓ MD ($p<0.003$) and ↑ RD ($p<0.002$) and ↑ AD ($p<0.002$) but the RD was the DTI that showed greatest differences between bvFTD and HC followed by MD. VBM bvFTD vs HC: • Moderate differences in the lt more than the rt but not statistically significant. • 4 possible bvFTD showed slightly significant cortical thinning compared to HC ($p=0.022$). Correlation between DTI and VBM: • In the lt hemisphere VBM correlated with FA ($p=0.002$) and MD ($p<0.001$), RD ($p<0.001$) and AD ($p=0.001$). Regression analysis between DTI and cortical integrity: • AUC of VBM and DTI parameters were larger in the lt hemisphere than the rt. Thickness can correctly classify 78% of cases, VBM 83%, FA 84%, MD 90%, AD 88% and RD 92%.	1. Yes. 2. Yes (part of Lund Prospective FTD study) 3. Yes. 4. Yes (mean +/- SD provided) 5. Yes. 6. Yes. 7. Yes. 8. Yes. 9. Yes (Rascovsky reference provided) 10. No (El Escorial known) 11. Yes. 12. Yes. 13. Unclear. 14. Unclear
Grapperon et al (2013)	14 ALS 6 LMN syndrom e 13 HC	1.5	FA, MD, RD,AD	SE-EPI, T2, FLAIR Tractography	NA	30	1000 s/mm3	0.2	CST	Structural CST impairment in ALS gp: o ↓ Z-MD ($p=0.004$) and Z-RD ($p=0.001$) o ↑ Z-FA ($p=0.055$). Correlation DTI vs clinical parameter: o Z-MD of lt.CST correlated with UMN scores ($p=0.014$) and Z-RD of lt.CST ($p=0.048$) No correlation with ALSFRS-R, DD, disease progression	1. Yes 2. Yes 3. Yes 4. Yes (2 years recruitment) 5. Yes 6. Yes 7. Yes 8. Yes 9. Yes

										(Unrelated results not reported)	10. No (El Escorial already known) 11. Yes (El Escorial applied before MRI was done) 12. Yes 13. Unclear 14. Unclear (no withdrawal)
Rajagopalan et al (2013)	12 HC; 87 ALS (4 subgroups)	1.5 T	FA, MD, AD, RD	Single-shot EPI Fiber tracking (FACT) ROI analysis	Not stated	12	1000 s/mm2	0.2	CST at four levels: 1. cerebral peduncle (CP) 2. PLIC 3. Centrum semiovale at top of lateral ventricle (CSoLV) 4. subPMC	• 4 subgroups: o UMN-CST hyperintense+ o UMN-CST hyperintense- o Mixed UMN/LMN o ALS-FTD • ALL ALS vs. controls: o ↓ FA at IC, CSoLV, subPMC (not CP) o ↓ FA at left subPMC only in UMN groups o ↓ FA at right PLIC in all 4 ALS groups o MD in lt and rt PLIC and lt. CSoLV in ALS-FTD, compared with both HC and other ALS groups o ↑ RD at lt.CP in all ALS groups o ↑ RD at lt. IC, and rt and lt.CSoLV in ALS-FTD • Correlation with clinical measures when all ALS patient pooled together, no FDR correction o MD and RD versus ALSFRS-R scores=-ve (p=0.001) at lt. CSoLV o AD and Disease duration=+ve (p=0.001) at lt CSoLV. o FA and disease progression rate=-ve at IC; rt (p=0.002) and lt. (p=0.001) o FA and disease progression rate=-ve at lt and rt CSoLV (p=0.001 and p=0.018) • No significant correlations when correction for multiple comparison	1. Yes 2. Incomplete (controls not specified appropriately) 3. Yes 4. Yes 5. Yes 6. Yes 7. Yes 8. Yes 9. Yes 10. No (El Escorial already known) 11. Yes 12. Yes 13. Yes 14. Unclear ** Note this study's presentation of data has some errors (e.g. comparing what is reported in the text and in the figures)

Trojsi et al (2013)	19 ALS, 19 HC	3T	GFA	GRE EPI TBSS VOI	NA	32	1000 s/mm3	>0.1	CST, CC, SLF,Unc. F, FOF	↓ GFA ALS vs HC ○ CST (p<0,05, corrected) within rostral WM below lt and rt.pre.CG, lt.PMJ, ant.Th, ant.Cg, splenium and body CC, Fx, lt. UncF, lt.ant.FOF, lt.SLF Correlation ALS vs clinical measures ○ ⇓ with UMN scores: along lt. CST at PMJ (p=0.031) ○ ⇓ with UMN scores: body CC, ant.Cg,SLF and bi.WM tracts from Central CC to pri.MC and preMC. ○ ⇓ with FrSBe: WM in lt. priMC, Body CC, lt.SLF, CST at PMJ ○ No sig between GFA and ALSFRS-R, DD, Disease progression in above tracts.	1. Yes 2. Yes 3. Yes 4. Yes (disease duration presented and consistent) 5. Yes 6. Yes 7. Yes 8. Yes 9. Yes 10. No (El Escorial already known) 11. Unclear 12. Yes 13. Unclear 14. Unclear (same number of pt.)
Zhang et al (2013)	13 bvFTD, 6 SD, 6 PNFA, 19 HC	4.0	FA AD RD	Twice-refocused spin-echo diffusion EPI, supplemented with GRAPPA SPM8; R; dTV; Volume-one	4mm FWHM Gaussian kernel	6 non-collinear directions	800 s/mm2	FA = 0.2	Whole brain, followed by specific TOI (ant CC, post CC, Cg, paraHpc, uncF, AF, Fx)	• Overall: DTI (especially RD) most accurate for FTD subtype classification • FA, RD and AD all presented visually in paper (in each subtype of FTLD vs. HC) TOI analysis bvFTD vs HC (supplementary data online) • ↑RD in rt.paraHpc (p<0.02), Fx, lt a.Cg, bilateral UncF,a.CC and p.CC (p<0.001), lt.paraHpc (p<0.04), rt.paraHpc (p<0.03), • ↓FA in the Fx, a.CC, lt.aCg (p<0.001), p.CC (p<0.03), lt.UncF (p<0.005), and rt.UncF (p<0.002). • ↑AD in the aCC, Fx, and bilateral UncF (p<0.001), pCC(p<0.03), and rt.paraHpc (p<0.05).	1. Yes 2. Yes (Neary criteria reference) 3. Yes 4. Yes 5. Yes 6. Yes 7. Yes 8. Yes 9. Yes 10. No (classification already known when analysing MRI data) 11. Yes 12. Yes 13. … 14. Unclear (no withdrawals)

Agosta et al (2013)	35 HC; 26 PLS; 28 ALS	3T	FA
			MD
			AD
			RD
				T2-weighted SE
				FLAIR
				T1 FFE
				Pulsed-gradient SE echo planar
				TBSS
				Probabilistic tractography
				ROI analysis
					Not stated	32	1000 s/mm2	0.2	CST
									CC
										• PLS vs. HC ($p < 0.05$, FWE):
										  ○ ↓FA in entire CST (pyramids – PLIC -preCG), CC (midbody, genu, splenium), ALIC, SLF, Fx, Th, parietal lobes
										  ○ ↑MD and RD in CST, CC (midbody), ALIC, thalamus, parietal lobes
										• ALS vs. HC ($p < 0.05$, FWE):
										  ○ ↓FA (and increased RD) in entire CST (pyramids –PLIC – preCG,CR), CC (midbody), SLF, parietal lobes
										  ○ No significant differences in MD
										• PLS vs. ALS ($p < 0.05$, FWE)
										  ○ ↓FA in CC (mid-body), patchy areas of motor, premotor, prefrontal and parietal WM, cerebellum
										  ○ ↑MD and ↑RD in CC (mid-body), cerebral peduncles, thalamic radiations, Fx, rt. PLIC, patchy areas of motor and premotor WM
										• TRACTOGRAPHY:
										  ○ PLS vs. HC:
										  ▪ CST, CC-PMC, CC-SMA
										  ○ ALS vs. HC:
										  ▪ CST, CC-PMC
										  ○ PLS vs. ALS:
										  ▪ CC-PMC, CC-SMA
											1. Yes
											2. Yes
											3. Yes
											4. Yes
											5. Yes (El Escorial, or reference for PLS diagnostic criteria)
											6. Yes
											7. Yes
											8. Yes
											9. Yes
											10. No (El Escorial / PLS already known)
											11. Yes
											12. Yes
											13. Yes (all non-significant results appear to be reported)
											14. Unclear (no withdrawals reported)

Barbagallo et al (2014)	24 ALS
	22 HC
		3T	FA, MD	SE-EPI
				
				Automated ROI (FSL) for DTI processing
				FIRST for T1-WI
					NA	27	1000 s/mm2	NA	Caudate, putamen, globus pallidus, Th, hippocampus, Amygdala (Ag), frontal	• DTI metrics were averaged due to absence of difference between lt vs rt .
										• DTI in ALS (n=24) vs HC:
										  ○ ↑ MD in patient at FC (p=0.023), caudate (p=0.01), Th (p= 0.019), Hpc (p=0.002) and Ag (p= 0.012).
										  ○ No sig in FA in any structure.
										• Correlation between clinical features and MD:
										  ○ +ve correlation with DD in caudate (p=0.004), Th (p=0.02), FC (p= 0.01).
											1. Yes
											2. Yes
											3. Yes
											4. Yes
											5. Yes
											6. Yes
											7. Yes
											8. Yes
											9. Yes
											10. Unclear
											11. Yes

									vortex (FC)	○ –ve correlation with ALSFRS-R scores in Th (p=0.02), Ag (p=0.02) and FC (p=0.001). • Correlation between neuropsychological scores (n=13) and MD: ○ -ve correlation with MSCT in caudate (p=0.007), hippocampus (p=0.001),Ag (p=0.015) and FC (p= 0.002). ○ -ve correlation with FAB scores caudate (p=0.017), hippocampus (p=0.001), Ag (p= 0.002) and FC (p=0.009).	12. Yes 13. Yes 14. Yes
Cardenas-Blanco et al (2014)	29 HC; 28 ALS (14 limb, 14 bulbar); FTD specifically excluded	3T	FA, MD, RD, axial diffusivity (AD)	Single-shot EPI GRAPPA TBSS (whole-brain) ROI analysis	Not stated	30	1000 s/mm2	FA >0.2	CST	• ALS-Bulbar (TBSS): ○ ↓FA in CST ($p < 0.05$ corrected); ○ RD ↑only at thalamic CST; ○ no changes in MD or AD • ALS-Limb (TBSS): ○ patchy ↓FA and ↑RD in CST (only at $p< 0.05$ uncorrected) ○ no changes in MD or AD • ROI Analysis: ○ also showed significantly ↑MD in ALS-Bulbar CST ○ significantly ↓AD in ALS-Limb CST	1. Yes 2. Yes 3. Yes 4. Unclear time-frame 5. Yes 6. Yes 7. Yes 8. Yes 9. Yes 10. No (El Escorial already known) 11. Yes 12. Yes 13. Unclear 14. Unclear (no withdrawals)
Christidi et al (2014)	21 ALS (not FTD); 11 HC	3T	FA ADC AD RD	Single-shot spin-echo EPI Quantitative tractography	NA	30	1000	0.15	Bilateral UncF	ALS vs. HC: • ↑ AD in the bilateral UncF in ALS, compared with HC ($p < 0.05$); other measures not significant	1. Yes. 2. Yes. 3. Yes. 4. Yes (mean +/- SD provided) 5. Yes. 6. Yes. 7. Yes. 8. Yes. 9. Yes 10. No (El Escorial known) 11. Yes. 12. Yes.

											13. Unclear. 14. Unclear (no withdrawals)
Crespi et al (2014)	20 HC; 19 non-demented ALS (a subset of the whole study cohort)	3T	FA MD Mode of anisotropy (MO)	Single-shot EPI TBSS Probabilistic tractography	Gaussian kernel (3mm)	32	1000 s/mm2	0.2	CST, SLF, ILF, IFOF, CC and other commissural fibers	• ALS vs. HC: o ↓FA ($p < 0.05$ FWE) in bilateral CST, body of CC o No significant differences in MD o Abnormal MO ($p < 0.05$ FWE) in right CST and ($p < 0.005$ uncorrected) in SLF, IFOF, ILF, genu of corpus callosum and forceps minor • Correlation between FA/MO of rt. ILF and IFOF, and emotional recognition (faces) in ALS o +ve global performance and mean FA in rt.ILF ($p=0.04$) o +ve relationship with cumulative scores of assessing negative emotions and the ability to recognize specific negative emotions with FA in rt.ILF ($p=0.004$) specifically with identification of fear ($p=0.03$), disgust ($p=.0.01$) and sadness ($p=0.008$) while in IFOF ($p=0.02$) correlated with fear ($p=0.008$), anger ($p=0.05$) and sadness ($p=0.02$) o No correlation between emotion recognition abilities and mean MO along tracts of interest. For additional analyses refer to the result section of the paper.	1. Yes 2. Yes 3. Yes 4. Yes (durations clearly specified) 5. Yes 6. Yes 7. Yes 8. Yes 9. Yes 10. No (El Escorial already known) 11. Yes 12. Yes 13. Yes 14. Yes (withdrawals from MRI stage clearly explained)

Floeter et al (2014)	28 HC; 25 PLS, 22 ALS	3T	FA MD	Single-shot EPI TBSS	Not stated	32 (Phillips) or 80 (GE)	1000 s/mm2 (Phillips) or 300 / 1100 s/mm2 (GE)	Not stated	Subcortical WM, middle cerebellar peduncles (MCP), pons, PLIC, ALIC, thalamus, CC	• All patients (ALS/PLS) vs. HC: o widespread decreased FA and ↑MD (subcortical WM, IC, CC) P<0.05 corrected • Patients with Pseudobulbar Affect (PBA+) vs. those without (PBA-) – ALS and PLS introduced as covariates: o reduced FA at left sub-preCG WM; Increased MD at bilateral MCP, transverse pontine fibers, bilateral PLIC, lt. ALIC, Th, frontotemporal subcortical WM, bilateral sub-preCG WM, and CC (P<0.05 corrected)	1. Yes 2. Yes 3. Yes 4. Yes (retrospective, so all diagnoses confirmed) 5. Yes 6. Yes 7. Yes 8. Yes 9. Yes 10. No (El Escorial already known) 11. Yes 12. Yes 13. Yes 14. Unclear (no withdrawals – retrospective)
Heimrath et al (2014)	11 HC; 9 ALS	3T	FA	Resting-state fMRI (EPI), with functional connectivity analysis Tensor imaging and fiber tracking (TIFT)	8mm FWHM (for fMRI); 6mm kernel (for DTI)	12	800 s/mm2	0.2	medial prefrontal cortex (MPFC), inferior PL (IPL), Posterior Cingulate cortex (PCC), Para hippocampus (paraHpc) Tracts to: anterior PC, orbitofrontal cortex (OFC), PreCG, superior	• ALS: ↑connectivity in resting (default-mode network) between: o Lt IPL – Rt IPL o Lt IPL – Lt paraHpc o Rt IPL – Lt paraHpc o Lt paraHpc – PCC • ALS: o significantly ↓FA (p<0.05) in tracts to Rt anterior PC, R OFC, PreCG (Rt and Lt), Lt superior PL, Lt SMG, Th.	1. Yes 2. Yes 3. Yes 4. Yes (time since Dx specified) 5. Yes 6. Yes 7. Yes 8. Yes 9. Yes 10. No (El Escorial already known) 11. Yes 12. Yes 13. … 14. Yes (reasons for withdrawals/ post-recruitment exclusions identified)

									PL, SMG, thalamus (Th)		
Irish et al (2014)	11 bvFTD. 10 SD, 15 AD, 14 HC	3 T	FA	TBSS , VBM	3 mm	32	100 s/mm^2	0.003	ILF, UncF, IFOF, SLF, Th, FMi, FMj, Cg, hippocampus	DTI bvFTD vs HC ($p<0.05$) ○ ↓ FA bilaterally in: • IFOF • UncF • ILF and SLF • Ant ThR • FMi and FMj • Cg VBM bvFTD vs HC • Rt cerebellum, rt inf TG, rt TP, bilateral para Hpc G, rt hippocampus, bilateral Ag, rt Th, rt and lt insular C, rt and lt OFC, rt mPFC, lt ant Cg, lt TP, lt inferior TG FA correlations with autobiographical memory performance • FA +ve correlation with ABM retrieval in the VBM analysis • FA for each WM tracts ($p<0.01$) show +ve correlations with recent and remote ABM. • Remote ABM retrieval correlated significantly with FA in lt UncF ($p=0.001$), lt Cg (CC part $p=0.008$ and hippocampus $p=0.002$) FMi ($p=0.001$). • Recent ABM retrieval associated significantly with FA values in FMi ($p=0.001$) and lt Cg (hippocampus part $p=0.01$).	1. Yes. 2. Yes. 3. Yes. 4. Yes (mean +/- SD provided) 5. Yes. 6. Yes. 7. Yes. 8. Yes. 9. Yes (Rascovsky reference provided) 10. No (El Escorial known) 11. Yes. 12. Yes. 13. Unclear. 14. Unclear

Kasper et al (2014)	72 ALS (49 no cognitive impairment and 23 with cognitive impairment); 65 HC	3T	FA MD AD RD	Twice refocused spin echo EPI TBSS and ROI methods both used	NA	30 non-collinear directions	1000	0.2	Whole brain (TBSS); 15 ROI's	(ROI methods gave less significant results than TBSS methods, p<0.05, corrected) ALS (no CI) vs. HC: • ↓ FA and ↑ RD in bilateral CST, PLIC and bCC ALS (CI) vs. HC: • As for the other ALS subjects, but <u>also</u> ↓ FA, ↑ RD and ↑ MD in the ant. ThR, Cg, IFOF, SLF, ILF and UncF (all changes bilateral but lt > rt hemisphere) ALS (no CI) vs. ALS (CI): • ↑ RD and ↑ MD in the ant. ThR, Cg, IFOF, SLF, UncF (in CI vs. non-CI patients) •	1. Yes. 2. Yes. 3. Yes. 4. Yes (mean +/- SD provided) 5. Yes. 6. Yes. 7. Yes. 8. Yes. 9. Yes (reference provided) 10. No (El Escorial known) 11. Yes. 12. Yes. 13. Unclear. 14. Unclear (no withdrawals)

Kassubek et al (2014)	111 ALS (A)78 scanned on 1.5 T, (B)33 scanned on 3 T) 74 HC (A)52 scanned on 1.5T, (B) 22 on 3 T)	1.5 T and 3 T	FA, AD, RD	(A) 1.5 T scanner
				
				(B) 3T
				
				Tract of interest-based fiber tracking (TFAS analysis vesus ROI-analysis)
					8 mm	(A) 31
						(B) 49
							1000 s/mm2	FA >0.2
								
								Eignvector = 0,9
									CST, corticorubral (CRT) and Cortico-pontine (CPT), cortico-striatal, prefrontal path	TFAS vs ROI
										• At 1.5 T:
										  ○ ROI (sensitivity 78%, specificity 69%)
										  ○ TFAS (sensitivity 79%, specificity 71%)
										• At 3 T
										  ○ ROI (sensitivity 82%, specificity 68%)
										  ○ TFAS (sensitivity 79%, specificity 73%)
										
										DTI metrics ALS vs HC:
										  ○ Bilateral CST FA=sig
										  ○ RD,AD both significant along CST
										• Correlation of ALS stages:
										  ○ ALSFRS-R and disease duration correlated significantly with the staging scheme
										  ○ (ALSFRS, p=0.0017)
										  ○ (Disease duration, p=0.0019)
											1. Yes
											2. Unclear (inclusion criteria clear but no exclusion criteria)
											3. Yes
											4. Unclear
											5. Yes
											6. Yes
											7. Yes
											8. No
											9. Yes
											10. Unclear
											11. Yes
											12. Yes
											13. Unclear
											14. Yes (no withdrawal)

Kim et al. (2014)	14 ALS 16 HC	3T	FA,	Double SE-EPI Whole brain. Seeding-based tractography	NA	25	NA	0.15	CC: BA4 (primary motor C) BA1/2/3 (primary sensory C) BA6 (supplementary motor area) BA11/47 (orbitofrontal C) BA44/45 (Broca's area)	↓ FA in ALS vs HC: ○ BA4 (p=0.003) ○ BA6 (p=0.01) ○ BA9/46 (p=0.0005) ○ No sig →BA1/2/3, BA11/47, BA44/45 Cortical ROI ↓ FA in ALS vs HC: ○ preCG (p=0.0036) ○ mid.FC (p=0.016) ○ sup.FC (p=0.0079)	1. Yes 2. Yes 3. Yes 4. Yes 5. Yes 6. Yes 7. Yes 8. Yes 9. Yes 10. No (El Escorial already known) 11. Yes 12. Yes 13. Yes (table with significant and non-significant results presented) 14. Unclear
Lam et al (2014)	12 bvFTD, 10 PNFA, 11 SD, 15 HC (2 time-points, 12 months apart)	3 T	FA, MD, RD, AD	TBSS, VBM	3 mm	32	1000 s/mm^2	0.2	Whole brain	DTI bvFTD vs HC ($p<0.05$): **At base-line**: ↓ FA, ↑ MD, ↑ AD, ↑ RD significant bilateral changes in frontotemporal regions, ant.ThR, ant.Cg, sup ILF, IFOF, UncF, gCC. Changes in MD and RD are most extensive in this group. **At 12 months**: bilateral changes sCC, longitudinal changes were most in FA and RD. rt ant Cg. Changes in MD obvious in Lt SLF. Changes in AD found bilateral ant Cg, SLF and ILF. Overlap between WM and GM changes (after 12 months): GM: Rt Frontotemporal region. WM: bilaterally.	1. Yes. 2. Yes. 3. Yes. 4. Yes (mean +/- SD provided) 5. Yes. 6. Yes. 7. Yes. 8. Yes. 9. Yes (references provided) 10. No (El Escorial known) 11. Yes. 12. Yes. 13. Unclear. 14. Unclear
Lu et al (2014)	8 bvFTD, 12 AD, 12 HC	1.5 T	FA, MD, RD, AD	SS-SE ROI	NA	12	800 s/mm^2	NA	Frontal WM, gCC, sCC	DTI group comparisons: • bvFTD showed significant group differences in all DTI metrics in frontal WM and gCC ($p<0.008$).	1. Yes. 2. Yes. 3. Yes. 4. Yes (mean +/- SD age at onset and at scan provided)

										• bvFTD had more WM changes compared to HC and AD in same regions. • No changes in sCC across all DTI metrics. Correlations with behavioral variables: • SEB rating associated with gCC DTI measures; FA (p=0.005), AD (p=0.007), RD (p=0.005) and MD (p=0.0006). Worse WM integrity associated with more symptoms of emotional blunting. • Examining two clinical groups separately showed a correlation between DTI measures and SEB rating in bvFTD.	5. Yes. 6. Yes. 7. Yes. 8. Yes. 9. Yes (Rascovsky reference provided) 10. No (El Escorial known) 11. Yes. 12. Yes. 13. Unclear. 14. Unclear
Mahoney et al (2014)	23 bvFTD, 18 HC	3 T	FA, MD, RD, AD	ROI VBM	NA	64	1000 s/mm^2	0.05	gCC, sCC, UncF, paraHpc Cg, CST, CP, Fx.	Cross DTI bvFTD vs HC • ↓ FA and ↑ MD (p< 0.02) in bCC, bilateral UncF and rt Cg (para Hpc part). • ↑ AD (p<0.01) in bCC and lt Cg (para Hpc part), Fx (p<0.04) and rt UncF (p<0.03) • ↑ RD (p<0.01) in bCC, bilateral Cg at para Hpc part and lt UncF abd rt UncF (p<0.001). Cross DTI MPAT carriers vs HC • ↓ FA rt Cg (p=0.009), ↑ MD in rt UncF (p=0.001)and rt Cg (p=0.01). Cross DTI sporadic bvFTD vs HC • ↓ FA lt UncF (p=0.01) and rt Cg (p=0.03). • ↑ MD in lt UncF (p=0.002) Cross DTI C9ORF72 carriers vs HC • ↓ FA bilater superior CP (p=0.03)	1. Yes. 2. Unclear (exclusion criteria not explicitly stated) 3. Yes. 4. Yes (disease duration introduced as nuisance covariate) 5. Yes. 6. Yes. 7. Yes. 8. Yes. 9. Yes (Rascovsky reference provided) 10. No (El Escorial known) 11. Yes. 12. Yes. 13. Yes. 14. Unclear

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<td>Longitudinal DTI bvFTD vs HC
• rate of changes in FA and MD were significant is bCC (p=0.003), sCC (p=0.001), bilateral Cg (p<0.001) and rt UncF (p<0.001) and lt UncF (=0.005).
• RD significant changes in subgroups
<table>
<tr><th>RD</th><th>bvFTD</th><th>MAPT</th><th>Sporadic</th><th>C9ORF72</th></tr>
<tr><td>bCC</td><td>0.002</td><td>0.001</td><td>-</td><td>-</td></tr>
<tr><td>sCC</td><td>0.001</td><td>0.01</td><td>0.01</td><td>-</td></tr>
<tr><td>Rt Cg</td><td>0.001</td><td>0.04</td><td>0.002</td><td>-</td></tr>
<tr><td>Lt Cg</td><td>0.001</td><td>0.01</td><td>0.001</td><td>-</td></tr>
<tr><td>Rt Cg Hpc</td><td>0.01</td><td>0.01</td><td>-</td><td>-</td></tr>
<tr><td>Lt Cg Hpc</td><td>0.03</td><td>0.001</td><td>-</td><td>-</td></tr>
<tr><td>Rt UncF</td><td>0.001</td><td>0.001</td><td>0.03</td><td>-</td></tr>
<tr><td>Lt UncF</td><td>0.01</td><td>0.001</td><td>-</td><td>0.02</td></tr>
</table>
• AD significant changes in subgroups
<table>
<tr><th>AD</th><th>bvFTD</th><th>MAPT</th><th>Sporadic</th><th>C9ORF72</th></tr>
<tr><td>gCC</td><td>-</td><td>-</td><td>0.03</td><td>-</td></tr>
<tr><td>bCC</td><td>-</td><td>-</td><td></td><td>0.003</td></tr>
<tr><td>sCC</td><td>0.02</td><td>0.04</td><td>-</td><td></td></tr>
<tr><td>Rt Cg para</td><td>0.001</td><td>0.001</td><td>-</td><td>-</td></tr>
<tr><td>Lt Cg Hpc</td><td>0.005</td><td>0.001</td><td>-</td><td>-</td></tr>
<tr><td>Rt UncF</td><td>0.001</td><td>0.001</td><td>-</td><td>-</td></tr>
<tr><td>Lt UncF</td><td>0.009</td><td>0.001</td><td>-</td><td>0.03</td></tr>
<tr><td>sCP</td><td>0.008</td><td>0.03</td><td>-</td><td>0.003</td></tr>
</table>
DTI metrics sensitivity and specificity
• Cross sectionally: RD best then MD followed by FA and AD
• Longitudinaly were best achieved by FA, followed by MD and RD then AD.</td>
<td></td>
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<tr>
<td>Menke et al 2014</td>
<td>60 ALS
36 HC</td>
<td>3T</td>
<td>FA, MD, RD, AD</td>
<td>MPRAGE, VBM, TBSS and ROI</td>
<td>3mm</td>
<td>60</td>
<td>1000 s/mm2</td>
<td>CC at 45
SLF at 10</td>
<td>CC, CST, SLF, FL and pre-motor cortex</td>
<td>In ALS gp at T0:
1. VBM ↓ GM
o lt. primary MC and lt. FL.
o Th, bilateral caudate head
2. DTI:
o ↑AD, MD in CC
o ↑AD in ltCST

UMN scores:
1. TBSS:</td>
<td>1. Yes
2. Yes
3. Yes
4. Yes
5. Yes
6. Yes
7. Yes
8. Yes
9. Yes</td>
</tr>
</table>

										○ ↓FA, ↑AD in CST ○ ↑MD, ↑RD in CST,SLF, CC **2. ROI → ↓FA in rt.SLF** 3. VBM → No sig ALSFRS: ○ TBSS, VBM→ no sig ○ **ROI→** RD –ve correlation in lt.SLF progression rate: 1. TBSS, VBM→ no sig 2. ROI→ ○ ↑RD , ↓FA in rt.SLF, CST ○ ↑AD in CC 3. **VBM ↓ GM** –ve correlation in lt. primary MC ACE: ○ TBSS, VBM →No sig ○ **ROI :** verbal fluency and AD →+ve correlation ○ **VBM:** verbal fluency and Broca's area, dorsolateral prefrontal cortex→+ve correlation	10. No (El Escorial already known) 11. Unclear 12. Yes 13. Yes (supplementary material available online) 14. Unclear
Prokscha et al (2014)	12 HC; 13 ALS	1.5 T	FA	Single-shot EPI Manual and atlas ROI approaches (with or without TBSS)	Not stated	12	1000 s/mm2	Not stated	CST	• ↓FA in bilateral CST in ALS (most significant using atlas ROI with TBSS approach; p = 0.0002) • ROC analysis showed best test performance was using atlas ROI with TBSS approach (AUC = 0.936, sensitivity 100%, specificity 91.67%)	1. Yes 2. Yes 3. Yes 4. Yes 5. Yes 6. Yes 7. Yes 8. Yes 9. Yes 10. No (El Escorial already known) 11. Yes 12. Yes 13. Unclear 14. Yes (reasons for withdrawals/ post-recruitment exclusions identified)

Romano et al (2014)	14 ALS; 14 HC	1.5 T	FA MD AD (PD) RD	SS-SE EPI (DTI performed to define the tracts as part of waveguide elastography (WGE) protocol)	NA	12 non-collinear directions	1000	NA	CST	ALS vs. HC: • In the CST's, there was significant ↓ FA (p = 0.011), ↑ MD (p = 0.023) and ↑ RD (0.007) No significant difference in AD	1. Yes 2. Unclear (exclusion criteria not specified) 3. Yes. 4. Unclear. 5. Yes. 6. Yes. 7. Yes. 8. Yes. 9. Yes (reference provided) 10. No (El Escorial known) 11. Yes. 12. Yes. 13. Unclear. 14. Unclear (no withdrawals)
Sarica et al (2014)	14 HC; 14 ALS	3T	FA MD RD AD	Spin-echo EPI Tractography (TRACULA) TBSS (to corroborate TRACULA results)	Not stated	27	1000 s/mm2	Not stated	CST, SLF (parietal and temporal), anterior thalamic radiation (ATH), Unc.F, supracallosal bundle, ILF, CC-forceps major (Fj)	• Tractography (ALS vs. HC, p<0.05, corrected for multiple comparisons): o Lt. cingulum-cingulate gyrus (supracallosal) bundle (RD, ↑MD) o Rt. CST (↓FA, ↑RD, ↑MD) o Rt. supracallosal bundle (↑MD, ↑AD) o Lt.ATR (↓FA, ↑RD, ↑MD) and rt Cg (↑AD and ↑MD) uncorrectep<0.05. • TBSS (ALS vs. HC, p<0.05, TFCE corrected): o Bilateral CST (FA, MD, RD) o Bilateral SLF (FA, MD) o Bilateral anterior thalamic radiation (FA, MD, RD) o Bilateral Unc.F (MD) o Bilateral CR (FA, MD, RD) o Cg (FA, MD, RD) o Bilateral ILF (MD, RD) o CC (FA, MD, RD) o No significant changes in AD • Correlations with clinical measure:	1. Yes 2. Yes 3. Yes 4. Yes (except one patient DD = 16 yrs with minimal disability) 5. Yes 6. Yes 7. Yes 8. Yes 9. Yes 10. No (El Escorial already known) 11. Yes 12. Yes 13. Yes (negative results also reported in table) 14. Unclear (no evidence of withdrawals)

										○ Rt. CST (RD and MD) correlated negatively with ALSFRS-R (p=0.0009) ○ Rt. CST (FA) showed a trend towards significant with ALSFRS-R (p=0.07)	
Tovar-Moll et al (2014)	20 bvFTD, 19 CBS, 15 HC	3 T	FA, MD	Voxel wise whole brain analysis ROI	Smoothing	Directions	1000 s/mm^2	FA Thresh	Whole brain ROIs: CC (gCC and sCC), IC, CR, Cg, UncF, medial forebrain bundle (MFB).	DTI ROI results (p<0.05): ○ Patients vs HC: • ↓ FA and ↑ MD in all tracts except IC and sCC. ○ bvFTD vs HC • Showed more changes in the Cg G, UncF in frontal and temporal parts. DTI VWA results (p<0.05): ○ bvFTD vs HC • Abnormalities in CC, AC, CR, UncF, IFOF, ILF, SFOF, SLF, Cg and MFB. • Ant limb of IC. ○ bvFTD vs CBS • WM damage restricted to FMi and gCC, UncF, MFB and rostral SLF. • Rt hemisphere was damaged more than the lt in gCC, UncF and rostral SLF. Correlations with clinical ratings In bvFTD: ○ Posterior Cg correlated with: • NPI-apathy subscores (MD, p<0.01) • Mattis total (MD and FA, p<0.05) ○ UncF: • NPI-aberrant motor behavior subscore with frontal part (MD and FA, p<0.05) and temporal part (FA, p<0.05) •	1. Yes. 2. Yes. 3. Yes. 4. Yes (mean +/- SD provided) 5. Yes. 6. Yes. 7. Yes. 8. Yes. 9. Yes (Neary & McKhann references provided) 10. No (El Escorial known) 11. Yes. 12. Yes. 13. Unclear. 14. Unclear

Woo et al (2014)	34 ALS 13 HC	3T	FA, MD	SS-SE GRAPPA Deterministic tractography		30	1000 s/mm3	0.2 inner 0.75	CST, forceps major (Fj)	• Between ALS and HC: o CST FA (p=0.01) and MD (p=0.003) o Linear relations with UMN score and ALSFRS-R. o No association with disease duration • Regression results: o MD and FA in CST was significant (p=0.02) o MD in Fj =no sig o UMN score (MD, p=0.005) (FA, p=0.003) and age (MD, p=0.03) both were significant predictor but not age and FA. o ALSFRS-R and disease duration = no sig o El Escorial, handedness and sex = no sig	1. Yes 2. Yes 3. Yes 4. Yes 5. Yes 6. Yes 7. – 8. yes 9. yes 10. yes 11. yes 12. Unclear 13. Unclear 14. Unclear
Zhang et al (2014)	20 ALS 21 HC	3T	FA, RD, AD,	SE-EPI TBSS AND PDT	NA	64	1000 s/mm2	2	Superior CR, CP, PLIC, PreCG, body CC, CST, SLF, CF	• VWA: ↓ FA in ALS vs HC o Bilateral Sup.CR o Caudal CST (rt.CP), Lt. PLIC. o Body CC. o Rt. preCG • PDT: ↓ FA and ↑ RD in ALS vs HC o Bilateral CST to preCG o CC fibers (bilateral SMA and preCG) o Rt.AF (SLF) – different result • Cortical thinning: o Lt.OFC o Rt.SMA o Bilateral preCG o Dorsal preMC. o Sup.PL o Lt. mid. OG o SMG o Rt. Prefrontal regions o Lt.OFC	1. Yes 2. Yes 3. Yes 4. Yes 5. Yes 6. Yes 7. Yes 8. Yes 9. Yes 10. No (El Escorial already known) 11. Yes 12. Yes 13. Unclear 14. Unclear
Agosta et al (2015)	21 ALS, 14 ALS-plus, 14 bvFTD, 12 SD, 11	3T	FA MD	Pulsed-gradient SE EPI TBSS – WM voxelwise analysis	8 mm FWHM	32 non-collinear directions	1000	0.2	Whole brain	• WM changes more widespread than GM (all groups) • Results $p < 0.05$, FWE corrected • Supporting figures on journal website	1. Yes. 2. Yes. 3. Yes. 4. Unclear. 5. Yes. 6. Yes.

	PNFA, 28 HC									ALS vs HC: • ↑ MD and ↓ FA in bilateral CR and posterior bCC. ALS-plus/bvFTD/PNFA vs. HC: • ↑ MD and ↓ FA in the CC, orbitofrontal WM, frontoparietal WM, occipital WM, IC, BS, temporal WM (all bilateral) SD vs. HC: • ↑ MD in the lt (> rt) IC and External capsule (ExC) , bCC, orbitofrontal, frontal, ant. temporal and inferior parietal WM (sparing of occipital lobes and BS) • ↓ FA in bCC and gCC, lt SLF, Cg, ExC and IC, ant. and middle temporal WM Between group ALS and FTD comparisons also performed	7. Yes. 8. Yes. 9. Yes (references provided) 10. No (El Escorial and FTD criteria known) 11. Yes. 12. Yes. 13. Unclear. 14. Unclear (no withdrawals)
Bae et al (2015)	25 ALS,17 bvFTD, 37 HC	3 T	NA	ROI VBM	NA	32	1000 s/mm^2	NA	WM Regions in Th, Cerebellum,motor cortex (pre CG, post CG, and supplementary motor area), BS and striatum (putamen, caudate, globus pallidus)	VBM and DTI: ALS vs HC ($p<0.05$): ○ Minor changes is the • Motor cortex • Cerebellum • Th • BS ○ WM showed greater changes is these regions including CST bvFTD vd HC ($p<0.05$): ○ Sever degeneration across • Motor system • Cerebellum ○ WM that connect these regions is severely affected. ALS vs bvFTD ($p<0.05$): ○ Minimal GM atrophy in the cerebellum. ○ WM changes in lt. Th in ALS. ○ While bvFTD patients showed more atrophy in:	1. Yes. 2. Yes (El Escorial and Rascovsky) 3. Yes. 4. Yes (mean +/- SD provided) 5. Yes. 6. Yes. 7. Yes. 8. Yes. 9. Yes (reference provided) 10. No (El Escorial known) 11. Yes. 12. Yes. 13. Yes. 14. Unclear.

										• Motor cortex • Cerebellum • Striatal • Th Changes in WM includes these regions found in VBM	
Daianu et al (2015)	20 bvFTD, 23 early-onset AD, 33 HC	1.5 T	FA,MD, RD, AD	SS-SE DWI autoMATE (automated multi-atlas tract extraction) Tractography	NA	30	1000 s/mm^2	0.2	**21 tracts:** Th, CC, Cg, CST, IFOF, ILF, SLF, UncF, paraHcp	bvFTD vs HC: • MD and RD detected most changes. • Frontal and temporal tracts are most impacted. • All 21 tracts were severly affected with ↑ MD (p=0.035). • ↓ FA (p=0.024) with ↑ AD (p=0.019), RD (p=0.034) and MD were significant (above 60%) in rt. Ant ThR. And bilateral UncF. • <u>Commissural fibers:</u> most significant is the frontal fibers in more than 99%. gCC shows late myelination • <u>Long association fibers:</u> more than 99% in bilateral UncF, lt. SLF and bilateral IFOF. • <u>Limbic system:</u> dorsal Cg and ant. ThR <u>Motor system:</u> CST was the least affected tract.	1. Yes. 2. Yes (Rascovsky) 3. Yes. 4. Unclear. 5. Yes. 6. Yes. 7. Yes. 8. Yes. 9. Yes (reference provided) 10. No (El Escorial known) 11. Yes. 12. Yes. 13. Unclear. 14. Unclear
Downey et al (2015)	29 bvFTD, 15 svPPA, 37 HC	3 T	Trace diffusivity (TR), FA, MD, RD, AD	SS-SE WM TBSS GM: VBM using DARTEL toolboxes of SPM8	6 mm	64	1000 s/mm^2	NA	Whole brain	Brain maps bvFTD vs HC ○ <u>GM:</u> bi hemisohereic atripgy in: • Ant TL, mTL structures, insular, prefrontal and orbitofrontal cortices. ○ WM (p<0.05): • Changes were dorsal and ventral mostly in Cg, bi UncF, CC • Less posteriorly in parieto-occipital ILF. Correlations with social cognition performance (p<0.05, corrected)	1. Yes. 2. Yes (Rascovsky and Gomo-Tempini references provided) 3. Yes. 4. Yes (mean +/- SD provided) 5. Yes. 6. Yes. 7. Yes. 8. Yes. 9. Yes (references provided) 10. No (El Escorial known)

										• -ve correlation betweem TASIT emotion identification score and DTI metrics (AD,TR and RD). • +ve correlated with FA in dorsal and ventral commissural WM bilaterally. • Frontal subcortical projection pathways (rt ant ThR) and Fx • Within bvFTD group, emotion identification impairment was associated with WM alteration in CC and Fx. • -ve correlation between TASIT total sarcasm with AD, RD and TR in the rt temporal WM, and inferior frontal WM • +ve correlation in sarcasm identification scores with rt side of WM but bilateral in Temporal inferior FWM, rt UncF and rt ant. ThR.	11. Yes. 12. Yes. 13. Unclear. 14. Unclear
Hubers et al (2015)	Subset of 30 ALS patients; 18 HC	1.5 T	FA	Voxelwise statistical comparison (WBSS) with manual ROI method	8 mm FWHM	31	1000	0.2	CST	ALS vs. HC: • ROI in the bilateral lower CST in both hemispheres Significant ↓ FA in ALS compared with HC ($p < 0.01$)	1. Yes. 2. Yes. 3. Yes. 4. Yes (mean +/- SD provided) 5. Yes. 6. Yes. 7. Yes. 8. Yes. 9. Yes (reference provided) 10. No (El Escorial known) 11. Yes. 12. Yes. 13. Unclear. 14. Yes (not all OCT patients consented to the MRI component)

Sheelakumari et al (2015)	17 ALS, 15 HC	1.5 T	FA MD	SS-SE EPI ROI-based analysis	NA	30 non-collinear directions	1000	NA	CST (ROI's in pons at level of CP and medullary pyramids)	ALS vs. HC: • PONS: ALS subjects had ↓ FA ($p = 0.001$) and ↑ MD ($p = 0.003$) MEDULLA: ALS subjects had ↑ MD ($p = 0.011$); FA not significant when corrected.	1. Yes. 2. Yes. 3. Yes. 4. Yes (online supplementary table) 5. Yes. 6. Yes. 7. Yes. 8. Yes. 9. Yes (reference provided) 10. No (El Escorial known) 11. Yes. 12. Yes. 13. Unclear. 14. Unclear (no withdrawals, retrospective cohort)
Steinbach et al (2015)	16 ALS, 16 HC	3T	FA MD	Spin echo EPI Probabilistic tractography → structural connectivity indices (CI) calculated	NA	12 non-collinear directions	1000	0.2	1. Intracranial CST (pons to PMC) 2. Visual cortex to medial temporal (ERC, PRC, PHC)	ALS vs. HC: • ↓ CI in the CST's ($p = 0.03$), negative correlation with disease duration ↓ CI in the VC-PRC tract ($p = 0.02$), no significant effect in the VC-ERC or VC-PHC tracts	1. Yes. 2. Yes. 3. Yes. 4. Yes 5. Yes. 6. Yes. 7. Yes. 8. Yes. 9. Yes (reference provided) 10. No (El Escorial known) 11. Yes. 12. Yes. 13. Unclear. 14. Unclear
Tang et al (2015)	69 ALS, 23 HC	3T	FA ADC	SS-SE EPI	NA	23	1000	Different thresholds used (ROC)	18 regions examined	• Data from rt and lt sides averaged <u>ALS vs. HC</u>: ($p < 0.05$)	1. Yes. 2. Yes. 3. Yes. 4. Unclear. 5. Yes. 6. Yes.

										• ↓ FA in centrum semiovale, deep frontal WM, deep parietal WM, CR, PLIC, gCC of CC, sCC, CP. ↑ ADC in centrum semiovale, deep frontal WM, deep parietal WM	7. Yes. 8. Yes. 9. Yes (reference provided) 10. No (El Escorial known) 11. Yes. 12. Yes. 13. Unclear 14. Unclear.
Zimmerman-Moreno et al (2015)	23 ALS, 18 HC	3T	FA MD RD AD	Novel method (FBC), compared with TBSS method	NA	15 (19 for 3 control subjects)	1000	0.2	Whole brain	ALS vs. HC (TBSS method): • Significant ($p \leq 0.05$ corr) ↓ FA and ↑ RD in the CST and bCC	1. Yes. 2. Yes. 3. Yes. 4. Unclear. 5. Yes (no El Escorial criteria/reference provided, but same patients scored in Ben Bashat et al (2011)) 6. Yes. 7. Yes. 8. Yes. 9. Yes 10. No (El Escorial provided in previous paper, as above) 11. Yes. 12. Yes. 13. Unclear. 14. Unclear
Vora et al (2016)	21 ALS, 13 HC	1.5 T	FA ADC MD	Single shot diffusion weighted EPI ROI-based methods	NA	30	NA	NA	ROI's at PMC WM, gCC and sCC, PLIC, medullary pyramid	ALS vs. HC: • ↓ FA and ↑ MD in bilateral PMC WM, PLIC, gCC and sCC ($p < 0.05$) • ↓ FA lt medullary pyramid ($p = 0.007$) CC and medullary pyramid changes were <u>not</u> significant when looking at the "possible ALS"	1. Yes. 2. Yes (divided into definite, probable and possible ALS) 3. Yes 4. Yes 5. Yes 6. Yes 7. Yes 8. Unclear (some details not included)

											9. Yes 10. No (El Escorial known) 11. Yes 12. Yes 13. Unclear 14. Unclear

Supplementary Table 2: FA correlations in ALS

Disease	Tracts	DTI measure	Correlation	ROI	Disease measure
ALS	Projection fibers	FA	-ve	CST	UMN scores
			-ve		DD
			+ve		CMCT
					Cortical_brain stem conduction time
					Trail making test scores
					Stroop test scores
					Letter fluency
			-ve		FrSBe
			-ve (weaker)	Rt CST	ALSFRS-R
			+ve	Rt. UncF	Sarcasm identification scores
				Bilateral IFOF ILF	Disease progression
			+ve	Bilateral IFOF ILF	Trail making
			+ve	Lt IFOF ILF	Stroop test scores
			-ve	SLF	• FBI A, FBI B and FBI AB • Personal neglect (eg, lack of personal hygiene, disorganization

					in planning and organizing complex activity, impulsivity or poor judgment and utilization behavior
			-ve	SLF	FrSBe
			-ve	SLF	• UMN scores • Trail-making Test B scoring
			-ve	Rt ILF	Cumulative scores of single emotions
			-ve	IFOF	Emotional recognition
	Commissural F	↓ FA	Weaker		ALSFRS-R
		FA			Trail making test scores
					Stroop test
			+ve	Dorsal and ventral CC	social cognition performance
	Limbic	FA		Fornix	Verbal learning
				Fornix	Memory test scores
				Para Hpc Lt Th	Increased Apathy scores on FrSBe
			+ve	Rt ant ThR	Sarcasm identification scores
	Whole Brain	FA		Lt hemisphere	VBM

Supplementary Table 3: MD correlations in ALS

Disease	Tracts	DTI measure	Correlation	ROI	Disease measure
ALS	Limbic	MD		Fornix	Verbal learning
					Memory test scores
			-ve	Th	ALSFRS-R
			+ve		DD
	Commissural	MD		CC	Trail making test scores
				CC	Stroop test
	Projection F	↑ MD		CST	↑ UMN scores
			-ve	Rt CST	ALSFRS-R

Supplementary Table 4: radial diffusivity and correlations in ALS

Disease	Tracts	DTI measure	Correlation	ROI	Disease measure
ALS	Commissural	↑ D_{radial}		CC	Clinical scores
	Projection	↑ D_{radial}	using ROI	CST	↑ UMN scores
					↓ CMCT
					↓ Cortical-brain stem conduction time
					↓ Trail making test scores
					↓ Stroop test scores
					↑ Disease progression
			-ve	Rt CST	ALSFRS-R
	Association Fibers		-ve	Rt SLF	ALSFRS-R
			+ve	Rt SLF	Disease progression
	Whole brain		Lt hemisphere		VBM

Supplementary Table 5: Axial diffusivity and correlations in ALS studies

Disease	Tracts	DTI measure	Correlation	ROI	Disease measure
ALS	Projection	↑ D_{axial}		CST*	↑ UMN scores
				CST*	↑ DD

Supplementary Table 6: FA correlations in FTD studies.

Disease	Tracts	DTI measure	Correlation	ROI	Disease measure
FTD	Projection F	↓ FA	+ve		Cortical thickness
	Association T			SLF IFOF ILF UncF	Cortical thickness
		FA and MD		UncF	NPI-aberrant motor behavior subscore
		FA	+ve	ILF, IFOF,UncF, SLF	Recent and remote autobiographical memory performance (ABM)
	Limbic	FA		Post Cg	Mattis total
		MD		Post Cg	NPI-apathy subscores
					Mattis total
		FA,MD,RD,AD	-ve	gCC	SEB rating
	Whole Brain		-ve		TASIT emotion identification score
	Whole brain		Lt hemisphere		VBM

The staging system proposed by Roche et al. for ALS is as following (211):

Stage 1: symptom onset;

Stage 2A: diagnosis;

Stage 2B: involvement of a second region;

Stage 3: involvement of a third region;

Stage 4A: need for gastrostomy;

Stage 4B: need for respiratory support