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Asymmetry and Vulnerability in Amyotrophic Lateral Sclerosis

Matthew Steven Devine

MBBS, BSc

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Abstract

Objectives:

Amyotrophic lateral sclerosis (ALS) is a progressive, fatal condition in which both upper (UMN) and lower motor neurons (LMN) undergo irreversible degeneration. Although the cause remains unknown and the disease is phenotypically diverse, certain functions and muscle groups are particularly vulnerable to early weakness, including the thenar eminence and foot dorsiflexion. It is also well established that disease onset and spread are typically asymmetric. Although this may reflect an additional source of vulnerability, the factors underlying this asymmetry are unknown. Furthermore, separate analysis of the asymmetry of UMN and LMN signs has not been performed, nor has this concept been applied to non-limb features of ALS such as bulbar involvement.

The aim of this project was to identify and characterise novel sources of vulnerability in a large cohort of ALS subjects, with particular focus on asymmetry, clinical phenotype and the effect of limb dominance on both onset and spread. Multiple modalities were employed, including structured questioning, direct clinical examination and scoring, and advanced magnetic resonance imaging (MRI) analysis. As a secondary objective, the burden of clinical LMN and UMN involvement was also correlated with patient survival across the study period.

Methods:

161 consecutive subjects with ALS were recruited from two tertiary centres. The effect of limb dominance on the onset and spread of weakness was assessed using a standardised structured questionnaire, followed by non-parametric statistical analysis. The clinical severity of UMN and LMN signs in each limb was then determined using a published scoring system. These scores were used to quantify UMN and LMN dysfunction in dominant and non-dominant limbs. The differential involvement of dysphagia and dysarthria in bulbar ALS was also investigated in a subgroup (n = 39).

To further investigate the laterality of central nervous system (CNS) changes, a novel voxel-based morphometry (VBM) MRI protocol was used, previously utilised to define normal cortical gray matter (GM) asymmetries in healthy subjects. In the current study, it was applied to a cohort of right-handed healthy controls (n = 17), and ALS subjects with first weakness in either a right-sided or left-sided limb (n = 15 each). Between-group voxelwise comparisons of GM density were performed. Subsequently, within-group comparisons were performed to assess areas of GM asymmetry between the two hemispheres in both healthy controls and ALS subjects.

Finally, the variables affecting survival of the original cohort (n = 161) were investigated, in particular the effects of UMN and LMN scores. Subjects were followed to a censorship date, after which univariate analysis was performed to screen potential predictor variables of survival to non-invasive ventilation (NIV) or death. Potentially useful predictor variables were then used in a Cox regression model.

Results:

Onset of weakness was more likely in the dominant upper limb ($p = 0.02$), but not in the dominant lower limb ($p = 0.78$). Furthermore, there was a significant effect of limb dominance on spread of weakness beyond the initial limb. For example, in subjects with initial weakness in a non-dominant limb, spread was more likely to be to the other ipsilateral limb ($p = 0.008$), suggesting an important role for central (UMN) factors in driving disease spread.

Both UMN and LMN scores were maximal in the limb of onset, suggesting focality of onset. UMN signs spread to other limbs relatively early. A significant effect of dominance was also identified, specifically that the distribution of UMN signs in the upper limbs was affected by whether weakness

had first occurred on the dominant or non-dominant side ($p = 0.03$). There was no significant effect of limb dominance on the distribution of LMN signs.

Voxelwise MRI analysis revealed multifocal clusters of reduced GM density in ALS subjects compared with controls, incorporating both motor and non-motor areas. Normal regions of cortical asymmetry in right-handed healthy controls were also identified, including leftward GM asymmetry ($p \leq 0.01$) corresponding to the dominant hand representation area in the motor cortex. Right-handed subjects with ALS showed complete loss of GM asymmetry in this area, irrespective of whether weakness had first occurred in a dominant or non-dominant limb ($p < 0.01$). However, asymmetric atrophy of the left somatosensory cortex and temporal gyri was only observed in ALS subjects with right-sided (dominant) onset of weakness.

Predictor variables associated with reduced survival included older age, bulbar and respiratory involvement, and shorter diagnostic delay (all $p < 0.05$). Whole-body LMN scores were strongly associated with survival to NIV or death, whereas UMN scores were poorly associated with survival.

Conclusions:

Both the clinical and imaging findings support limb dominance as a significant factor underlying both onset and spread of ALS, with central (UMN) processes playing an important role in disease asymmetry. This effect of limb dominance on the presentation of ALS may reflect underlying developmental CNS vulnerabilities, which become exposed by the disease process. However, ultimately LMN factors more closely correlate with survival.

Declaration By Author

This thesis *is composed of my original work, and contains* no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, financial support and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my higher degree by research candidature and does not include a substantial part of work that has been submitted *to qualify for the award of any* other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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Publications Included In This Thesis

Devine MS, Woodhouse H, McCombe PA, Henderson RD. The relationship between limb dominance, disease lateralization and spread of weakness in amyotrophic lateral sclerosis (ALS). Amyotroph Lateral Scler Frontotemporal Degener 2013;14:150-1.

Devine MS, Farrell A, Woodhouse H, McCombe PA, Henderson RD. A developmental perspective on bulbar involvement in amyotrophic lateral sclerosis. Amyotroph Lateral Scler Frontotemporal Degener 2013;14:638-9.

Devine MS, Kiernan MC, Heggie S, McCombe PA, Henderson RD. Study of motor asymmetry in ALS indicates an effect of limb dominance on onset and spread of weakness, and an important role for upper motor neurons. Amyotroph Lateral Scler Frontotemporal Degener 2014;15:481-7.

Devine MS, Pannek K, Coulthard A, McCombe PA, Rose SE, Henderson RD. Exposing asymmetric gray matter vulnerability in amyotrophic lateral sclerosis. Neuroimage Clin 2015;7:782-7.

Devine MS, Ballard E, O'Rourke P, Kiernan MC, McCombe PA, Henderson RD. Targeted assessment of lower motor neuron burden is associated with survival in amyotrophic lateral sclerosis. Amyotroph Lateral Scler Frontotemporal Degener 2016;17:184-90.

Submitted Manuscripts Included In This Thesis

No further manuscripts submitted for publication.

Other Publications During Candidature

Conference Abstracts:

"Asymmetry in ALS (Clinical and Neuroimaging)", ANZAN Annual Scientific Meeting, 18-22 May 2014, Adelaide, Australia.

"Unravelling Asymmetry in MND: Combining Clinical Observations with Novel Imaging", RBWH Research Symposium, 18 October 2013, Brisbane, Australia.

"Asymmetry in ALS: The Effect of Limb Dominance", International Symposium on ALS/MND, 6-8 December 2013, Milan, Italy.

Contributions By Others To This Thesis

A/Prof Robert Henderson and Professor Pamela McCombe both contributed to the conception, design and review of the project, as conjoint principal advisors of the candidate. Both principal advisors performed the clinical assessment of UMN and LMN scores, along with Professor Matthew Kiernan. Professor Stephen Rose contributed to the design of the MRI protocol and image analysis (Devine et al, Neuroimage Clin 2015;7:782-7). Professor Peter O'Rourke contributed to statistical analysis for the survival paper (Devine et al, Amyotroph Lateral Scler Frontotemporal Degener 2016;17:184-90).

Statement Of Parts Of The Thesis Submitted To Qualify For The Award Of Another Degree

No works submitted towards another degree have been included in this thesis.

Research Involving Human Or Animal Subjects

Approvals were obtained from the relevant Human Research Ethics Committee prior to commencing the research. The following ethics approvals were obtained:

- HREC/11/QRBW/206 (Appendix 1.1)
- LNR/13/POWH/29 (Appendix 1.2)
- 2008/098 (Appendix 1.3)

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Keywords

Amyotrophic lateral sclerosis, anterior horn cell disease, motor neuron disease, handedness, limb dominance, voxel-based morphometry, asymmetry, survival, prognostic, clinical phenotype

Australian And New Zealand Standard Research Classifications (ANZSRC)

ANZSRC code: 110904, Neurology and Neuromuscular Diseases, 70%

ANZSRC code: 110320, Radiology and Organ Imaging, 30%

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Allows for categorisation of the thesis according to the field of research.

FoR code: 1109, Neurosciences, 70%

FoR code: 1103, Clinical Sciences, 30%

Dedications

I would like to dedicate this work to my wife Shuyang and daughter Isabelle, who have both supported me through many years of study, absences and late nights.

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1.1. Tables (located in Appendix 2)

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2. List of Abbreviations

ALS: amyotrophic lateral sclerosis

CNS: central nervous system

FDI: first dorsal interosseous (muscle)

GM: gray matter

LL: lower limb

LLL: left lower limb

LMN: lower motor neuron

LUL: left upper limb

NIV: non-invasive ventilation

MRI: magnetic resonance imaging

POWH: Prince of Wales Hospital

PNS: peripheral nervous system

RBWH: Royal Brisbane and Women's Hospital

RLL: right lower limb

RUL: right upper limb

UL: upper limb

UMN: upper motor neuron

VBM: voxel-based morphometry

WM: white matter

3. Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative condition, of which the primary cause in the majority of cases remains unknown although both genetic and environmental factors likely contribute (1,2). There is typically degeneration of both upper motor neurons (UMN) in the brain and spinal cord, and lower motor neurons (LMN) supplying the peripheral skeletal musculature. Patients present with weakness and wasting of limb muscles, dysphagia (difficulty swallowing), dysarthria (slurring of speech) and ultimately respiratory failure secondary to diaphragmatic insufficiency (3).

A key feature of ALS is significant variability in clinical presentation, especially in terms of the relative degree of UMN and LMN involvement, the disease duration and the effects of age and gender (1-5). However, despite this variability, certain characteristic and intriguing clinical patterns have been observed in ALS. For example, certain "clusters" have been described, including younger males presenting more often with upper limb onset and older females with bulbar onset (3). Clinical patterns have also been noted, including preferential weakness of the thenar eminence (the "split hand") (6) and early loss of ankle dorsiflexion (7). It is important to identify and investigate any other patterns within the ALS population, since these provide key clues regarding the pathophysiology of the disease. In particular, the existence of these patterns suggests that certain components of the nervous system have properties which make them particularly vulnerable to degeneration in ALS.

Turner et al (8) first hypothesised a link between limb dominance and self-reported onset of limb weakness in ALS. This effect has also been apparent in the datasets of some other previous studies (9,10). If so, this may represent another inherent vulnerability within the motor system which becomes exposed during the disease process. However, this finding has not been reported or

replicated in all large-scale population studies (11,12), and the effect (if any) of limb dominance on spread of weakness beyond the limb of onset has not been explored. Furthermore, it is not known whether any such effect of limb dominance is mediated through UMN and/or LMN processes (8). If limb dominance is shown to affect the presentation of ALS, this may provide important clues regarding why particular neuronal pathways are susceptible to the underlying disease process.

The central aim of this current project has been to identify and characterise novel sources of vulnerability in ALS, using multiple modalities. Of particular interest has been the relationship between limb dominance and the asymmetry of the disease. The initial stage involved assessment of a large cohort of subjects from two tertiary referral centres (the Royal Brisbane and Women's Hospital (RBWH) and the Prince of Wales Hospital in Sydney (POWH)). Conducting this study at these specialist tertiary sites allowed unique access to a large number of subjects with ALS, which has an incidence of 1.5 to 2.5 per 100,000 per year (13). To address the key question of whether limb dominance affects the onset and spread of limb weakness, a structured questionnaire was used, incorporating as variables the site(s) of onset and spread (upper limb, lower limb, bulbar) and side of the body, as well as standard limb dominance assessment tools. Next, to delineate separately the effect of limb dominance on objective UMN and LMN dysfunction, the published clinical scoring system of Ravits et al (9) was applied to each subject.

Since UMN and cortical factors are thought to play an important role in patterns such as the "split hand" (6,14,15), it is also important to specifically investigate the relationship between asymmetry of cortical degeneration in ALS and hemispheric motor dominance. Since clinical examination assessment of UMN dysfunction is complex (16), and there remains no unifying marker of UMN loss in ALS (17), this current project has applied a novel VBM protocol, previously used in healthy subjects (18) to visualise cortical gray matter asymmetry in subjects with ALS. The aim of this

phase of the project was to determine whether certain cortical areas are more significantly affected in the dominant (compared with non-dominant) hemisphere in subjects with ALS, as compared with the normal cortical asymmetries present in healthy members of the population (18).

In addition to the significant variability in clinical phenotype of ALS, there is also wide variation in survival. For example, subjects with more "pure" UMN and LMN syndromes typically have more prolonged survival (5), whereas others undergo a more fulminant rapid disease course (19). Variables such as age, gender and body region of onset are known to influence survival (4,5,19). Both UMN and LMN factors are also considerations in predicting survival (1,2), however the specific relative importance of each has not been previously investigated. Hence, the final phase of this project was to determine factors predicting survival of the original cohort, with particular interest in the UMN and LMN clinical scores.

4. Literature Review

Although the primary cause of ALS is not known, several large-scale observational studies have provided insight into the onset and spread of both UMN and LMN dysfunction in the disease. Ravits and colleagues performed a key study of 100 subjects (9) in which they identified that both clinical UMN and LMN signs show focal onset in the same body region, and appear to spread from that site along anatomical axes. In Ravits' study, a scoring system was used to retrospectively assess the severity of clinical UMN and LMN signs in each limb as documented in clinic notes, from 0 (no involvement) to 3 (significant and severe involvement). UMN signs include increased muscle tone, hyperreflexia, clonus and an upgoing plantar response and LMN signs include wasting and muscle weakness (3,9). Muscle fasciculations are variable in severity and generalisation (3), do not appear to correlate with the degree of muscle weakness (20), and may have a central as well as peripheral component (21).

Focal onset and contiguous spread of motor neuron degeneration has been proposed to occur along somatotopic planes in the nervous system, with UMN pathology initially spreading through the ipsilateral motor cortex and LMN pathology crossing early to the contralateral anterior horn (9,22). The concept of contiguous spread of LMN pathology is also supported by histological spinal cord specimens showing radial degrees of motor neuron loss from the spinal segments of onset (23), and other large-scale phenotypic studies, including those of Körner et al (10) and Gargiulo-Monachelli et al (12), have supported the finding of initial onset in a single body region. Another common observation has been that spread of pathology favours a rostro-caudal rather than caudo-rostral direction (9,10,12).

There remain, however, some discrepancies between the results of different studies of UMN and LMN signs in ALS. Although some authors have proposed that spread of UMN and LMN

pathology becomes independent after initial focal onset (9,24), Körner et al (10) observed at least some ongoing correspondence between UMN and LMN signs as the disease progresses. However, the same authors also described a greater propensity for UMN signs to be present early in the lower limbs, regardless of the site of onset of weakness (10). One potential explanation for this finding is that the UMN supplying the lower limbs are more susceptible to ALS pathology due to greater axonal length. Several studies have also reported subjects with spread of weakness between anatomically disparate regions (10,12), and an electrophysiological study found that 39% of examined subjects showed non-contiguous EMG changes suggestive of multifocal onset of LMN pathology (25).

Another unknown aspect of ALS is whether the primary onset of disease occurs in the UMN and/or LMN neuronal populations. Both “dying back” (26) and “dying forward” (27) hypotheses have been proposed for the directionality of ALS spread between neurons, however neither theory has been definitively proven (2,9). Assessment of the onset of ALS is also complicated by the fact that the disease is likely to have a prolonged pre-clinical asymptomatic phase of uncertain duration, most likely masked by compensatory mechanisms (28). Known pre-symptomatic carriers of pathogenic familial SOD1 mutations have been shown to have abnormal cortical hyperexcitability 3-8 months before symptom onset, as measured using transcranial magnetic stimulation (TMS) (29). Cortical hyperexcitability is also a known early feature of subjects with symptomatic ALS, and it is likely that calcium and glutamate-mediated excitotoxicity plays a role in this pathway (21). Loss of GABA-ergic inhibitory drive in the CNS (from interneurons) is also likely to contribute to cortical hyperexcitability in ALS (21,30). However, hyperexcitability, in terms of altered sodium and potassium conductances, has also been demonstrated in LMN axons in ALS subjects (21), and dysfunction of inhibitory interneurons within the spinal cord also may occur (30).

ALS also remains a highly heterogeneous disease, both at the patient level and the microscopic level. Some authors have even proposed that it is not a single disease entity, but rather a group of related disorders (2). Subjects present across a wide spectrum from those with UMN-predominant to LMN-predominant signs, although for inclusion in clinical studies they are usually required to fulfil the revised El Escorial criteria (31). 'Flail-arm' and 'flail-leg' variants have also been described, which initially present as LMN-predominant disorders of the proximal upper limbs or distal lower limbs respectively (32,33). Pure UMN and LMN disorders, termed primary lateral sclerosis (PLS) (34) and progressive muscular atrophy (PMA) (35) also exist, and subjects with these conditions are usually excluded from ALS studies. At the microscopic level, specimens from ALS subjects do not show a common pathological aggregate across the whole population, although TDP-43, SOD1, FUS and ubiquilin aggregates have all been described (2).

Subjects with ALS also show marked variability in survival from onset of symptoms. Those with bulbar or respiratory onset of disease have poor mean survival, whereas those with predominant or pure UMN or LMN features tend to present with more slowly progressive disease (5). Analysis of a cohort from the Australian Motor Neurone Disease Registry (AMNDR), also showed that subjects with a "global" phenotype (mixed UMN and LMN signs in at least two body regions at the time of presentation) had reduced survival compared with those having only LMN or UMN signs at this time (36). Other factors which have been associated with increased survival include greater time to spread beyond the limb of onset (37) and contiguous and caudo-rostral spread of weakness (12). In order to describe ALS study cohorts, while accounting for this significant phenotypic variability, it is important to use an inclusive but easily applicable disease staging system. One such system has been proposed by Roche et al (38). This system describes involvement of general body regions (upper limb, lower limb, bulbar), with Stage 1 showing involvement of one region, Stage 2A being disease diagnosis, Stage 2B showing involvement of two regions, and Stage 3 three regions.

Factors such as age and gender have also been shown to affect the phenotype and progression of ALS. The overall incidence of ALS is higher in males (3,11), especially among the younger spectrum of subjects (4). Males are also more likely to present with limb-onset disease, whereas females predominate in bulbar-onset ALS (4), however the reason for this is not known. Although there is no definite independent effect of gender itself on survival (4), certain "clusters" have been observed in the clinical population which share common survival traits. For example, presentation with an isolated bulbar palsy most commonly occurs in older females (39) who tend to show prolonged survival.

Despite the significant variability of ALS, several clinical patterns have been observed across a wide range of phenotypic groups. One such example is the "split hand" phenomenon, in which there is disproportionate wasting and weakness of the thenar/first dorsal interossous (FDI) complex, as compared with the hypothenar complex (6). There is evidence that this phenomenon has both central (UMN) and peripheral (LMN) components. Weber et al (14) reported that cortical motor inputs, as measured by TMS, were disproportionately reduced to the thenar complex as compared with the hypothenar complex in ALS subjects. Other studies have also described differences in the excitability of peripheral nerves supplying thenar and hypothenar muscles (40,41). However, recent evidence has suggested that cortical factors are more likely to be the primary driver of the "split hand" phenomenon (15). Disproportionate weakness of dorsiflexion over plantar-flexion has also been described (7). In contrast, there is typically preservation of extraocular and sphincter muscles throughout the clinical course of ALS (42).

A novel perspective on these patterns has been proposed – that they represent developmental vulnerabilities within the nervous system. It has been proposed that certain networks and pathways within the nervous system are more vulnerable to the disease process in ALS, due to more recent

development in evolution and therefore greater complexity (7,43). For example, the thenar/FDI complex has developed a significant function in the human pincer grip and fine manipulative movements, and consequently its somatotopic representation has disproportionately increased (7). Similarly, dorsiflexion has gained increasing importance as humans developed upright and bipedal gait (7). Several authors have proposed that gaining this complexity of function has come at the expense of greater vulnerability to accumulation of toxic products or errors, as is likely to occur in ALS (7,43).

Whilst most of these theories imply a cortical basis for this effect, there is also evidence that peripheral nerve and muscle populations are differently vulnerable in ALS. For example, it has been shown that fast-twitch fatigable motor units degenerate earlier in the disease course than slow units, including those controlling extraocular and sphincter muscles (42). It is also important to note that the neural input to extraocular and sphincter muscles is polysynaptic rather than direct (monosynaptic) as occurs with UMN and LMN to limb skeletal muscles (43), which may have implications for a "dying forward" or "dying back" hypothesis for disease spread. Although not studied to the same degree as the "split hand", it has also been suggested that since complex vocalisation is another recently evolved human function, onset of dysarthria in ALS may also represent another developmental vulnerability which becomes exposed by the disease process (7). Although several studies have reported the sequence of muscular involvement in bulbar ALS (44,45), this has not been examined in the context or developmental theories of the disease, and comparisons have not been made between subjects with UMN-predominant, LMN-predominant and mixed bulbar clinical signs. Furthermore, since frontotemporal dementia (FTD) has now been established as closely linked with ALS (2,46), it has been suggested that human "Theory of Mind" represents yet another area of susceptibility in the nervous system which undergoes early dysfunction in ALS (7).

Unilateral limb dominance is another function which has undergone significant development during human evolution. Although other primates and other vertebrates do show some lateralisation of limb function, humans are unique in having a strong, consistent species-level bias towards right upper limb dominance (47). This increased specialisation of upper limb activities has also been facilitated by the concurrent development of bipedal gait (7). The concept of limb dominance has been studied in great detail, yet it remains incompletely understood. Several MRI studies have demonstrated asymmetry of gray matter (GM) and white matter (WM) in dominant (compared with non-dominant) motor areas using voxel-based morphometry (VBM) (48,49). Others have also found significant differences in corticomotor white matter (WM) pathways in the dominant and non-dominant hemispheres using diffusion-weighted MRI imaging (49-51). However, not all studies have demonstrated a significant relationship between limb dominance and asymmetry of cortical motor areas and pathways (52,53). It is also important to control for other factors which influence cortical asymmetry, including age (50) and gender (52).

A study from the RBWH (18) applied a novel VBM asymmetry analysis protocol to structural MRI from healthy right-handed subjects, and was able to define an area of significantly greater GM density in the left motor cortex, corresponded to the thenar hand representation area localised by a previous population-based TMS study (54). Furthermore, the authors were able to demonstrate asymmetry of the cortical WM pathways projecting from this area, some of which correlated with handedness measures (18). As well as MRI imaging, other studies have demonstrated increased neuropil (55) and horizontal connecting fibres (56) in the dominant motor cortex, as well as asymmetric excitability of the dominant and non-dominant hemispheres (57). Despite these findings, the concept of dominance has only recently been mentioned in the ALS literature, and has not been thoroughly investigated.

The results of several recent studies have suggested that limb dominance is of importance in ALS. Ravits et al (9) and Körner et al (10) documented that onset of upper limb weakness was more likely to occur on the right side. However, this finding was not further explored, and other large-scale phenotypic studies have either not reported side of weakness (12) or failed to replicate this tendency toward upper limb onset (11). Turner et al (8) published a study of 343 subjects who completed a short survey via an online support community, in which they were asked to nominate the side of onset of limb weakness and whether they were right or left-limb dominant. The authors found a significant association between handedness and side of upper limb onset, but no relationship between footedness and lower limb onset (8). Potential explanations for this finding provided in the paper included asymmetric physical stress of LMN supplying the dominant limb, or greater vulnerability of the dominant hemisphere corticomotor networks (8). However, the authors acknowledged that it remains unclear whether any effect of limb dominance on onset of weakness is mediated via UMN and/or LMN factors (8), and also spread of weakness beyond the initial limb was not analysed.

Another shortcoming of this study was the inability to diagnostically screen participants or apply standardised limb dominance tools. Several validated dominance tools have been widely used in the literature, including the Edinburgh Handedness Inventory (58) which has recently been revised (59) and the Waterloo Footedness Questionnaire (60). Although physical measures such as grip strength and finger tapping may be more accurate than self-reported questionnaires (18), these are not applicable in an ALS population in which muscle weakness may bias the result.

Overall, the current ALS literature supports an asymmetric, focal spreading disorder of both UMN and LMN, which is highly heterogeneous yet also presents with characteristic clinical patterns such as the “split hand”. There are theories that pre-existing developmental vulnerabilities within the

nervous system become exposed during the disease process, yet the effect of limb dominance in ALS has not been explored in detail.

5. Hypotheses

The overall aim of this project has been to identify and characterise novel sources of vulnerability in a large cohort of ALS subjects, with a particular focus on asymmetry, clinical phenotype and the effect of limb dominance. Multiple modalities have been employed in addressing this question, including structured questioning, direct clinical examination and scoring, and advanced imaging analysis. The major hypotheses are as follows:

1) That the side of onset of limb weakness in ALS is affected by limb dominance.

2) That spread of weakness beyond the initial limb of onset is also affected by dominance.

3) That any effect of limb dominance on onset and spread of weakness is also reflected in the asymmetry of carefully scored clinical UMN and/or LMN signs on limb examination.

4) That any significant asymmetry of clinical UMN involvement may be studied in more detail using novel MRI imaging approaches, which have been previously used to define normal cortical asymmetries in healthy subjects.

5) That the concept of vulnerability can be extended more broadly to other clinical aspects of ALS, including bulbar disease, and that the differentiation between clinical UMN and LMN involvement is also important in this case. Specifically, it is hypothesised that speech is more vulnerable to early dysfunction in ALS, compared with swallowing, due to greater evolutionary complexity.

6) That by following the original cohort of ALS subjects over time, phenotypic factors affecting survival can be assessed. In particular, an association is predicted between limb LMN scores and survival to death or non-invasive ventilation (NIV), potentially due to the association with diaphragmatic weakness.

6. Asymmetry in Onset and Spread of Limb Motor Involvement in ALS

6.1. Preface

This chapter incorporates the text of two peer-reviewed publications in their entirety:

(1) Devine MS, Woodhouse H, McCombe PA, Henderson RD. The relationship between limb dominance, disease lateralization and spread of weakness in amyotrophic lateral sclerosis (ALS). Amyotroph Lateral Scler Frontotemporal Degener 2013;14:150-1. (Section 6.2). (61).

(2) Devine MS, Kiernan MC, Heggie S, McCombe PA, Henderson RD. Study of motor asymmetry in ALS indicates an effect of limb dominance on onset and spread of weakness, and an important role for upper motor neurons. Amyotroph Lateral Scler Frontotemporal Degener 2014;15:481-7. (Section 6.3). (62).

The first paper was a pilot questionnaire-based study of 95 subjects, which identified some initial trends (Chapter 6.2). These trends were further investigated and expanded in a larger study of 158 screened subjects, incorporating both questionnaire-based assessment and direct clinical examination and scoring (Chapter 6.3). As detailed subsequently, some trends only became statistically significant upon analysis of the larger second cohort of subjects. For example, the relationship between handedness and upper limb onset, as well as the effect of dominance on disease spread.

As first author of both publications, the author directly contributed to the conception and design of the project, data collection, interpretation and statistical analysis of data, and drafting and revising both publications. Clinical assessment of subjects' UMN and LMN scores, as well as professional editorial guidance, were contributed to by neurologists A/Prof Henderson and Prof McCombe from RBWH in Brisbane, and Prof Kiernan from POWH in Sydney.

6.2. Preliminary Assessment of Limb Dominance and ALS Symptoms

Amyotrophic lateral sclerosis (ALS) is a heterogeneous disorder, and the mechanisms governing its site of onset and spread of weakness remain unknown. Turner et al. (8) proposed that onset of ALS in an upper limb is likely to be concordant with handedness. This finding has implications for theories regarding ALS pathogenesis. It is important to further explore this, and assess whether limb dominance affects spread of disease.

A questionnaire regarding the initial site (and side) of disease onset and the location of subsequent weakness, was completed by 95 consecutive patients with ALS. The questionnaire included the Edinburgh Handedness Inventory (EHI) (59), with an added question regarding footedness. Statistical analysis was performed using SPSS (V.15, SPSS Inc).

The mean age of the patients was 61 years (SD 11 years) and 58% were males. All patients had clinically probable or definite ALS according to Airlie House criteria. The majority (91%) had no known family history of ALS. Ninety percent of patients were right-handed, and the remainder either left- or mixed-handed. The right lower limb (LL) was dominant in 88% of cases, with 12% being left- or mixed-footed.

If the side of onset of upper limb (UL) ALS is unrelated to handedness, the following null hypothesis should hold true: a right-handed individual should have a 50% probability of disease occurring in either the right or left UL. A binomial test was performed using data from all right-handed patients who reported unilateral UL onset (n = 29). Of these, 17 (59%) had onset in the RUL, and 12 (41%) in the LUL; p = 0.46. Of the patients who were non-right-handed (i.e. left-

handed or ambidextrous), UL onset was reported as bilateral or poorly lateralized for three of five subjects, whereas this was the case for only seven of 52 right-handed subjects.

The present study confirmed the finding of Turner et al. (8) that there was no concordance between footedness and side of ALS onset in the LL. In 13 (46%) of right-footed patients with LL onset, the onset was in the RLL, compared with the LLL in 15 cases (54%); $p = 0.85$.

We also studied the spread of weakness. Of the patients with unilateral UL onset whose weakness spread to involve another limb (n = 22), 10 subjects (45%) showed spread to the contralateral UL and 10 (45%) showed spread to the ipsilateral LL. A Fisher's exact test showed that the spread of weakness in right-handers was not significantly different for onset on the right (dominant) or left (non-dominant) side; $p = 1.0$.

The pattern of spread was different for the LL, where the most frequent secondary site for patients with unilateral disease onset (n = 25) was the contralateral LL (n = 18, 72%), with the ipsilateral UL less likely (n = 5, 20%). This predilection for LL onset ALS to spread to the contralateral LL has been previously recognized (37). The progression of LL onset ALS in right-footed patients was not significantly affected by whether first onset of symptoms was on the right or left side (Fisher's exact test; $p = 0.60$).

Irrespective of limb dominance, it was found that spread of weakness differed between UL- and LL onset disease. Spread from a UL site of onset was more evenly balanced between the contralateral UL and the ipsilateral LL, whereas spread from an LL site of onset was strongly to the contralateral

LL (χ2 contingency test; χ 2 (1, n = 43) = 3.76; p = 0.052). Our finding was consistent with the notion that ALS spread favours a 'rostro-caudal' rather than 'caudo-rostral' pattern (12).

There was a novel suggestion that right-dominant individuals with ALS starting in a non-dominant limb (i.e. left UL or LL) were more likely to show ipsilateral spread (Table 1, Appendix 2). This was non-significant, χ2 (1, n =38) = 0.208; p = 0.65, but based on small patient numbers, and it would be useful to explore this in a larger study.

In summary, we found no significant effect of handedness on the onset or subsequent pattern of disease spread. We confirmed the results of Turner et al. (8), showing no concordance between footedness and side of LL ALS onset. We found that secondary spread of UL-onset ALS was evenly balanced between the contralateral UL and ipsilateral LL, but this was not the case with LL-onset ALS which strongly showed spread to the contralateral LL. Further investigation could include differentiation of upper and lower motor neuron features of ALS, which have focal onset and independent spread (22).

6.3. Laterality of ALS Symptoms and Clinical Signs in a Larger Cohort

Abstract

In amyotrophic lateral sclerosis (ALS), onset and spread of upper motor neuron (UMN) and lower motor neuron (LMN) dysfunction is typically asymmetric. Our aim was to investigate the relationship between limb dominance and the onset and spread of clinical UMN and LMN dysfunction in ALS. We studied 138 ALS subjects with unilateral and concordant limb dominance, from two tertiary centres. A questionnaire was used to determine the pattern of disease onset and spread. The clinical severity of UMN and LMN signs in each limb was quantified using a validated scoring system. Results showed that onset of weakness was more likely to occur in the dominant upper limb ($p = 0.02$). In subjects with initial weakness in a non-dominant limb, spread of weakness was more likely to be to the other limb on that side ($p = 0.008$). The relative distribution of upper limb UMN signs was affected by whether weakness first occurred on the dominant or non-dominant side ($p = 0.03$). These findings support limb dominance as a significant factor underlying onset and spread of ALS, with UMN processes playing an important role. The effect of limb dominance on the presentation of ALS may reflect underlying neuronal vulnerabilities, which become exposed by the disease.

Introduction

Amyotrophic lateral sclerosis (ALS) is characterized by progressive degeneration of upper motor neurons (UMN) and lower motor neurons (LMN) (1,2). Previous studies have demonstrated that both UMN and LMN degeneration typically show focal onset and subsequent spread along anatomical axes (9,10,12). However, there is marked phenotypic variability, including the site of onset and the degree of UMN and LMN involvement.

A further unexplained feature of ALS is the asymmetric onset of weakness. There is preliminary evidence suggesting that limb dominance may influence the side of upper limb onset (8-10); however, other large-scale phenotypic studies have reported only slight differences (11) or did not specify the side of the body (12). It is also unknown whether such an effect would be mediated though UMN or LMN processes (8). Furthermore, the factors underlying the direction of disease spread remain poorly understood. Carefully studying the asymmetry of ALS may improve understanding of why certain components of the nervous system are particularly vulnerable to the degenerative process.

The present study investigated the relationship between limb dominance and the onset and spread of limb weakness in 138 subjects with ALS. This study prospectively expanded upon the original cohort from our observational pilot study (61), incorporating both questionnaire-based assessment and objective scoring of clinical UMN and LMN signs. Our hypothesis was that the asymmetry of ALS onset and spread would be associated with limb dominance.

Materials and Methods

Conduct of this study was approved by the Royal Brisbane and Women's Hospital Ethics Committee and the South Eastern Sydney Local Health District Ethics Committee. All subjects provided written informed consent.

We screened 158 consecutive subjects from outpatient ALS clinics at the Royal Brisbane and Women's Hospital and Prince of Wales Hospital, Sydney. One hundred and thirty-eight of these subjects were included in the final cohort, based on a history of unilateral dominance for motor tasks (prior to onset of ALS), which was concordant for the upper limb (UL) and lower limb (LL).

Twenty subjects with discordant UL and LL dominance, or mixed dominance in either region, were excluded. Limb dominance was determined using a revised version (59) of the Edinburgh Handedness Inventory (58) with an added question regarding footedness for kicking a ball (a commonly lateralized LL activity).

All subjects had diagnoses of clinically probable (or probable laboratory-supported) or definite ALS, according to the revised El Escorial criteria (31). Some subjects met published definitions of flail arm (32) and flail leg ALS (33) at the time of assessment, but fulfilled El Escorial criteria at a subsequent visit. Subjects presented across the spectrum from UMN-predominant to LMN-predominant ALS; however, those with primary lateral sclerosis or progressive muscular atrophy were specifically excluded.

Onset and spread of weakness

Each subject was asked to complete a standard questionnaire, which included demographic information (age, gender, family history). Subjects were asked to specify the site and side of onset of weakness, by ticking a single box in a table. The available options to tick in the table were: the UL (right or left), LL (right or left) or bulbar region. There was also the option to tick a box indicating that onset of weakness (in the UL or LL) was difficult to lateralize. Where applicable, subjects were also able to specify the order of up to two sites of subsequent disease spread, again by ticking a single box in each of two additional tables. In each case, subjects' written responses (regarding the sequence of weakness) were confirmed by careful review of the history documented by the treating neurologist. If any discrepancy was found, the relevant subject was questioned to clarify the exact sequence of weakness, without using any potentially leading or biased phrases.

UMN and LMN scores

Clinical UMN and LMN involvement were quantified using the scoring system of Ravits et al. (9), which assigned a separate UMN and LMN score from 0 (no involvement) to 3 (significant and severe involvement) to each limb. This scoring system was applied by an experienced ALS clinician (MK, PM, or RH) within 12 months of diagnosis of ALS or the time of first limb involvement. For subjects who presented outside this window, chart review was conducted to apply the scoring system to an earlier visit, provided a thorough and consistent clinical examination was documented by one of the ALS clinicians. Clinical scoring of UMN and LMN signs was able to be performed in 130 of the 138 subjects (94%).

Statistics

Statistical analysis was performed using SPSS (Version 20, SPSS Inc.). To determine the relationship between limb dominance and the onset and spread of weakness, non-parametric tests of the binomial and $\chi 2$ families were used. For all calculations involving disease spread and clinical UMN and LMN scores, we defined the first limb affected by weakness (either as the initial site of onset or immediately after bulbar onset) as the Index Limb. All other limbs were termed Non-Index Limbs. For simplicity in determining the effect of dominance on disease onset and spread, the right- and left dominant subjects were combined.

To assess the cohort as a whole, subjects were divided into two groups according to which was the Index Limb (UL or LL), irrespective of dominance. Following the approach of Ravits et al. (9), we calculated Total UMN and LMN scores for each limb. In order to quantify the relative contribution of clinical UMN (compared with LMN) dysfunction, we also calculated UMN% and LMN% scores for each limb of each individual subject, using the following formulae:

UMN% Score = UMN Score / (UMN Score + LMN Score) x 100

LMN% Score = LMN Score / (LMN Score + UMN Score) x 100

Individual scores were then used to calculate mean UMN% and LMN% scores (± SE) for the Index Limb and each Non-Index Limb. These mean scores were then compared using ANOVA.

To determine whether limb dominance influenced the distribution of UMN and/or LMN dysfunction, the cohort was further subdivided into four groups, according to the dominance of the Index Limb: dominant UL, non-dominant UL, dominant LL and non-dominant LL. In each individual subject, the asymmetry of UMN and LMN signs was quantified using 'proportional scores'. A pair of proportional scores defined a comparison between two limbs (the Index Limb and a Non-Index Limb), with both scores adding to give 100%. The following formulae were used:

Proportional Score for Index Limb = (Score in Index Limb/Combined Score for Both Limbs) x 100.

Proportional Score for Non-Index Limb = (Score in Non-Index Limb/Combined Score for Both Limbs) x 100

Proportional scores were calculated separately for UMN and LMN signs. Individual proportional scores were used to calculate mean scores, which were compared using Student's *t* -tests.

Results

Cohort characteristics

Of the cohort of 138 subjects, 52% were males and 93% had no known family history of ALS. The mean (± SD) age at presentation was 61 (± 11) years (range 29 – 85 years). Disease onset was in the UL in 43 subjects (31%), LL in 57 subjects (41%) and bulbar region in 38 subjects (28%). One hundred and thirty-four subjects (97%) were right-dominant for both UL and LL, and four subjects (3%) were concordantly left-dominant.

Defining the Index Limb

Across the entire cohort, 115 subjects (83%) were able to lateralize initial weakness to a single Index Limb. These subjects either had initial onset in that limb (37 in an UL, 51 in a LL), or had bulbar-onset disease that then spread to one limb (20 to an UL, seven to a LL). The remaining 23 subjects either had symptoms confined to the bulbar region ($n = 9$) or were unable to lateralize the onset of limb weakness ($n = 14$). The distribution of all 138 subjects, according to site of onset and subsequent spread of weakness, is shown in Figure 1 (Appendix 3).

Effect of limb dominance on initial onset of weakness

In the 37 subjects with initial weakness in one UL, onset was more likely to occur in the dominant UL ($n = 26$, 70%) than in the non-dominant UL ($n = 11$, 30%) ($p = 0.02$ using a binomial test). This laterality of UL onset was not significantly affected by gender ($\chi 2$ (1, $n = 37$) = 0.11; $p = 0.74$). In the 51 subjects with unilateral LL onset of weakness, there was no tendency toward onset in the dominant LL ($n = 24$, 47%) over the non-dominant LL ($n = 27$, 53%) ($p = 0.78$).

Effect of limb dominance on spread of weakness

Of the 115 subjects with a unilateral Index Limb (either as the initial limb of onset or the first limb after bulbar onset), 99 reported subsequent spread to a second discrete limb. When considering these subjects, we found that spread of weakness was influenced by whether the Index Limb was on the dominant or non-dominant side. As shown in Figure 2 (Appendix 3), when the Index Limb was dominant, weakness most commonly spread to the contralateral limb. When the Index Limb was on the non-dominant side, weakness was more likely to spread ipsilaterally to the other limb on that side; ($\chi 2$ (1, n = 97) = 7.10; p = 0.008). There was no confounding effect of gender on these patterns of spread; p = 0.49 using logistic regression.

Distribution of limb UMN and LMN scores

One hundred and eight subjects reported weakness in a unilateral Index Limb, and also underwent clinical scoring of UMN and LMN signs. These subjects were initially divided into two groups according to the Index Limb, irrespective of whether this was on the dominant or non-dominant side (55 in an UL, 53 in a LL). As shown in Figure 3 (Appendix 3), when individual subject scores were summated together, the Total UMN and Total LMN scores were greatest in the Index Limb, suggesting initial focal onset of both UMN and LMN dysfunction.

In the 55 subjects with an Index UL (for which the Total UMN score was 84), the next highest Total UMN score (80) was in the ipsilateral LL. In the 53 subjects with an Index LL (for which the total UMN score was 105), the next highest Total UMN score (93) was in the contralateral LL and both sides of the body demonstrated a rostro-caudal gradient in Total UMN scores. For all subjects, the Total LMN score was always second highest in the limb contralateral to the Index Limb, supporting radial spread.

To quantify the relative contribution of UMN and LMN to the clinical deficit in each limb, we next calculated mean UMN% and LMN% scores (± SE) as outlined in the Methods. A key finding from our cohort was the presence of greater UMN (relative to LMN) dysfunction in limbs more anatomically distant from the Index Limb. Specifically, as shown in Figure 4 (Appendix 3), mean UMN% scores were consistently lowest in the Index Limb and highest in the contralateral limb most distant from the Index Limb. In our cohort, the clinical evidence for UMN dysfunction in these Non-Index limbs was most commonly hyperreflexia.

Effect of limb dominance on UMN and LMN scores

To delineate the effect of limb dominance on the distribution of clinical UMN and LMN signs, the subjects shown in Figure 3 were further subdivided into four groups according to the dominance of the Index Limb (35 subjects in the dominant UL, 20 in the non-dominant UL, 25 in the dominant LL and 28 in the non-dominant LL). Using mean proportional scores, we found that the relative distribution of UMN signs in the UL was affected by limb dominance (Figure 5, Appendix 3). In subjects with a dominant Index UL, the proportional UMN scores were well balanced between the two upper limbs (51%:49%). In contrast, subjects with a non-dominant Index UL showed more asymmetric proportional UMN scores in the upper limb region (59%:41%) ($p = 0.03$). Practically, this suggested that subjects with onset of weakness in the non-dominant UL exhibited more pronounced and asymmetric UMN deficits involving that limb.

There were no significant effects of limb dominance on the relative distribution of UMN scores in subjects with an Index LL. Limb dominance did not significantly affect the distribution of LMN scores following onset of weakness in an Index UL or Index LL.

Discussion

This study investigated the relationship between limb dominance and the onset and spread of motor dysfunction in ALS, in a cohort of 138 well characterized subjects. Using a validated clinical scoring system and limb dominance tools, we established that limb dominance influences the onset and spread of weakness in ALS. We also found that the distribution of UMN signs was affected by limb dominance, and showed greater evidence of spread between body regions compared to LMN signs. To the authors' knowledge, this is the first study linking the objective clinical motor neuron phenotype with limb dominance, and expands on the work of Ravits et al (9,22) and Turner et al. (8).

Rather than onset being entirely random, the present study suggests that it is more likely for weakness to begin in the dominant UL, and also supports the previous finding (9,22) that UMN and LMN dysfunction appear maximal in the limb of onset. Preferential onset of weakness in the dominant UL could reflect greater use-dependent stress of LMNs supplying that limb (2). Another possibility is that the cortical networks associated with the dominant UL are particularly vulnerable to disease triggers in ALS.

Intriguingly, limb dominance also appears to influence the spread of weakness. This novel finding was supported by objective clinical data, showing that limb dominance influenced the asymmetry of clinical UMN signs in the upper limbs. In contrast, the distribution of LMN signs was not significantly affected by the location and dominance of the Index Limb, with a predictable preference for contralateral spread. Such a finding would be consistent with histological evidence suggesting a radial pattern of LMN degeneration in the spinal cord (23). Furthermore, clinical evidence from the current study demonstrated a higher frequency of UMN involvement in anatomically distant limbs. This suggests that spread of dysfunction at the UMN level occurs

relatively early (compared to LMN), although another potential explanation for this finding is the greater physical distance across which LMN pathology must spread, compared with UMN in the motor cortex (22). Overall, the findings from this study are clinically useful in predicting patterns of spread of weakness beyond the Index Limb, and suggest that the UMN plays an important role in this process.

It has been proposed that certain cortical networks are more vulnerable to early degeneration in ALS, due to more recent development in evolution and a disproportionate increase in complexity (43). A well-studied example is the 'split hand' (6). We hypothesize that the cortical networks involved in limb dominance may be similarly vulnerable in ALS. Although primates and other vertebrates show some lateralization of limb motor function, humans are unique in having a strong, consistent species-level bias towards right UL dominance (47). MRI studies have also shown significant leftward asymmetry in cortical hand representation and projecting white matter pathways in right-handers (18), and disproportionate evolutionary expansion of these networks could make them more vulnerable to stressors in ALS. In contrast, we found no effect of footedness on the onset of LL weakness. This may reflect reduced asymmetry of cortical control of the LL compared with the UL (66), or may imply lesser overall complexity of cortical representation of the lower extremities, irrespective of hemispheric dominance. However, an alternative explanation for this finding is that the LMNs supplying the LL are subjected to more symmetrical physical stressors during daily activities (such as ambulation).

If the effect of limb dominance on spread of weakness is mediated though cortical (UMN) factors, the direction of spread may reflect underlying differences between the two hemispheres and their communication. Corpus callosal involvement is an early feature of ALS (67), and inherent directionality within this structure (68) may explain why ALS onset in a non-dominant limb is more

likely to spread ipsilaterally. Alternatively, the connections between motor areas within the non-dominant hemisphere may be more vulnerable to contiguous spread of ALS pathology. Pre-existing differences in excitability of the dominant and non-dominant hemispheres (57) may also be important in ALS, since neuronal hyperexcitability occurs early in the cortical compartment during the disease (21). Since the formation and accumulation of aberrant gene products has been implicated in the pathogenesis of ALS (2), it is conceivable that the disproportionate complexity of the dominant UL representation area makes it more vulnerable to the effects of abnormal gene expression, although this requires further study.

In terms of limitations of the current study, although care was taken with administration of the questionnaire, collection of subjective information is limited by recall bias. For example, subjects may have been more aware of early weakness in a dominant limb. From a clinical perspective, it is accepted that both UMNs and LMNs undergo subclinical degeneration of uncertain duration prior to clinical signs being apparent (21,28), and that EMG would have been helpful for LMN assessment. Assessment of UMN dysfunction via clinical examination may also be complicated by concurrent changes in interneurons (30) and other descending pathways (16). There are also limitations in grouping right- and left-dominant subjects, since it is known that left-handers do not exhibit the same degree of population-wide bias toward dominance of the contralateral hemisphere (for both motor and language functions) as that found in right-handers (69). Therefore, we predict that the effect of limb dominance in non-right-dominant ALS subjects may be more complex. Although subjects with initial bulbar onset of disease were not analysed separately in this study, the authors intend to perform a further prospective study of bulbar-onset subjects, expanding upon our recent pilot series (61).

To further investigate the issues arising from the present study, neuroimaging, electrophysiology and assessment of asymmetric frontotemporal deficits will be useful.

7. Applying Vulnerability Concepts to Non-Limb Presentations of ALS

7.1. Preface

This chapter incorporates the text of one peer-reviewed publication in its entirety:

(1) Devine MS, Farrell A, Woodhouse H, McCombe PA, Henderson RD. A developmental perspective on bulbar involvement in amyotrophic lateral sclerosis. Amyotroph Lateral Scler Frontotemporal Degener 2013;14:638-9. (63).

The two papers in Chapter 6 established a link between limb dominance and asymmetry of both the onset and spread of ALS in the limbs (61,62). These findings were intriguing, especially in the context of the broader theory of selective developmental vulnerability in ALS (7). Although these studies also included subjects with bulbar-onset ALS with subsequent spread to a limb, they did not separately analyse the bulbar subgroup in more detail. The aim of the paper in Chapter 7 was to explore the bulbar ALS group in more detail, in particular the differential involvement of speech and swallow dysfunction in the context of UMN and LMN degeneration.

As first author of this publication, the author directly contributed to the conception and design of the project, collection, interpretation and statistical analysis of data, and drafting and revising the paper. Clinical assessment of subjects as well as professional editorial guidance, were contributed to by neurologists A/Prof Henderson and Prof McCombe from RBWH. Dr Anna Farrell, speech pathologist at RBWH, also assisted with data collection.

7.2. Developmental Perspectives on Bulbar ALS

There has been recent interest in the developmental and evolutionary aspects of amyotrophic lateral sclerosis (ALS). The overall theme has been that certain cortical circuits are vulnerable to early degeneration because they have developed more recently in human evolution (43). Examples of vulnerable circuits include preferential weakness of the thenar/FDI complex important in the pincer grip ('split hand'), and the ankle dorsiflexors that assist in upright gait. Examples of less vulnerable functions are the extraocular and sphincter muscles. Although Eisen (43) identified speech as another potentially vulnerable function in ALS, due to complexity of cortical representation, this has not been quantified in a clinical cohort.

To evaluate whether speech function is vulnerable to early involvement in ALS, the differential onset of dysarthria and dysphagia was investigated in a small series of consecutive patients (n = 39) from the Royal Brisbane and Women's Hospital ALS clinic. All patients met El Escorial criteria for probable or definite ALS, and all had definite bulbar involvement. Fifty-nine percent were males and mean age was 61 years (SD 11 years). In 56%, disease onset was in the bulbar region, and the remainder had limb onset with bulbar spread. We included six recently deceased patients with clearly documented and verified history and examination. Patients were asked the date (in months) when they first noticed speech change (slurring, or altered quality), and swallow change (coughing, choking, or dietary change). Verification was also sought from family or accompanying members.

We inquired about motor speech function, rather than language generation and semantics. Patients were divided into three groups according to clinical examination of the bulbar region: as upper motor neuron (UMN)-predominant, lower motor neuron (LMN)-predominant, or having balanced bulbar UMN and LMN signs. This was similar to a classification published by our group (70) but applied specifically to the bulbar region, and based on examination of UMN signs (brisk jaw jerk,

tongue spasticity, spastic dysarthria) and LMN signs (tongue wasting, fasciculations, flaccid dysarthria) at the last clinical encounter. Statistical analysis was performed using SPSS (Version 20, SPSS Inc.).

The reported onset of dysarthria occurred before dysphagia in 29 patients (74%), concurrent with dysphagia in six patients (16%), and after dysphagia in four patients (10%). Of the 29 patients reporting dysarthria first, the mean delay until reported onset of dysphagia was 8.3 months (SD 6.1 months; range 1 – 24 months), with no significant differences according to gender ($p = 0.864$), age ($p = 0.874$), or whether the initial onset site was bulbar or limb ($p = 0.491$).

When considering patients with onset of dysarthria before dysphagia ($n = 29$), the mean delay between the two symptoms appeared to be affected by clinical phenotype. The mean dysarthria-dysphagia delay in the UMN-predominant patients ($n = 11$) was 11.8 months (SD 7.8 months), whereas for patients with both UMN and LMN signs ($n = 16$) it was 5.9 months (SD 3.7 months); $p = 0.035$ corrected. However, two patients with LMN-predominant bulbar signs also described dysarthria occurring before dysphagia (by six and 10 months). All patients who reported onset of dysphagia before dysarthria ($n = 4$) had evidence of balanced bulbar UMN and LMN signs.

Our findings suggest a tendency towards onset of dysarthria occurring before dysphagia in patients with ALS. This observation occurred across all clinical phenotypes, but most prominently and consistently in patients with UMN-predominant bulbar signs. In general, patients with UMN-predominant disease have slower progression than those with equal UMN and LMN signs (5). The occurrence of dysarthria preceding dysphagia also concurs with previous reports, including a population-based study by Traynor et al. (71).

However, we also found evidence of wide variability in the dysarthria-dysphagia delay between individuals of all phenotypes. The limitations of this study include small patient numbers, potential bias towards personal recall of dysarthria (due to easier recognition by patients and families), and the lack of formal cognitive assessment. However, our findings appear to support Eisen's theory that the complex neocortical networks involved in the mechanics of human speech are vulnerable to earlier degeneration in ALS compared with other functions (43). This is compared with deglutition, which has a greater component of subconscious brainstem-mediated reflex activity, and which is shared with other animals. However, it is important to consider that peripheral factors could also play a role in selective vulnerability of certain functions, including altered nerve excitability, which may contribute to the 'split hand' (6).

8. Imaging Cortical Asymmetry in ALS

8.1. Preface

This chapter incorporates the text of one peer-reviewed publication in its entirety:

(1) Devine MS, Pannek K, Coulthard A, McCombe PA, Rose SE, Henderson RD. Exposing asymmetric gray matter vulnerability in amyotrophic lateral sclerosis. Neuroimage Clin 2015;7:782-7. (64)

The findings presented in Chapter 6 revealed a link between limb dominance and asymmetry of limb onset of ALS, as well as the spread of the disease to other limbs. In particular, the relatively early spread of UMN signs beyond the limb of onset (compared with LMN signs), as well as the patterns of ipsilateral spread, imply an important role for UMN factors in driving this process. Imaging remains a valuable tool in assessing CNS abnormalities in ALS, particularly given the complexity of clinical examination (16) and lack of an alternative unifying central biomarker (17). The peer-reviewed study presented in Chapter 8 further investigated the asymmetry of cortical gray matter in both healthy controls and ALS subjects, including analysis of the effect of limb dominance. The 30 ALS subjects included some from the clinically examined cohort, as well as others who only participated in the imaging study.

As first author of this publication, the author directly contributed to the conception and design of the project, collection, interpretation and statistical analysis of data, and drafting and revising the paper. The author personally performed the processing of the raw MRI data and statistical analysis. Original design of the MRI protocol and assistance with advanced imaging analysis were also contributed by Prof Stephen Rose and Dr Kerstin Pannek.

8.2. Assessment of Gray Matter Asymmetry in ALS using Novel MRI Techniques

Abstract

Limb weakness in amyotrophic lateral sclerosis (ALS) is typically asymmetric. Previous studies have identified an effect of limb dominance on onset and spread of weakness, however relative atrophy of dominant and nondominant brain regions has not been investigated. Our objective was to use voxel-based morphometry (VBM) to explore gray matter (GM) asymmetry in ALS, in the context of limb dominance. 30 ALS subjects were matched with 17 healthy controls. All subjects were right-handed. Each underwent a structural MRI sequence, from which GM segmentations were generated. Patterns of GM atrophy were assessed in ALS subjects with first weakness in a right-sided limb (n = 15) or left-sided limb (n = 15). Within each group, a voxelwise comparison was also performed between native and mirror GM images, to identify regions of hemispheric GM asymmetry.

Subjects with ALS showed disproportionate atrophy of the dominant (left) motor cortex hand area, irrespective of the side of first limb weakness ($p < 0.01$). Asymmetric atrophy of the left somatosensory cortex and temporal gyri was only observed in ALS subjects with right-sided onset of limb weakness. Our VBM protocol, contrasting native and mirror images, was able to more sensitively detect asymmetric GM pathology in a small cohort, compared with standard methods. These findings indicate particular vulnerability of dominant upper limb representation in ALS, supporting previous clinical studies, and with implications for cortical organisation and selective vulnerability.

Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative condition affecting upper (UMN) and lower motor neurons (LMN) (1,2). Understanding the pathophysiology of ALS is challenging, due to significant variability of clinical phenotype, patient characteristics and disease progression (2,5).

Despite this variability, common patterns have been observed across a wide range of ALS subjects. A well-studied example is the "split hand" phenomenon, in which there is disproportionate weakness of the thenar/first dorsal interosseous muscle group (6,7). Early weakness of ankle dorsiflexors (7) and speech (63) has also been observed. These findings have prompted suggestions that functions which humans have evolved more recently, such as the pincer grip and upright stance, are more susceptible to ALS (7,43).

Onset of weakness in ALS is also typically asymmetric. However, the factors determining the side of onset and direction of spread remain unclear. Since humans have evolved strong population-wide upper limb dominance (47), it is important to explore this as another potential source of vulnerability in ALS. It has been shown that the dominant upper limb, but not lower limb, is more susceptible to onset of weakness (8). We have also described that spread of weakness and UMN signs are affected by dominance, suggesting importance of central factors (62).

The aim of this study was to investigate gray matter (GM) asymmetry in ALS, and thus identify regions asymmetrically affected by the disease. Applying voxel-based morphometry (VBM) analysis of structural MRI, we performed direct comparisons between ALS subjects and controls, as well as using a novel asymmetry protocol to assess interhemispheric differences (18). Our

hypothesis was that this asymmetry protocol would detect patterns of disproportionate atrophy in ALS, which would be affected by whether weakness first occurred in a dominant or non-dominant limb.

Materials and Methods

Subjects and recruitment

Ethical approval was obtained from the Royal Brisbane and Women's Hospital (RBWH) Human Research Ethics Committee. All subjects provided written informed consent, and all research was conducted in accordance with the Declaration of Helsinki.

Thirty right-handed subjects were recruited from ALS outpatient clinics at the RBWH (2008–2013). All had diagnoses of clinically probable or definite ALS, according to revised El Escorial criteria (31). We chose to study only right-handed subjects due to their predominance in the population (72), as well as greater uniformity of motor and language lateralisation (69). Handedness was confirmed using the Edinburgh Handedness Inventory (58). Subjects were grouped according to the index limb, defined as the first limb affected by weakness (either the limb of onset, or the first limb affected after bulbar onset) (62). Fifteen subjects had a right-sided (dominant) index limb and 15 had a left-sided (non-dominant) index limb. Each subject was administered the ALS Functional Rating Scale—Revised (ALSFRS-R) as a measure of disability. To adjust the degree of disability for the disease duration, we calculated "disease progression" as: (48 − ALSFRS-R score) / (disease duration). Seventeen right-handed healthy controls were closely age and sex-matched with each group of 15 ALS subjects. None of the control or ALS subjects had a history of cerebrovascular events, intracranial pathology, or other neurological diseases.

MRI acquisition

Each subject underwent an MRI scan acquired with a 3T Siemens TimTrio (Siemens, Erlangen, Germany), using sequences from VB17 Neuro applications and a 12-channel head coil. A high-resolution structural image was acquired for each subject using a $1mm^3$ isotropic 3D T1 MPRAGE (FOV 24 × 25.6 × 17.6 cm, TR/TE/TI 2300/2.26/900 ms, flip angle 9). Slice thickness was 1 mm and image acquisition time was 9:14 min.

Image processing

Structural images were processed according to the protocol previously reported (Rose et al., 2012). The software package FSL-VBM (Version 4.1), an optimised VBM protocol (73) carried out with FSL tools (74), was used for all image processing and analysis. The brain was extracted using BET (75). GM segmentation was performed using FAST (76), with the segmentations then aligned toMNI152 standard space using affine registration, FLIRT (77), followed by non-linear registration using FNIRT (78). The resulting images were averaged to create a study-specific GM template. Each image was non-linearly re-registered to that template, before being modulated by dividing by the Jacobian determinant of the warp field and smoothed with an isotropic Gaussian kernel (sigma = 4mm). Mirror images were then generated for each of the smoothed, modulated GM images in standard space, for each of the 47 subjects.

Statistical analysis

All statistical comparisons were performed using Randomise (79), and adjusted for multiple comparisons using threshold-free cluster enhancement (TFCE) (80).

ALS and controls

Firstly, a voxelwise unpaired t-test was performed, comparing the GM density of all ALS subjects (n = 30) with all controls (n = 17). ALS subjects were then subdivided into two groups (15 with a right-sided index limb, and 15 with a left-sided index limb), and each group was compared with 15 age and sex-matched controls. Finally, the ALS subjects with a right-sided index limb were compared directly with those having a left-sided index limb. For each test, age and disease progression were introduced as nuisance covariates.

Asymmetry analysis

In order to identify areas of hemispheric asymmetry, a voxelwise paired t-test was performed between the native and mirror images. This was performed separately for each of the three groups of subjects (17 controls, 15 ALS subjects with a right-sided index limb and 15 with a left-sided index limb). The limb subscore (questions 4–9) of the ALSFRS-R was introduced as a covariate. The anatomical location of each cluster of GM asymmetry was determined using the Talairach Daemon. The threshold for statistical significance was set at $p \leq 0.01$ (TFCE-corrected).

Results

ALS and controls

Specific subject characteristics are presented in Table 2 (Appendix 2). Compared with controls (n=17), subjects with ALS (n=30) showed a multifocal cluster of reduced GM density, involving the left precentral gyrus and adjacent regions of the left middle frontal gyrus and bilateral medial frontal gyri (2087 voxels; centre-of-gravity: −22, −11, 52; $p \leq 0.05$). There was a separate cluster of reduced GM density involving bilateral anterior cingulate gyri (425 voxels; centre-of-gravity: 1, 39, 6; $p \leq 0.05$). These patterns of atrophy are illustrated in Figure 6A (Appendix 3).

Across all 47 subjects, there was a negative correlation ($p \leq 0.05$) between age and GM density in widespread regions of the frontal, parietal, temporal and occipital lobes, representative of age-related atrophy. However, there was no confounding effect of age or disease progression on the patterns of atrophy in ALS.

ALS (according to index limb) and controls

As illustrated in Figure 6B (Appendix 3), ALS subjects with a right (dominant) index limb (n = 15) showed patchy reductions in GM density affecting the left precentral gyrus, at a threshold of $p \leq 0.05$. These changes were not significant at a higher threshold of $p \leq 0.01$. Subjects with a left (non-dominant) index limb (n = 15) did not demonstrate any significant reductions in GM density at either the left or right precentral gyri at a threshold of $p \leq 0.05$ (Figure 6C, Appendix 3).

Direct voxelwise comparison between ALS subjects with either a right or left index limb also did not reveal any significant differences in GM density.

GM asymmetry in controls

In the 17 right-handed control subjects, multiple statistical clusters of both rightward and leftward asymmetries were identified (Table 3 Appendix 2; Figure 7A Appendix 3). Of particular note was an area of leftward asymmetry ($p \leq 0.01$) encompassing a dorsolateral region of the precentral and postcentral gyri. This area corresponded closely with the centre-of-gravity of the dominant thenar representation area, previously defined using transcranial magnetic stimulation (TMS) (54). Control subjects also demonstrated significant leftward asymmetry of a region of the superior and transverse temporal gyri, adjacent to the Sylvian fissure. There were no significant asymmetries of lower limb

or bulbar representation areas, indicating that these regions were of a similar density in the right and left hemispheres.

GM asymmetry in ALS

In the 15 ALS subjects with a right-sided (dominant) index limb, there was complete absence of leftward asymmetry at the precentral gyrus hand representation area, at the threshold of $p \leq 0.01$ (Table 3 Appendix 2; Figure 7B Appendix 3). This indicated disproportionate loss of GM supplying the dominant hand, relative to the remainder of the bilateral motor strip (including lower limb and bulbar representation areas). Leftward asymmetry was also lost at the adjacent region of the postcentral gyrus, as well as the superior and transverse temporal gyri and anterior insula.

In contrast, leftward asymmetry of the dorsolateral postcentral gyrus, superior and transverse temporal gyri and anterior insula was preserved in the 15 ALS subjects with a left-sided (non-dominant) index limb (Table 3 Appendix 2; Figure 7C Appendix 3). However, these subjects still showed complete absence of leftward asymmetry at the precentral gyrus hand representation area. This disproportionate loss of GM density in the left motor cortex occurred despite first weakness occurring in a limb controlled by the right hemisphere. These subjects also demonstrated a new cluster of leftward asymmetry involving the middle frontal gyrus (MFG), which was not present in controls (Table 3). This may indicate either relative gain of GM density at the left MFG or GM loss at the right MFG, with the latter favoured.

Other regions of GM asymmetry were preserved across all ALS subjects and controls. These included rightward asymmetry of the inferior frontal, rectal and orbital gyri, posterior thalamus, posterior cingulate gyrus and cuneus, and leftward asymmetry of the posterolateral cerebellum and

occipital lobes. Several of these regions, including rightward asymmetry of the antero-inferior frontal lobes and leftward asymmetry of the occipital lobes, have been previously reported in larger populations of healthy subjects (53,81).

Discussion

The objective of this study was to identify ALS-related changes to the normal patterns of GM asymmetry, and interpret these in the context of limb dominance. We have established that a VBM asymmetry protocol applied to structural T1 MRI (18) is a useful tool for assessing GM changes in both healthy and disease states, especially in smaller cohorts in which standard voxelwise comparisons are less sensitive. Using this protocol, we found that right-handed ALS subjects with dominant limb onset disproportionately lost GM in left hemispheric sensorimotor (upper limb representation) and language regions. However, unlike other regions, disproportionate atrophy of the left motor cortical hand area occurred independent of whether onset of weakness was in a dominant or non-dominant limb. These findings support previous clinical studies of ALS laterality, and have implications for cortical organisation, its evolution, and selective vulnerability.

Multiple large studies have demonstrated motor cortical atrophy in ALS, which may initially be left-hemisphere predominant (82). Furthermore, motor impairment has been shown to be focal, using both clinical examination (9) and neuroimaging (83). However, in the current study, major differences between ALS and controls were only apparent when analysing all 30 subjects together, and at a more relaxed statistical threshold ($p \leq 0.05$). Comparisons involving 15 ALS subjects only detected minor, patchy reductions in motor cortical GM density, and were unable to differentiate subjects with right or left-sided disease onset. In contrast, the VBM asymmetry protocol was sufficiently powered to identify differences in GM between the two clinical groups of ALS subjects,

at a stricter threshold ($p \leq 0.01$). Therefore, this method is ideal for exposing subtle asymmetric changes in less common diseases such as ALS.

Despite previous authors correlating the size or density of motor areas with handedness (18,48,49), some have not demonstrated this (52). In the current study, we defined a significant cluster of leftward GM asymmetry in right-handed controls, incorporating the cortical hand representation area. This indicates greater size and complexity of this area in the left hemisphere. In contrast, the absence of this asymmetry across all right-handed ALS subjects suggests that the left hemisphere hand area disproportionately loses GM density relative to the remainder of the bilateral motor cortex. Our results also suggest that this region of motor cortex is particularly vulnerable to atrophy, regardless of whether the patient or clinician had noticed first weakness in a right or left-sided limb.

Vulnerability of the dominant upper limb in ALS has been previously described using history and clinical examination (8,62). Our study provides neuroimaging evidence to support this phenomenon, and suggests an important role for central factors in driving this effect. Our findings also confirm the lack of preferential involvement of either lower limb, which has been observed clinically (8). It has been proposed that certain functions, such as the “split hand”, are more vulnerable to the pathology of ALS due to more recent development in human evolution (7). Another key feature of human development has been a population-wide bias toward right-handedness (47), which is likely to have been facilitated by the onset of upright gait (72). The current results suggest that the increased complexity of left hemispheric motor networks in right-handers may lead to greater susceptibility to ALS. The cause of neuronal vulnerability in ALS remains unknown, although potential contributory factors include cellular density, excitability, and hormonal influences (55,57,84).

In our study, subjects with dominant limb onset of weakness also showed disproportionate atrophy of areas involved in language and communication (left superior and transverse temporal gyri). It is known that 98% of right-handers have left hemispheric language lateralisation (Adamo and Taufiq, 2011). Although these subjects did not undergo formal cognitive testing, this finding may reflect the occurrence of language dysfunction as part of the ALS–FTD spectrum, including milder language deficits in subjects without frank dementia (85). Alternatively, some authors have proposed that the dominant upper limb and speech form a single network for communication, with gestures and vocalisations being linked (72,86).

This study has limitations. Due to the current cohort being restricted to right-handers, we were unable to assess changes to GM asymmetry in left-handed or ambidextrous subjects. However, this would require recruitment of a larger cohort, since hemispheric lateralisation of motor and language is less predictable in non-right-handed individuals (69). The size of our cohort also limited the ability to separately analyse smaller subgroups, for example only those subjects with upper limb onset of weakness. Formal neuropsychological testing was also not performed, therefore the cognitive correlates of some changes (such as those affecting the left frontal and bilateral cingulate gyri) remain unclear. Finally, it remains to be seen whether the current results can be translated to individual subjects, for example in a diagnostic setting.

Overall, this study provides evidence that certain areas of GM are disproportionately and asymmetrically vulnerable to the pathology of ALS, and that these regions can be identified using a sensitive VBM protocol. In particular, we have identified the dominant hand area as being particularly susceptible to atrophy, supporting previous history and examination-based studies. This finding warrants further investigation, including assessment of associated white matter asymmetry using diffusion tensor imaging.

9. Clinical Phenotype and Survival in ALS

9.1. Preface

This chapter incorporates the text of one peer-reviewed publication in its entirety:

(1) Devine MS, Ballard E, O'Rourke P, Kiernan MC, McCombe PA, Henderson RD. Targeted assessment of lower motor neuron burden is associated with survival in amyotrophic lateral sclerosis. Amyotroph Lateral Scler Frontotemporal Degener 2016;17:184-90. (65)

The previous chapters have elucidated a connection between limb dominance and asymmetry of ALS, using the multiple modalities of structured questioning, clinical assessment of UMN and LMN phenotype, and novel MRI imaging techniques. Throughout the duration of these studies, the patient cohort was also able to be followed longitudinally for assessment of the factors affecting their survival. Of particular interest was the effect of both UMN and LMN clinical burden on survival to non-invasive ventilation (NIV) or death. The peer-reviewed study presented in Chapter 9 analysed the factors determining survival of the entire original cohort of recruited subjects with particular reference to clinical phenotype.

As first author of this publication, the author directly contributed to the conception and design of the project, collection, interpretation and statistical analysis of data, and drafting and revising both publications. Clinical assessment of subjects' UMN and LMN scores were contributed to by neurologists A/Prof Henderson and Prof McCombe from RBWH in Brisbane, and Prof Kiernan from POWH in Sydney. Guidance regarding statistical analysis was provided by Prof Peter O'Rourke and Dr Emma Ballard (QIMR).

9.2. Correlation between LMN Burden and Survival in ALS

Abstract

Estimating survival in amyotrophic lateral sclerosis (ALS) is challenging due to heterogeneity in clinical features of disease and a lack of suitable markers that predict survival. Our aim was to determine whether scoring of upper or lower motor neuron weakness is associated with survival. With this objective, 161 ALS subjects were recruited from two tertiary referral centres. Scoring of upper (UMN) and lower motor neuron (LMN) signs was performed, in addition to a brief questionnaire. Subjects were then followed until the censorship date. Univariate analysis was performed to identify variables associated with survival to either non-invasive ventilation (NIV) or death, which were then further characterized using Cox regression.

Results showed that factors associated with reduced survival included older age, bulbar and respiratory involvement and shorter diagnostic delay (all $p < 0.05$). Whole body LMN score was strongly associated with time to NIV or death ($p \leq 0.001$) whereas UMN scores were poorly associated with survival. In conclusion, our results suggest that, early in disease assessment and in the context of other factors (age, bulbar, respiratory status), the burden of LMN weakness provides an accurate estimate of outcome. Such a scoring system could predict prognosis, and thereby aid in selection of patients for clinical trials.

Introduction

Amyotrophic lateral sclerosis (ALS) is a heterogeneous disorder with the common underlying feature being progressive degeneration of motor neurons in the cortex, the brainstem and the spinal cord (1,2). Patients typically exhibit clinical signs indicating dysfunction of upper (UMN) and lower motor neurons (LMN). There is also great variability in individual survival times (19). These range from fulminant disease with survival less than three years to a 'tail' of 10–20% of patients with a very slowly progressive or plateau course (19,87,88). Estimating prognosis is of great importance to patients, with older age, lower score on the ALSFRS-R, shorter diagnostic delay with more rapid progression, bulbar onset, presence of frontotemporal dementia (FTD), smoking and low body-mass index all portending a shorter survival (19,89-95). Some factors, such as site of disease onset (bulbar versus limb), have a more complex effect on survival that is influenced by age and gender (4,5). Survival can be prolonged through initiation of non-invasive ventilation (NIV) (96), which is sometimes considered as a secondary endpoint for disease progression.

The relative involvement of UMN and LMN also appears to affect survival (1,2). Typically, patients with pure-UMN or pure-LMN forms of ALS have longer average survival, compared with patients with disease affecting both UMN and LMN (19,36,97). However, there has not been direct comparison between UMN and LMN involvement, in terms of which is more predictive of patient survival. In some models, they are assumed to be relatively equal contributors to disease severity (95). Although various electrophysiological and imaging parameters show promise as biomarkers of severity of UMN and LMN involvement (98), these are not widely established in clinical practice and are difficult to administer at the bedside. Clinical assessment remains the primary method of quantifying UMN and LMN involvement in large cohorts. While detailed clinical scoring systems have been developed and correlate with survival (11,99), these often do not discriminate between UMN and LMN signs, and are lengthy and not easily administered during a clinic appointment. A

practical prognostic tool should ideally quantify UMN and LMN signs in a short clinical examination, in addition to being able to estimate survival based on these scores. With this in mind, the aim of the present study was to investigate factors affecting survival in a cohort of ALS subjects from two specialized multidisciplinary clinics, focusing on the effects of increasing UMN and LMN signs. This cohort had been previously recruited and examined at a single time-point using a concise limb UMN and LMN scoring system (9,62). The hypothesis was that this score, able to be performed within a standard clinic appointment, would be predictive of survival. We also hypothesized that limb LMN scores would show greater association with survival (compared with UMN scores), since diaphragmatic weakness, which leads to death in ALS, is probably driven by LMN factors (100,101).

Materials and Methods

Conduct of this study was approved by the Royal Brisbane and Women's Hospital Ethics Committee and the South Eastern Sydney Local Health District Ethics Committee. All subjects provided written informed consent. A cohort of 161 consecutive patients, with clinically-definite or clinically-probable ALS according to the El Escorial criteria (31), were recruited from the Royal Brisbane and Women's Hospital and the Prince of Wales Hospital, Sydney, over the period 2011–2014. Clinical assessment and scoring was originally performed for a study of motor asymmetry in ALS (62), with this cohort then being followed for analysis of survival.

In the current study, several key time-points were recorded. Onset Date (OD) was defined as the time at which ALS symptoms (limb weakness, dysathria or dysphagia) were first recognized. Diagnosis Date (DD) was the time at which a diagnosis of ALS was made by the treating neurologist. Examination Date (ED) was the point at which the questionnaire and clinical scoring were performed, as outlined below. Due to the focal, asymmetric nature of early ALS (9), all

subjects were examined and scored within 12 months of the DD. If subjects presented more than 12 months from the DD, careful retrospective scoring based on examination notes was performed, as previously described (62).

On the ED, basic demographic information (age, gender, family history) was collected using a standardized questionnaire. Subjects were also asked about the site of disease onset (bulbar, upper limb, lower limb). A scoring system based on that of Ravits et al. (9) was applied by the examining neurologist (MK, PM or RH). This involved assigning a separate UMN and LMN score to each limb, ranging from 0 (no involvement) to 3 (significant, severe involvement) (Table 4). A whole body UMN or LMN score was calculated by summating the scores in each limb (giving a maximum value of 12). Subjects were also assessed for bulbar involvement (Yes/No), defined as at least one symptom (dysphagia, dysarthria) with at least one clinical sign (tongue wasting, fasciculations, brisk jaw jerk). Respiratory involvement (Yes/No) was defined as significant dyspnoea or orthopnoea, not present prior to ALS.

All subjects were followed until December 2014. By this date, subjects had either had an ‘Event’, or were ‘Censored’. Subjects were assigned the outcome of ‘Censor’ if they were alive at the end of December 2014, or had died from a cause unrelated to ALS. An ‘Event’ was defined as either Death (from ALS) or initiation of non-invasive ventilation (NIV), whichever occurred first. For the purposes of this study, NIV was defined as initiation of regular (either nocturnal or intermittent) bilevel positive airway pressure ventilation due to ALS related respiratory failure under the guidance of a respiratory physician. Subjects who commenced NIV, and subsequently died before December 2014, were therefore considered to have had an ‘Event’ at the initiation of NIV but were still followed until the date of death. For each subject, use of riluzole (Yes/No) was also recorded, which was defined as taking the drug regularly for at least six months.

Statistical analysis was performed using SPSS (Version 22, SPSS Inc.). The four outcomes of interest were 'Onset to Event' (OD to Event), 'Exam to Event' (ED to Event), 'Onset to Death' (OD to Death) and 'Exam to Death' (ED to Death). Univariate analysis was completed using Kaplan-Meier estimates with the log-rank test used to identify useful predictors for both Event and Death outcomes. Potential predictor variables included age, gender, family history, site of onset, bulbar involvement, respiratory involvement, diagnostic delay and UMN and LMN scores. Whole body UMN and LMN scores were subdivided according to severity (0–2, 3–6 and 7–12). UMN and LMN scores were also analysed separately for the upper limb and lower limb regions. Variables were screened as being potentially useful predictors of survival, during univariate analysis, using a significant log-rank test p-value (< 0.05) for both Event and Death outcomes. These variables of interest were then used in a Cox regression model for each outcome (Event or Death). Estimated hazards ratios and 95% confidence intervals were reported.

Results

Of the 161 subjects, 56% were males and 93% had no family history of ALS. Mean age at ED was 61 years (SD, 11 years). Disease onset was in the upper limb in 54 subjects (33%), lower limb in 64 subjects (40%) and bulbar region in 43 subjects (27%). At the censorship date, 59% had died due to ALS, while 66% had undergone an Event (NIV or death due to ALS, whichever occurred first). Univariate analysis was performed to identify which variables were potential predictors of death or an Event (Tables 5 and 6). Gender, family history, site of onset and riluzole use were screened out as not being associated with survival. Increasing age and shorter delay between onset and diagnosis were both strongly associated with shorter time to NIV or death (Table 5).

Subjects with evidence of either bulbar or respiratory involvement were more likely to progress to NIV or death from that point in time (Exam to Event), as well as showing reduced survival overall

(Onset to Event) ($p < 0.01$). Increasing the LMN score, in the upper limbs, lower limbs or whole body, was associated with reduced time to NIV or death ($p \leq 0.001$ for whole body score). In contrast, increasing UMN scores were not significantly associated with survival (Table 6).

Variables identified as significant using univariate analysis (age, bulbar involvement, diagnostic delay and LMN score) were further assessed using a Cox regression model. The covariate for respiratory involvement had a singularity with time to Event, and therefore was not considered further for Cox regression. LMN upper limb and lower limb scores were both colinear with the whole body LMN score, and therefore were also not included separately for Cox regression.

Older age, bulbar involvement and shorter diagnostic delay were all associated with reduced covariate-adjusted survival (from onset or exam, to either NIV or death; Table 7). For example, a patient 65 years of age or older has approximately twice the risk of death or NIV compared with a patient younger than 55 years of age. A patient with bulbar involvement at the time of clinical examination has a two- to three-fold increase in risk of death or NIV, compared with a patient in whom bulbar function is preserved. For all variables except diagnostic delay, hazard ratios were similar across all of the four endpoints (Onset to Event, Onset to Death, Exam to Event and Exam to Death). With diagnostic delay, hazard ratios were higher when survival was measured from an earlier time-point (Onset vs. Exam), probably related to the longer observation period.

Using Cox regression, it was confirmed that higher whole body LMN score is strongly associated with survival. A subject with a whole body LMN score of 7–12 has four- to six-fold increased risk of death or NIV, compared to subjects with mild LMN involvement (Table 7, Figure 8). At 18 months from examination, the duration of a typical ALS clinical trial (102), 73% of subjects with an

LMN score of 7–12 were deceased, whereas only 26% of those with an LMN score of 3–6 were deceased.

Discussion

A key challenge facing ALS clinicians is the ability to provide patients and carers with accurate prognostic information. This is especially challenging given the significant heterogeneity of ALS, including diverse combinations of UMN and LMN clinical signs (1,2). Furthermore, many assessment tools and prospective biomarkers are either too time-consuming or experimental to be applied in a busy clinical setting (2,98). In this study, using a simple bedside scoring system at a single time-point, we have demonstrated that a higher burden of LMN signs is strongly associated with lower survival from the time of examination to an event (either NIV or death). In contrast, the severity of UMN signs was not associated with patient outcome.

Although there has been recent interest in ALS staging systems from the perspective of clinical trials (103,104), these population-based scores typically do not differentiate UMN and LMN features of the disease. Earlier scoring systems (11,99), while very comprehensive, are also time-consuming and difficult to adopt in a clinical setting. Other studies (9,10,12), while quantifying UMN and LMN burden using similar scoring systems, were targeted toward patterns of spread rather than effects on survival. The scoring system used in the current study, modified from that of Ravits et al. (9), is based around a standard neurological examination and therefore is practical to perform within a clinic appointment. While UMN factors appear important in the onset and spread of ALS (2,62), our results emphasise the importance of LMN in driving survival, in both upper and lower limb regions as well as the body as a whole. From a practical viewpoint, LMN signs (weakness in combination with wasting) are readily apparent on examination and easily graded.

We found close association between total LMN burden and time to NIV or death, thus allowing better identification of patients more likely to die within 12–18 months. Such subjects are likely to be informative in a clinical trial compared to more slowly progressive, atypical patients, which may skew the dataset and mask the true beneficial effect of a therapy within the trial period (102,105).

Our study also confirmed the known detrimental effects of increasing age and bulbar involvement on survival (19,36,87,89). Poorer prognosis in subjects with a shorter diagnostic delay has also been reported (106) and probably reflects overall more fulminant disease that comes to medical attention more rapidly. We observed no association between severity of UMN signs and survival, suggesting that these have minimal utility as a predictor of outcome. UMN signs also generalize early (62,104) and may be more difficult to interpret on examination (16). Furthermore, UMN-predominant forms of ALS often have a favourable prognosis, forming a relatively large proportion of the long-surviving 'tail' (19,36,87,97). The lack of association between riluzole usage and survival in our study probably reflects the smaller numbers in this cohort.

A key mechanism underlying the effect of LMN burden on survival (to NIV or death) is probably diaphragmatic weakness. In our cohort, respiratory involvement at the time of examination was a strong predictor of poor survival. While use of NIV may prolong survival (5,96), it does not alter the underlying neurodegenerative process. Adoption of NIV is also influenced by local practices – for example, use of NIV in our cohort was relatively modest (22%). Diaphragmatic involvement in ALS is thought to be driven by loss of anterior horn cells (LMN) in the cervical cord, adjacent to the LMN supplying paraspinal muscles (100,101). Cervical-level LMN signs are associated with reduced survival in ALS (107), probably related to contiguous spread of LMN degeneration at the cord level (11). In contrast, UMN involvement in diaphragmatic control is complex (108) but does not appear to have the same effect on dysfunction as LMN.

In terms of study limitations, it is accepted that although our scoring system specifies that LMN weakness should be in a typical muscle distribution and accompanied by wasting, it remains difficult to completely delineate this from UMN weakness on examination (16). For example, clinical detection of UMN signs is more difficult in a limb with significant LMN involvement, and it is acknowledged that precise grading of UMN severity (e.g. tone) on clinical examination is difficult even for experienced neurologists. This highlights the ongoing need for an objective UMN biomarker (98). EMG would also have been helpful to corroborate the clinical assessment of LMN burden. Finally, the absence of formal cognitive assessment is also a limitation of the current study. Although there was no association between UMN signs and survival, FTD as another marker of central involvement is linked with poorer prognosis. This effect is incompletely understood; however, the presence of FTD may reflect more widespread multisystem dysfunction (95).

Overall, our results indicate that a short, targeted bedside assessment of total limb LMN burden, combined with other concomitant features (age, bulbar and respiratory involvement) is strongly associated with ALS survival. While this requires validation, such a tool would be useful in clinical prognostication as well as appropriate recruitment for ALS clinical trials. Our findings could also be further explored using a larger model, incorporating numerical measures such as ALSFRS-R, forced vital capacity and formal cognitive scoring.

10. Conclusion

Amyotrophic lateral sclerosis remains an incompletely understood entity, with likely complex interplay between genetic and environmental factors (109-112). A complete understanding of the pathogenesis and pathophysiology of ALS is further hampered by the wide variability in multiple facets of the disease, including the relative presentation of clinical UMN and LMN phenotypes and the broad range of clinical trajectories ranging from fulminant rapidly progressive disease to atypical slowly progressive forms (19,87,88). It is therefore important that when certain recurring patterns or "clusters" are observed within the ALS population that these are studied closely. Understanding why these patterns occur will likely provide more broad insight into why the disease occurs in certain individuals, why certain muscle groups and functions are particularly susceptible, and how the disease spreads and progresses over time.

Evolutionary perspectives on ALS have become increasingly topical (7,43,112) and offer an intriguing theory as to why such phenotypic patterns occur. ALS only occurs in humans (112). During their evolution, humans have developed and refined multiple unique functions, including complex hand manipulation and thumb opposability, upright gait navigating complex landscapes, speech, social interactions and executive functioning. Whilst these functions have disproportionately gained complexity, and therefore increased cortical representation, during evolution, this is "mirrored" by their disproportionately early loss of function in ALS (112). This gives rise to distinctive patterns such as the "split hand" and "split foot" (weakness in dorsiflexion), which are often observed in ALS subjects. Indeed, a "split elbow" has been more recently described in the literature, comprising disproportionate weakness of the biceps brachii muscle relative to triceps (113), and furthermore the finger extensors are typically weaker than the finger flexors in ALS (114). Impairment in social interactions and executive function manifests as frontotemporal dementia spectrum disorders. Furthermore, one of the publications in the current thesis has revealed

disproportionate impairment of speech compared with swallow function in bulbar ALS (63). The nature of these patterns implies that CNS factors play an important role in ALS onset and spread. Concordant with this theory, the white matter pathways which have developed to sustain these complex functions have been shown to undergo widespread breakdown during the disease process, with involvement of both motor and extramotor areas (115).

The core objective of the current project has been to identify and characterise novel sources of vulnerability in ALS in a large cohort of subjects, with a particular focus on limb dominance and clinical phenotype. Humans are unique in developing a consistent, population-wide laterality of upper limb function with predominance of right-handedness (47,112). This has presumably necessitated development of more complex "wiring" in the dominant hemisphere, both within the primary motor pathways and their communications with other brain regions. In turn, we predicted that this introduces another source of vulnerability of the nervous system to ALS, which would manifest as asymmetry in both disease onset in the limbs and subsequent direction of disease spread through the neuraxis.

The finding that onset of weakness is more likely in the dominant upper limb is concordant with several earlier studies (8-10), however interestingly only became statistically significant upon analysis of a larger patient cohort (62). The symptomatic weakness, clinical UMN and LMN signs were all maximal in the Index Limb which also confirmed the well-described focality of onset of ALS (9). Whilst the current findings also support the previously described phenomena of contiguous disease spread beyond the Index Limb and tendency to rostro-caudal spread (9,10,12), the novel finding was that the direction of spread of limb weakness is also affected by limb dominance. In particular, there was a tendency for weakness in a non-dominant limb to spread to the other ipsilateral limb, which anatomically suggests an important role for UMN factors. Clinical

UMN signs were also found on average to spread to other limbs earlier than LMN signs, again reinforcing their importance in the process of disease spread (62). A strength of the current study was also the integrated assessment of both symptomatic weakness, as reported by the subjects on structured questionnaires, and objective clinical assessment of UMN and LMN dysfunction.

The asymmetry of both ALS onset and spread, as revealed by both structured questioning and clinical examination, suggests importance of central (UMN) factors in these processes. Unfortunately, there remains no widely available and accepted biomarker for UMN dysfunction in ALS although advanced imaging is likely to play a key role (98,116,117). This is compounded by significant variability between the published imaging studies. Whilst many authors have demonstrated cortical atrophy of the precentral gyrus in ALS compared with healthy controls (116, 118), not all studies have replicated this finding (119). Of the studies that did show atrophy of the precentral gyrus, many revealed this to be predominantly unilateral (82,118,120-125) however some authors reported bilateral atrophy without significant asymmetry (126) or bilateral but asymmetric atrophy (127). Furthermore, of the studies that showed unilateral atrophy, some demonstrated predominant involvement of the right precentral gyrus (118,120-122) whilst in contrast others showed predominantly left-sided precentral gyrus atrophy (82,123-125). Some, but not all, of these studies were controlled for patient handedness.

Many factors likely account for this variability in study findings, including differences in subject selection, sample sizes, timing of imaging, and the specific choice of imaging and analysis protocols (116). Imaging too late in the disease course risks obscuring asymmetric changes within the more diffuse advanced atrophy. Furthermore, several studies have also revealed that gray matter and white matter changes in ALS are not always concordant (116,128), and that longitudinally the gray matter changes become more severe whereas white matter changes may not progress to the

same extent (82,129). The degree of gray matter atrophy in ALS does appear to correlate with the extent of clinical UMN involvement phenotypically (116,130) and therefore assessment of gray matter may serve as a more useful future biomarker.

The key advantage of the novel imaging protocol employed in the current study (64) was the comparison of gray matter density between the two hemispheres in both ALS subjects and healthy controls, rather than just comparing the two groups of subjects with each other. This allowed subtle areas of cortical asymmetry to be accentuated in a relatively modest sample size, controlled for handedness (all subjects right-handed) and with many subjects at a relatively early disease stage. There was disproportionate atrophy of the left precentral gyrus hand representation area, regardless of whether onset had occurred in a right or left-sided limb. This suggests that this area of cortex is particularly vulnerable to the pathology of ALS, again with implications for the evolutionary theories of ALS and handedness (112) and supporting the findings of the earlier clinical scoring assessment which was also performed at a relatively early disease stage (62).

The phenomenon of differential ALS spread depending on the dominance of the limb of onset is also intriguing. The inherent directionality of the corpus callosum is likely an important factor (112), in particular the interhemispheric fibres connecting the primary motor cortices which have been shown to be the most severely affected callosal pathways in both imaging and pathological studies of ALS subjects (131,132). The relative degree of cortical excitability is also likely relevant. Normal healthy right-handed subjects have been shown to have asymmetry in the balance between excitation and inhibition when comparing the two motor cortices (133), with left-handed subjects likely more complex (69,112,133). Healthy individuals also appear to have topographical differences in excitability within the motor cortex itself (134). Abnormal cortical excitability and dysfunction of inhibitory interneurons are thought to play an important role in ALS pathogenesis

(21,30,111,135), therefore the inherent physiological asymmetry in excitability may account for some of the findings in the current studies.

It is also likely that the central nervous system attempts to compensate for the deficits caused by ALS, ultimately without success. For example, one combined VBM and functional MRI (fMRI) study found that in right-handed ALS subjects there was asymmetric hyper-activation of fronto-parietal pathways in the left hemisphere regardless of which hand the motor task was performed with (127). This contrasted with the abnormal hypo-activation in the primary motor area. The authors hypothesised that this activation represented compensatory changes within the dominant hemisphere. Another more recent study found increased gray matter volume in both cerebellar hemispheres of ALS subjects (123), also potentially reflecting secondary compensatory change. Therefore, the robustness of this compensation may influence the asymmetry of disease onset and spread in ALS subjects, but requires further investigation.

Whilst asymmetric changes in ALS have been assessed using quantitative measures of both gray matter and white matter, other more novel imaging techniques may also prove valuable in future studies. For example, cortical iron deposition has been described in ALS including in the precentral gyrus (136,137). A recent study using susceptibility-weighted imaging (SWI) in right-handed subjects with ALS found greater degree of hypointensity in the left hemisphere cortical hand representation area, thought to reflect iron deposition (138). This finding provides further support for the vulnerability of this area to the ALS disease process. Iron accumulation has been demonstrated histologically within the microglia and macrophages (138) and although its role in disease is not fully understood it likely occurs as part of an oxidative stress process (139). MR spectroscopy may be another useful tool for detecting asymmetric abnormalities in ALS. For example, in a recent study it was found that the precentral gyrus contralateral to the most clinically

affected limb(s) showed asymmetrically reduced NAA/Cr ratio compared with the contralateral precentral gyrus (140). Subtle early changes in gray and white matter are also detectable with ultra-high field strength magnets, however only applicable to animal models such as mice at this stage (141). Integration of multiple different imaging modalities is likely to prove useful in future studies of brain asymmetry in ALS.

Whilst central nervous system and UMN factors appear to play a key role in the onset and spread of ALS, the findings of the current study suggest that ultimately LMN factors correlate more closely with patient survival (65). This is of importance in selecting appropriate subjects to participate in clinical trials (102,105), and helpful given that LMN signs are typically more readily assessed and scored at the bedside. Subsequent authors have also found that the number of body regions with clinical LMN involvement shows significant negative correlation with survival; $p < 0.0001$ (142). Given the importance of LMN involvement in influencing survival, it is imperative to develop a reliable, readily measurable quantitative LMN biomarker, for both clinical prognostication and recruitment of clinical trials. Methods of assessing motor unit numbers such as MUNIX may show promise in this regard (143), particularly given the limitations of patient-reported assessments such as the ALSFRS-R. For example, it has been found that the ALSFRS-R can underestimate the true extent of deficit in right-handed subjects with onset of weakness in the left upper limb (144), a problem which would be avoided with the use of an objective quantitative biomarker.

Another difficulty in validating a potential biomarker in ALS is determining whether it is causative or a consequence of the disease process (109). For example, whilst hypermetabolism has been well described in ALS patients, it is unclear whether this is related to the underlying cause of the disease or secondary consequence for example due to denervation (145). Study of large patient populations is likely required, due to the significant disease heterogeneity as previously described (109).

Finally, the concept of limb dominance and asymmetric vulnerability in ALS may possibly be extrapolated to other neurodegenerative diseases. In particular, recent studies of Parkinson's disease have shown some intriguing findings, including some similarities with the current ALS studies. Between 50 and > 80 percent of Parkinson's disease patients show asymmetry of motor features in published series, with some studies showing a correlation between handedness and the earliest and most severely affected side of the body (146). Uitti et al (147) described an overall population trend toward greater deficit in the right-sided limbs in Parkinson's disease, likely due to the predominance of right-handedness in general. However, they also found that left-handed patients had more severe symptoms in the left side of the body. Furthermore, similar to the findings in ALS, the degree of asymmetry in Parkinson's disease is more pronounced in the earlier stages of the disease suggesting initial focality (147,148). Claassen et al (148) also found disproportionate left frontal lobe and insular cortical gray matter thinning in Parkinson's disease, irrespective of handedness or the side of the body with greatest clinical motor deficit. This is finding is intriguing, as it appears analogous to the disproportionate atrophy of the left hand motor cortex area in ALS, regardless of the side of onset of weakness (64). It is possible that a number of neurodegenerative diseases, which share asymmetry as a clinical feature, may manifest similar sources of vulnerability as seen in ALS (148) however this hypothesis requires further study.

In conclusion, the current studies have identified and characterised novel sources of vulnerability in a large cohort of ALS subjects, addressing the core hypotheses via multiple modalities including structured questioning, direct clinical examination and scoring and advanced neuroimaging. In particular, a link has been established between limb dominance and asymmetry of both onset and spread of disease. The importance of cortical factors in driving asymmetric patterns of disease onset and spread, having been initially identified in the clinical studies of both limb and bulbar ALS (61,62,63), was further confirmed with novel MR imaging techniques (64). These findings have implications for the "dying forward" and broader evolutionary theories regarding ALS. However, it

seems that ultimately it is LMN factors which correlate with patient survival (65). It will be important to extend these findings in future studies, in particular with specific evaluation of left-handed subjects and incorporation of cognitive assessment.

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12. Appendices

Appendix 1 (Ethics Documentation)

Appendix 1.1: HREC/11/QRBW/206

Appendix 1.2: LNR/13/POWH/29

Appendix 1.3: 2008/098

Appendix 2 (Tables)

Table 1: Spread of ALS in right-dominant patients following unilateral limb onset

		Site of spread	
	Site of onset	Contralateral upper limb	Ipsilateral lower limb
Right-handed patients with upper limb onset (n = 19)	Right upper limb (dominant)	6 (50%)	6 (50%)
	Left upper limb (non-dominant)	3 (43%)	4 (57%)
	Total	9	10
		Contralateral lower limb	Ipsilateral upper limb
Right-footed patients with lower limb onset (n = 19)	Right lower limb (dominant)	7 (88%)	1 (12%)
	Left lower limb (non-dominant)	8 (73%)	3 (27%)
	Total	15	4

Table 2: Demographics and clinical features of ALS subjects and controls

	Controls	ALS with Right Index Limb	ALS with Left Index Limb	
Number of subjects	17	15	15	–
Handedness (right:left)	17:0	15:0	15:0	–
Age (mean ± SD; range)	56 ± 13 years (33–74 years)	59 ± 13 years (30–76 years)	56 ± 11 years (29–73 years)	–
Sex (male:female)	11M:6F	11M:4F	10M:5F	–
Sporadic:familial	–	14:1	11:3	–
Onset site	–	• 9 right upper limb • 5 Right lower limb • 1 bulbar	• 7 left upper limb • 5 left lower limb • 3 bulbar	–
Disease duration, months (mean ± SE; range)	–	23.8 ± 6.4 months (4–104 months)	28.7 ± 10.5 months (7–173 months)	$p = 0.69$
ALSFRS-R score (mean ± SD)	–	40 ± 4	39 ± 6	$p = 0.58$
Disease progression[a] (mean ± SE)	–	0.53 ± 0.08	0.55 ± 0.11	$p = 0.93$

[a] Disease progression = (48 − ALSFRS score) / disease duration.

Table 3: Statistical clusters of GM asymmetry in controls and ALS subjects

	Cluster Centre of Gravity (MNI)			GM Regions Encompassed	Cluster Size (voxels) [a]	T-value[b]
	x	y	z			
Controls	**Leftward Asymmetries (L hemisphere > R hemisphere)**					
(n = 17)	-39	-74	-26	• Cerebellum (posterolateral) • Occipital lobe (posterolateral)	5523	12.3
	-34	-14	35	• Precentral gyrus (dorsolateral) • Postcentral gyrus (dorsolateral) • Superior and transverse temporal gyri • Anterior insula	4120	9.6
	Rightward Asymmetries (R hemisphere > L hemisphere)					
	11	-53	12	• Posterior thalamus • Posterior cingulate gyrus • Cuneus and precuneus	2007	10.9
	9	-61	-40	• Cerebellum (inferomedial)	1334	10.1
	12	15	-17	• Inferior frontal, rectal and orbital gyri	452	8.2
ALS with Right Index Limb	**Leftward Asymmetries (L hemisphere > R hemisphere)**					
(n = 15)	-39	-73	-32	• Cerebellum (posterolateral) • Occipital lobe (posterolateral)	2938	10.7
	Rightward Asymmetries (R hemisphere > L hemisphere)					
	10	-65	-28	• Cerebellum (inferomedial)	1791	9.4
	13	19	-18	• Inferior frontal, rectal and orbital gyri	1011	12.4
	10	-62	13	• Posterior cingulate gyrus • Cuneus and precuneus	769	8.5
	12	-27	6	• Posterior thalamus	452	6.9
ALS with Left Index Limb	**Leftward Asymmetries (L hemisphere > R hemisphere)**					
(n = 15)	-34	-67	-44	• Cerebellum (posterolateral)	2285	7.5
	-42	-25	13	• Superior and transverse temporal gyri	971	9.0
	-47	-77	-6	• Occipital lobe (posterolateral)	617	9.7
	-29	19	4	• Anterior insula	609	7.3
	-29	40	30	• Middle frontal gyrus	498	7.5
	-47	-23	55	• Postcentral gyrus (dorsolateral)	185	6.9
	Rightward Asymmetries (R hemisphere > L hemisphere)					
	10	-68	-17	• Cerebellum (inferomedial) • Posterior cingulate gyrus • Cuneus and precuneus	3564	9.3
	13	18	-18	• Inferior frontal, rectal and orbital gyri	556	10.3
	13	-24	10	• Posterior thalamus	550	8.2

[a] Only clusters of ≥180 voxels are reported.
[b] All regions are significant, after correction for multiple comparisons (TFCE), at a threshold of $p \leq 0.01$.

Table 4: Scoring system used to quantify clinical UMN and LMN involvement in each limb

UMN Score	
0	No involvement
1	Definite, but trace involvement • Reflexes preserved in a limb with weakness, or • Slight increase in tone
2	Moderate involvement • At least one reflex brisk (≥ 3 on a 4-point scale), or • Moderate increase in tone, or • Presence of an extensor plantar response (in lower limb)
3	Significant and severe involvement • All reflexes pathologically brisk with significant transmission or clonus, or • Significant spasticity, limiting ability to move limb or walk
LMN Score	
0	No involvement
1	Definite, but trace involvement • Weakness $\geq 4/5$, involving one or more segments (with no segments $<4/5$), and • Mild wasting
2	Moderate involvement • Weakness $\geq 3/5$, involving one or more segments (with no segment $<3/5$), and • Moderate wasting
3	Significant and severe involvement • Little or no movement (LMN weakness $\leq 2/5$) involving one or more segments, and • Severe wasting

Table 5: Utility of demographic covariates in predicting survival to an event or death

				Outcomes (log-rank test p-value)			
Covariate of Interest	Total subjects	Event, n (%)	Death, n (%)	Onset to Event	Exam to Event	Onset to Death	Exam to Death
Age							
<55	42	21 (50%)	20 (48%)	0.026	0.005	0.028	0.008
55 – 64	53	36 (68%)	31 (59%)				
≥ 65	66	49 (74%)	44 (67%)				
Gender							
Male	90	60 (67%)	51 (57%)	0.94	0.80	0.28	0.28
Female	71	46 (65%)	44 (62%)				
Family history							
Sporadic	150	100 (67%)	89 (59%)	0.35	0.45	0.56	0.69
Familial	11	6 (55%)	6 (55%)				
Site of onset							
Upper Limb	54	32 (59%)	27 (50%)	0.29	0.36	0.041	0.063
Lower Limb	64	44 (69%)	38 (59%)				
Bulbar	43	30 (70%)	30 (70%)				
Diagnostic delay							
≤ 12 Months	97	70 (72%)	64 (66%)	<0.001	0.070	<0.001	0.025
>12 Months	64	36 (56%)	31 (48%)				
Riluzole use (>6 months)							
Yes	88	59 (67%)	53 (60%)	0.47	0.47	0.41	0.45
No	73	47 (64%)	42 (58%)				

Table 6: Utility of phenotypic covariates in predicting survival to an event or death

				Outcomes (log-rank test *p*-value)			
Covariate of Interest	Total subjects	Event, *n* (%)	Death, *n* (%)	Onset to Event	Exam to Event	Onset to Death	Exam to Death
Bulbar involvement							
Yes	77	56 (73%)	54 (70%)	0.005	0.002	<0.001	<0.001
No	84	50 (60%)	41 (49%)				
Respiratory involvement							
Yes	17	17 (100%)	14 (82%)	<0.001	<0.001	<0.001	<0.001
No	144	89 (62%)	81 (56%)				
UMN Score (Upper limbs)							
0 – 1	35	24 (69%)	21 (60%)	0.73	0.90	0.66	0.69
2 – 6	126	82 (65%)	74 (59%)				
UMN Score (Lower limbs)							
0 – 1	28	20 (71%)	15 (54%)	0.96	0.95	0.31	0.46
2 – 6	133	86 (65%)	80 (60%)				
UMN Score (Whole body)							
0 – 2	32	25 (78%)	21 (66%)	0.096	0.24	0.066	0.12
3 – 6	44	25 (57%)	20 (46%)				
7 – 12	85	56 (66%)	54 (64%)				
LMN Score (Upper limbs)							
0 – 1	58	31 (53%)	30 (52%)	0.012	0.003	0.057	0.013
2 – 6	103	75 (73%)	65 (63%)				
LMN Score (Lower limbs)							
0 – 1	90	50 (56%)	44 (49%)	0.025	0.003	0.048	0.009
2 – 6	71	56 (79%)	51 (72%)				
LMN Score (Whole body)							
0 – 2	43	22 (51%)	20 (47%)	0.001	<0.001	0.001	<0.001
3 – 6	92	61 (66%)	54 (59%)				
7 – 12	26	23 (89%)	21 (81%)				

Table 7: Cox regression analysis of variables affecting survival in ALS

Variable of Interest	Hazard ratios for each outcome (95% CI)			
	Onset to Event	Exam to Event	Onset to Death	Exam to Death
Age				
<55	1	1	1	1
55–64	1.4 (0.8–2.5)	1.7 (1.0–3.0)	1.2 (0.7–2.0)	1.3 (0.7–2.2)
≥65	2.2 (1.3–3.8)	2.5 (1.5–4.4)	2.0 (1.2–3.5)	2.2 (1.3–3.8)
p-value (Wald)	0.008	0.003	0.015	0.008
Bulbar				
No	1	1	1	1
Yes	2.0 (1.3–3.1)	2.4 (1.5–3.7)	2.6 (1.7–4.0)	2.8 (1.8–4.4)
p-value (Wald)	0.001	<0.001	<0.001	<0.001
Diagnostic delay				
>12 Months	1	1	1	1
≤12 Months	3.4 (2.2–5.2)	1.7 (1.1–2.5)	3.9 (2.4–6.3)	1.9 (1.2–3.0)
p-value (Wald)	<0.001	0.019	<0.001	0.004
Whole body LMN score				
0–2	1	1	1	1
3–6	1.8 (1.0–3.1)	2.4 (1.4–4.1)	1.7 (1.0–3.0)	2.0 (1.2–3.5)
7–12	4.5 (2.4–8.3)	4.9 (2.6–9.3)	5.1 (2.7–9.9)	5.7 (3.0–11.0)
p-value (Wald)	<0.001	<0.001	<0.001	<0.001

Appendix 3 (Figures)

Figure 1: Onset and spread of weakness in all ALS subjects

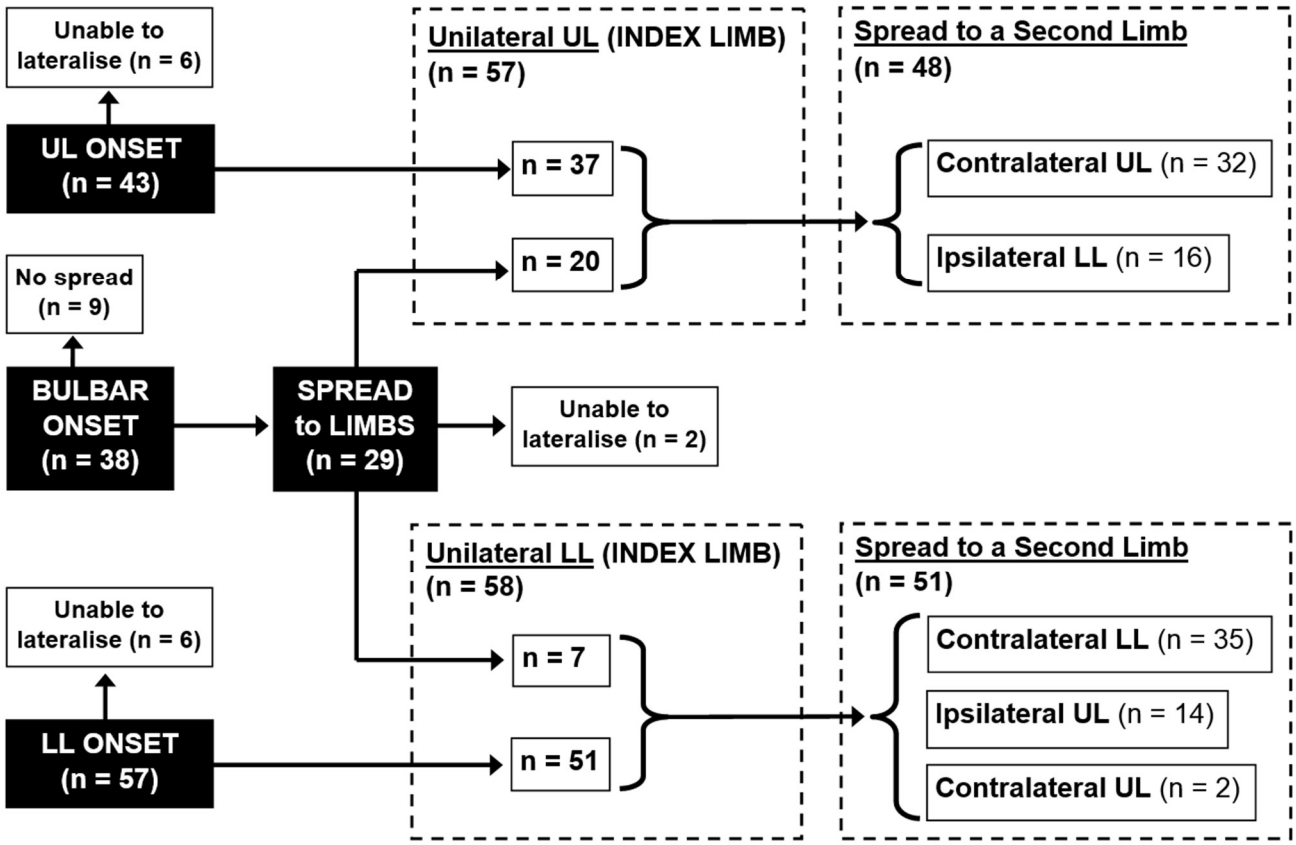
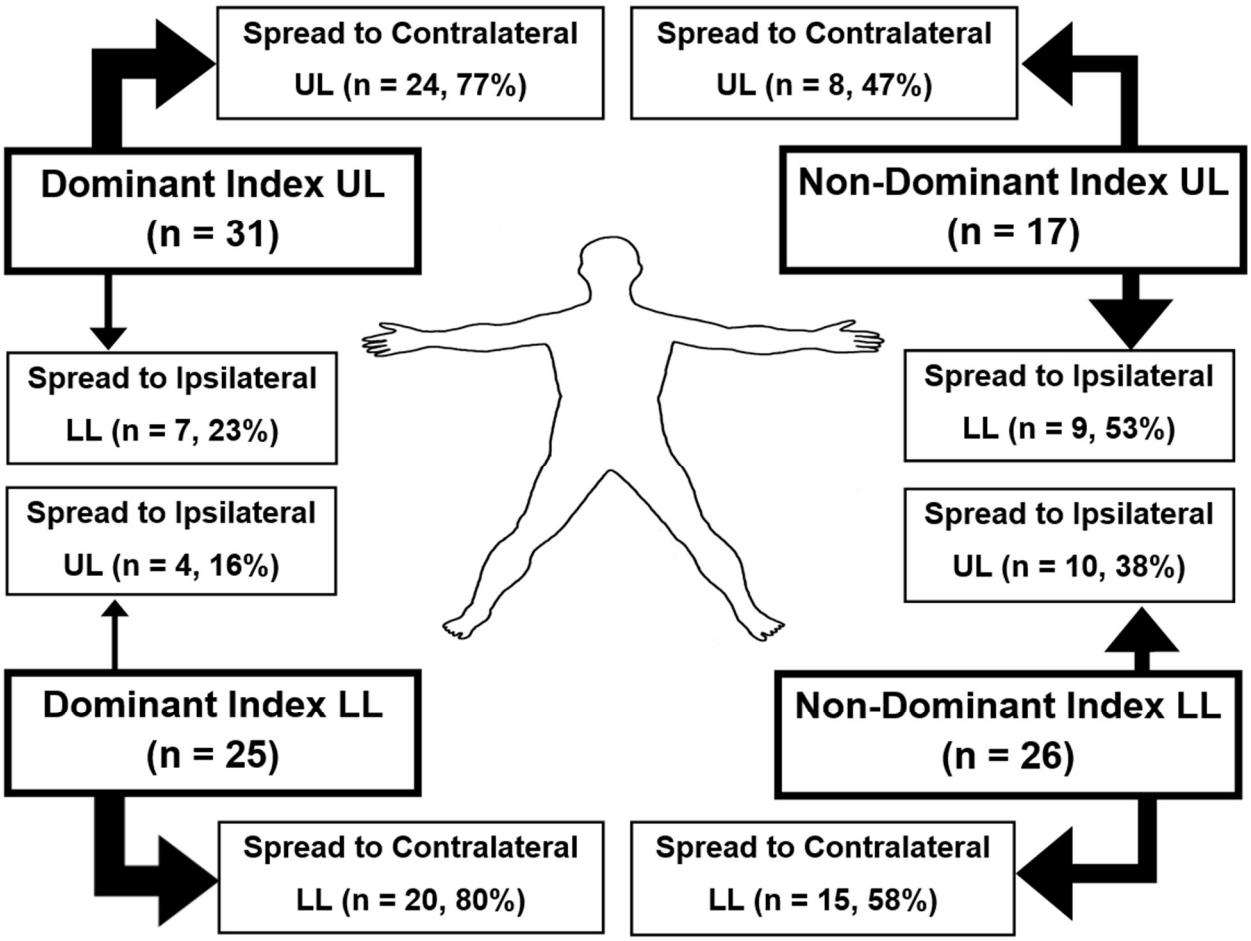
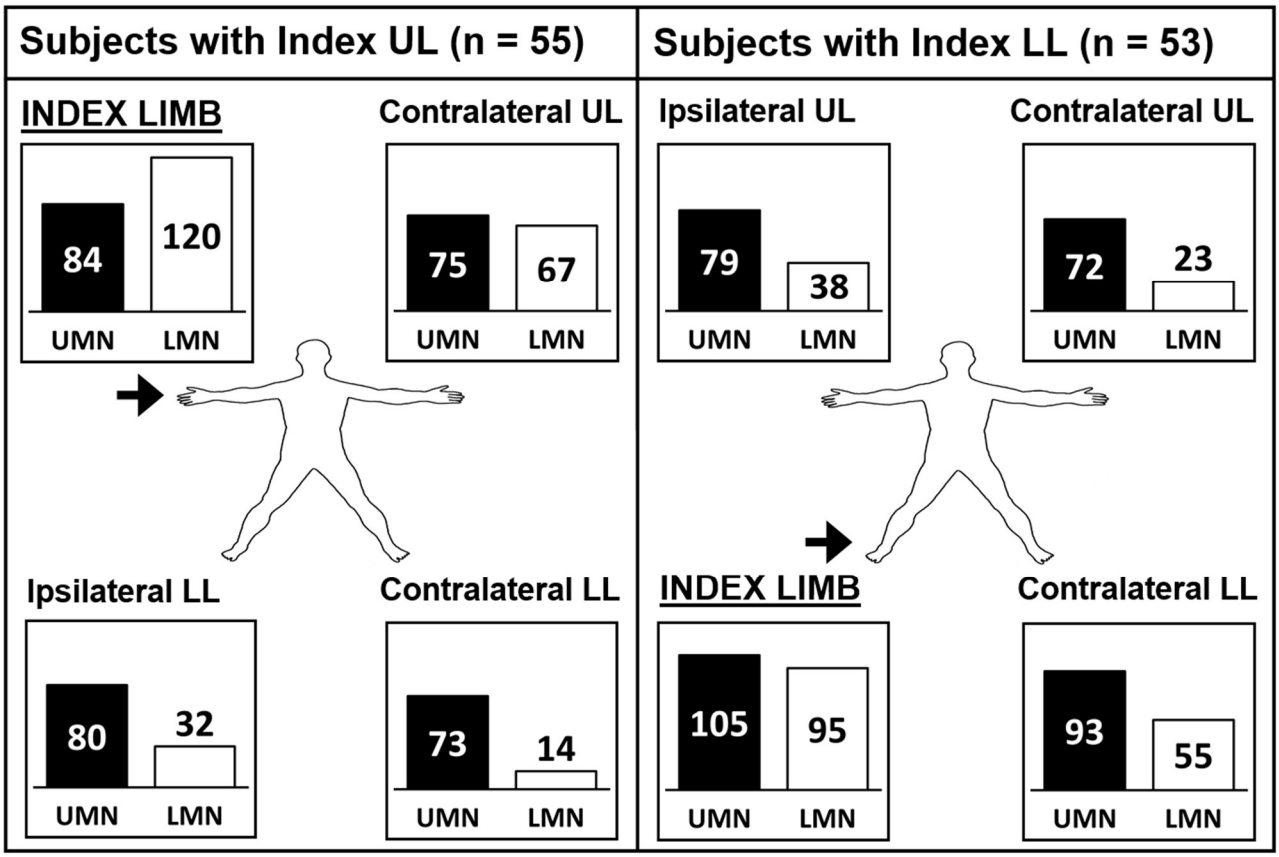


Figure 2: The effect of limb dominance on spread of weakness



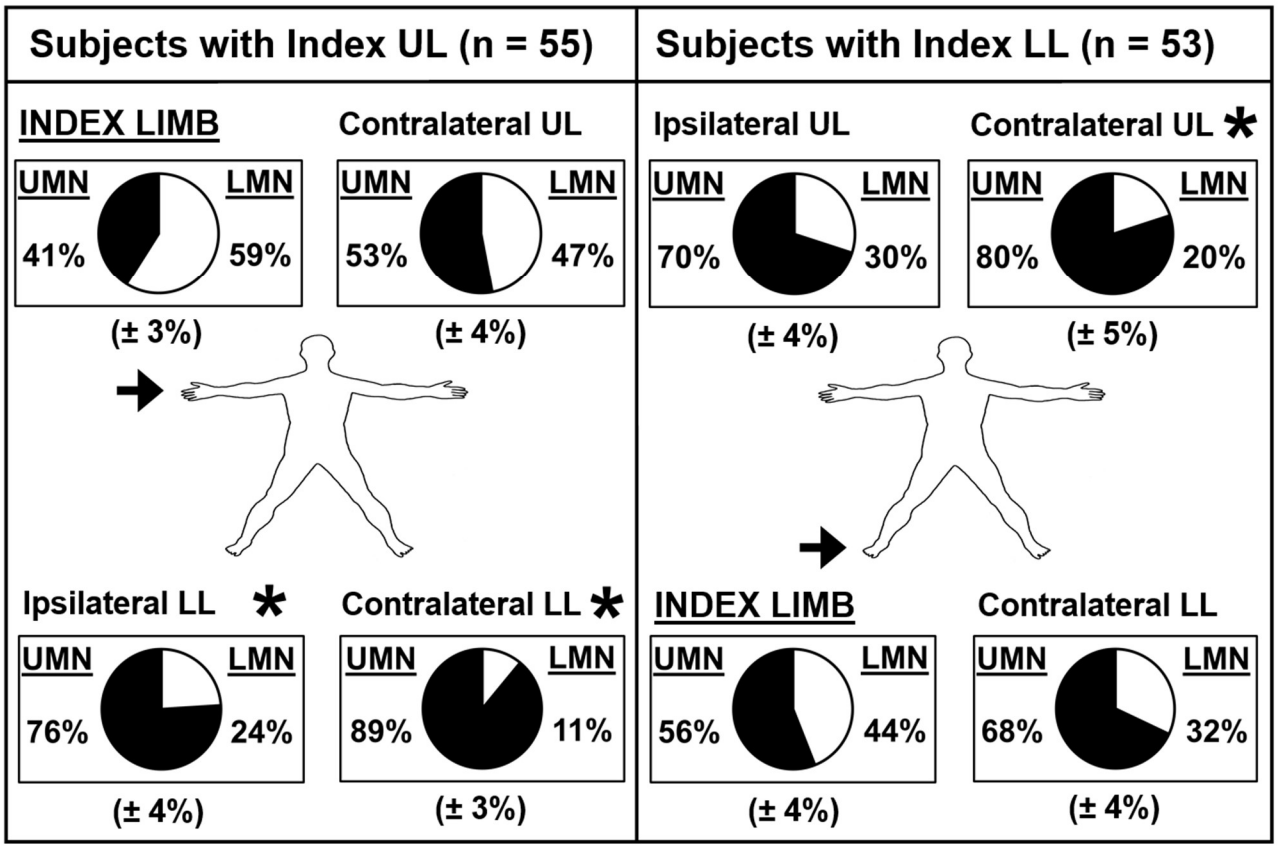
This diagram illustrates the spread of limb weakness, according to whether the Index Limb was on the dominant or non-dominant side. Subjects included in this diagram reported onset of weakness in a unilateral Index Limb, and then reported spread of weakness to another discrete limb (n = 99 in total). Spread of weakness from a non-dominant Index Limb was more likely to be in an ipsilateral direction (p = 0.008). Two subjects (one of 25 subjects with a dominant Index LL, and one of 26 subjects with a non-dominant Index LL) reported spread of weakness to the contralateral UL. These two subjects are not included in the spread patterns shown in this diagram.

Figure 3: Total UMN and LMN scores for each limb



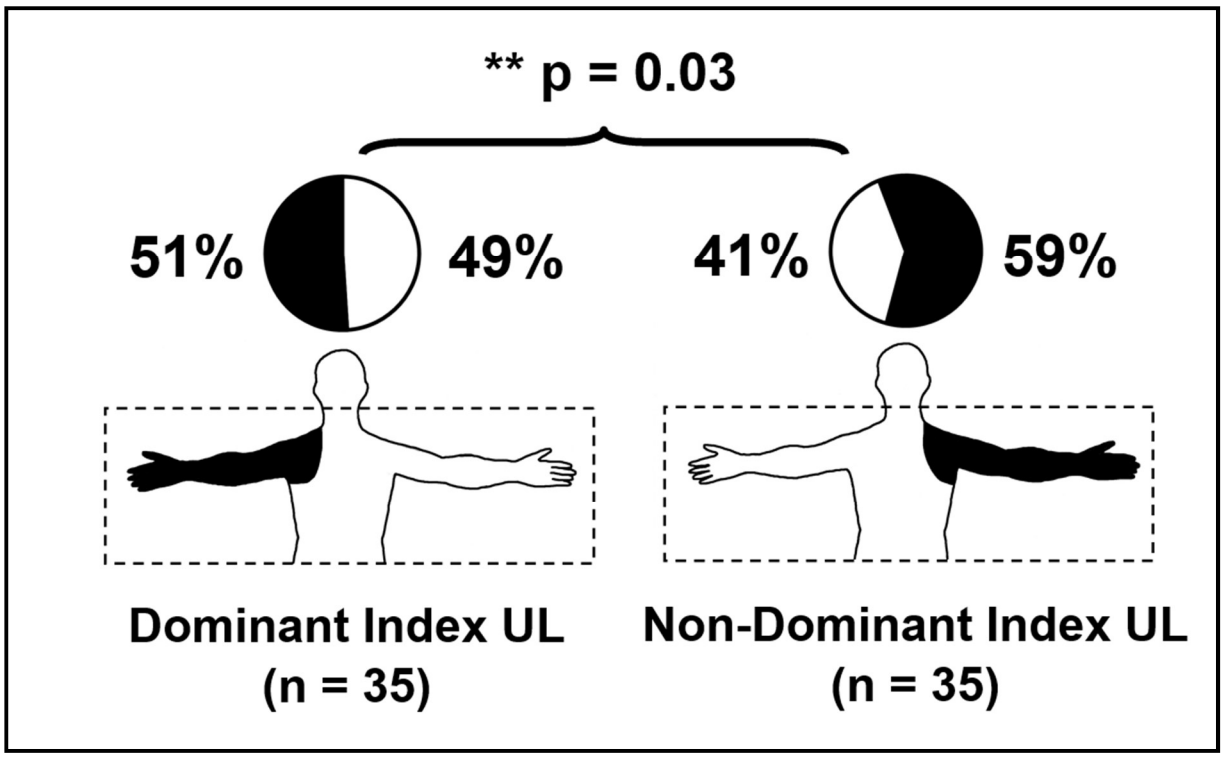
This diagram shows the distribution of Total UMN scores (black bars) and Total LMN scores (white bars) relative to the Index Limb. Subjects are grouped according to whether the Index Limb was an UL (n = 55) or a LL (n = 53), irrespective of limb dominance. In both groups, the Total scores were highest in the Index Limb (which is indicated with an arrow).

Figure 4: Mean UMN% and LMN% scores for each limb (± SE)



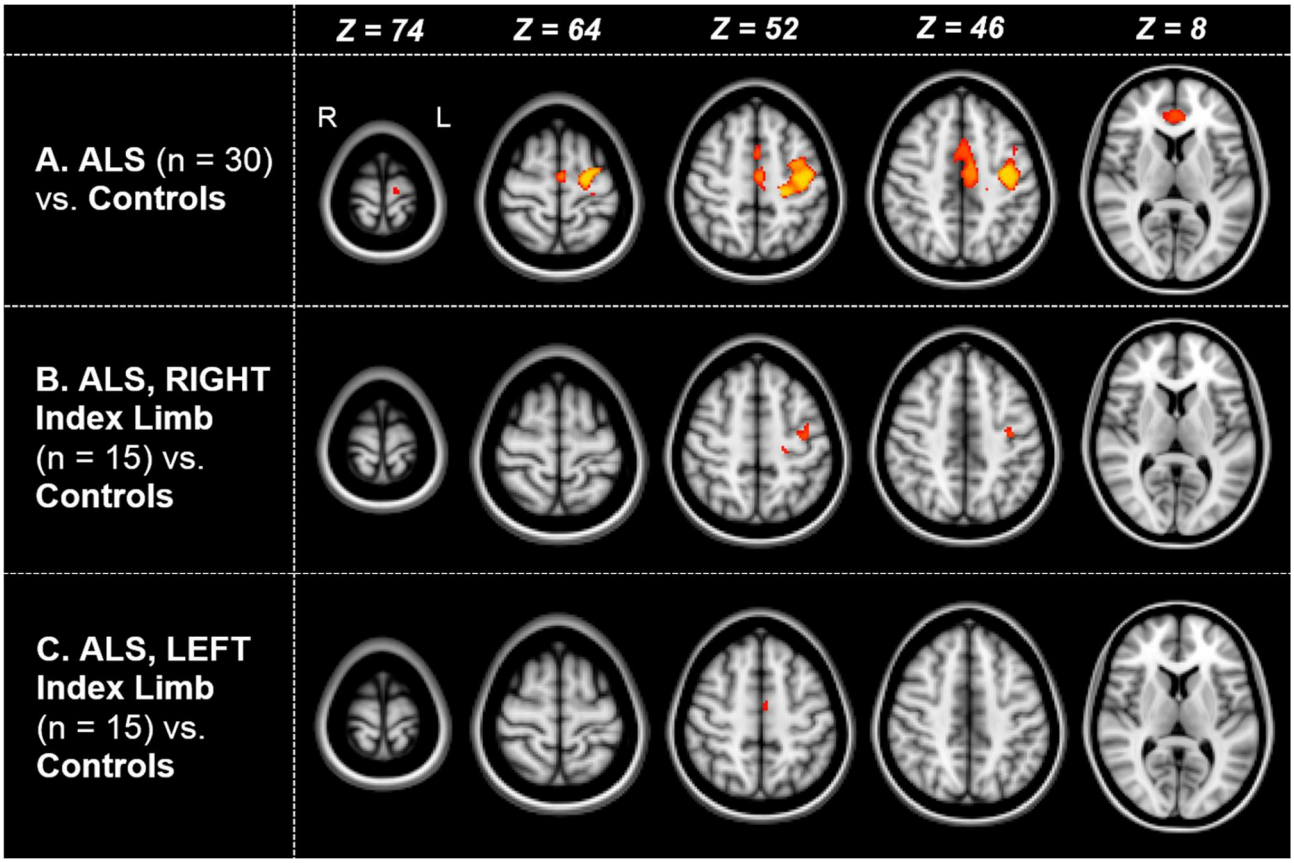
These scores represent the proportion of the total clinical deficit in each limb attributable to UMN and LMN dysfunction (with both scores adding to give 100%). Subjects are grouped according to whether the Index Limb was an UL (n = 55) or a LL (n = 53), irrespective of limb dominance. Mean UMN% scores were greatest in the limb most anatomically distant from the Index Limb. Significant differences ($p < 0.01$) between the UMN% score in the Index Limb and each Non-Index Limb, using ANOVA with post hoc comparisons (Bonferroni), are indicated by an asterisk (*).

Figure 5: Mean proportional UMN scores for the upper limbs



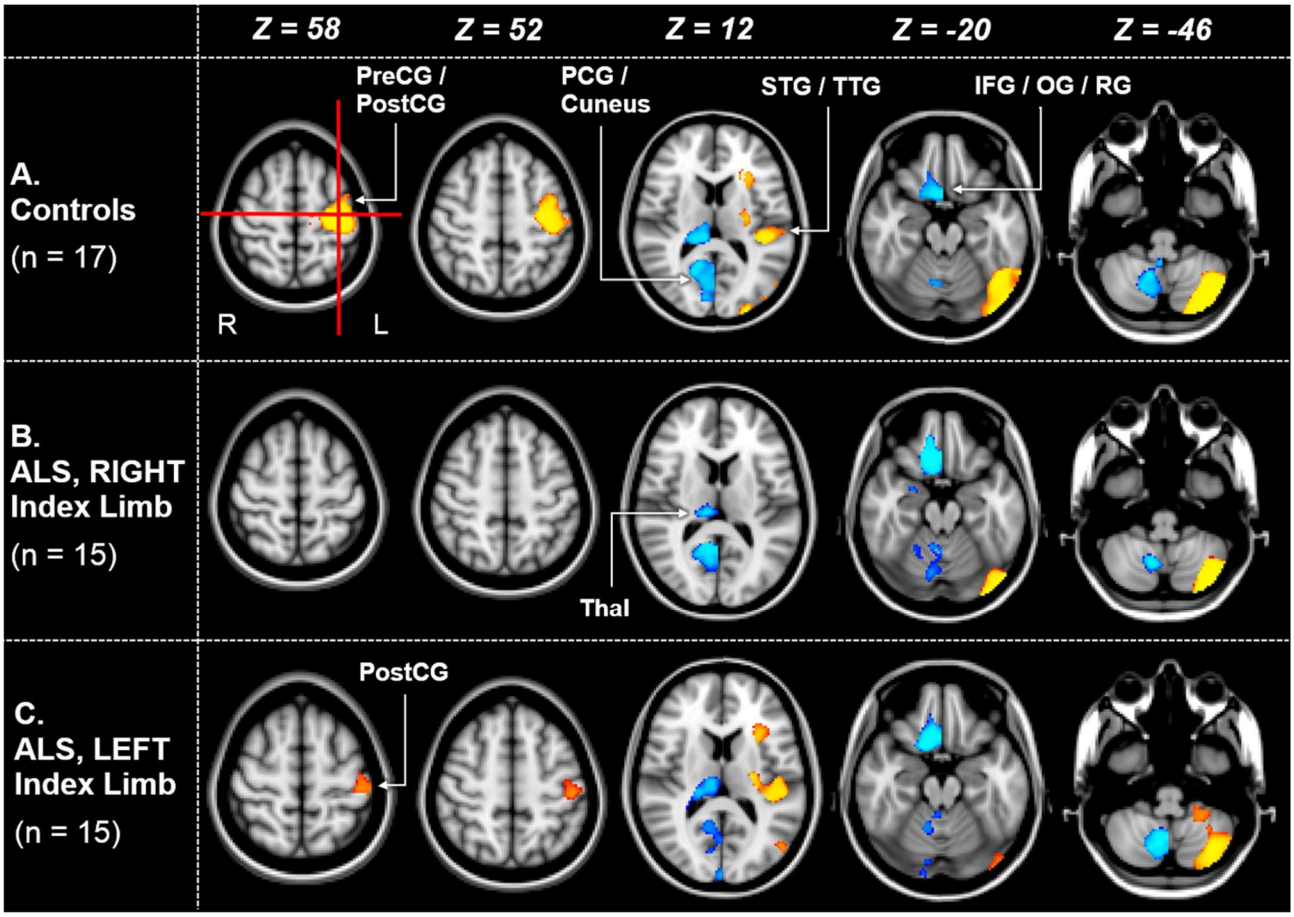
This diagram compares the mean proportional UMN scores in subjects with an Index upper limb on either the dominant side or non-dominant side. Proportional scores represent a comparison between the Index Limb (coloured in black) and a Non-Index Limb (coloured in white), and each pair of scores add to give 100%. A Student's t-test was used to compare mean proportional UMN scores with a dominant versus non-dominant Index upper limb. This comparison was significant ($p = 0.03$), suggesting greater asymmetry of UMN signs in the upper limbs, when the Index Limb was on the non-dominant side.

Figure 6: Gray matter atrophy in ALS versus controls



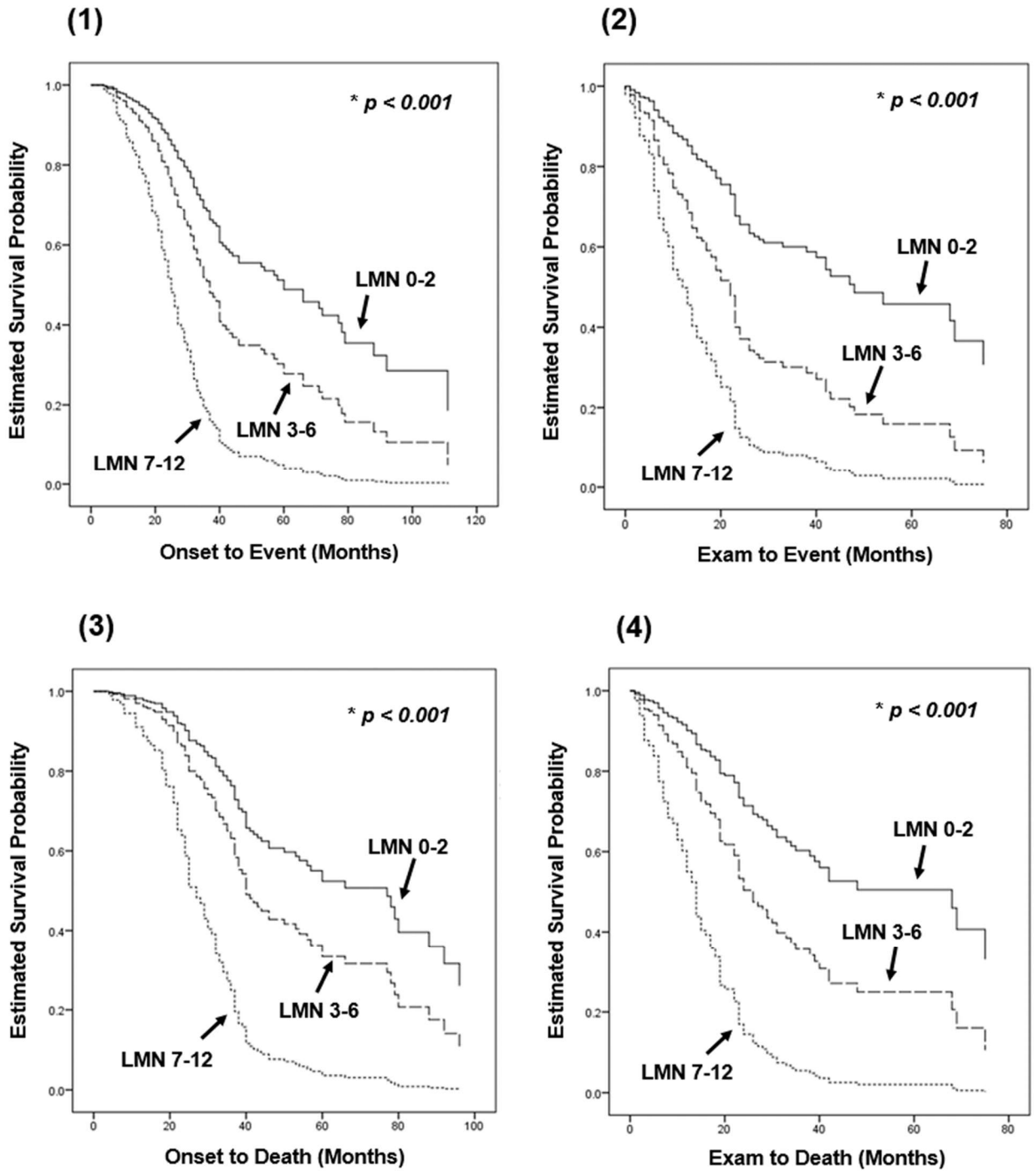
Selected axial plane reconstructions showing regions of reduced GM density in subjects with ALS, compared with controls. Regions of significantly reduced density ($p \leq 0.05$, TFCE-corrected) are coloured in orange-yellow. Row A shows a comparison between all ALS subjects (n=30) and controls (n=17). Rows B and C show the patterns of atrophy in ALS subjects with a right-sided or left-sided index limb, respectively (n = 15 in each group).

Figure 7: Patterns of gray matter asymmetry in ALS and control subjects



Selected axial plane reconstructions from 17 right-handed controls (Row A), 15 ALS subjects with a right-sided index limb (Row B), and 15 ALS subjects with a left-sided index limb (Row C). Significant regions of GM asymmetry ($p \leq 0.01$, TFCE-corrected) are shown. Regions coloured in orange-yellow represent leftward asymmetry (i.e. higher GM density in the left hemisphere), whereas blue clusters signify rightward asymmetry. In control subjects, there is a cluster of leftward GM asymmetry which incorporates the centre-of-gravity of the dominant thenar representation area (shown by the intersection of the two red lines). PreCG=precentral gyrus; PostCG=postcentral gyrus; PCG=posterior cingulate gyrus; STG=superior temporal gyrus; TTG=transverse temporal gyrus; IFG=inferior frontal gyrus; OG=orbital gyrus; RG=rectal gyrus; Thal=thalamus.

Figure 8: Covariate-adjusted survival function for each category of LMN score (whole body)



Covariate- adjusted survival function for each category of LMN score (whole body), for each of the four outcomes: (1) Onset to Event; (2) Exam to Event; (3) Onset to Death; and (4) Exam to Death.