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DISSERTATION

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Cortical and subcortical grey matter atrophy in Amyotrophic Lateral Sclerosis correlates with measures of disease accumulation independent of disease aggressiveness

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Abbreviations

ALS	amyotrophic lateral sclerosis
ALSFRS-R	amyotrophic lateral sclerosis functional rating scale – revised
CAT12	computational anatomy toolbox 12
cFL	calculated functional loss-rate
cFS	calculated functional state
CT	cortical thickness
D50	parameter of disease aggressiveness
ECAS	Edinburgh cognitive and behavioral ALS screen
GM	grey matter
HC	healthy controls
MRI	magnetic resonance imaging
PrCG	precentral gyrus
rD50	relative D50; parameter of disease progression
ROI	region of interest
SBM	surface-based morphometry
SPM	statistical parametric mapping
VBM	voxel-based morphometry

Zusammenfassung (Deutsch)

Die beiliegende Publikation mit dem Titel „Cortical and subcortical grey matter atrophy in Amyotrophic Lateral Sclerosis correlates with measures of disease accumulation independent of disease aggressiveness“ untersucht die Möglichkeit strukturelle MRT-Untersuchungen als Surrogat für die klinische Krankheitsprogression bei Amyotropher Lateralsklerose zu verwenden.

Die Amyotrophe Lateralsklerose ist eine neuromuskuläre Erkrankung des neurodegenerativen Spektrums, die durch eine fortschreitende muskuläre Atrophie und einen motorischen Funktionsverlust im Krankheitsverlauf gekennzeichnet ist. Die starke Heterogenität der Erkrankung mit interindividuell variablen Spontanverläufen stellt eine Herausforderung, für das therapeutische Management der Patienten, aber auch für die Durchführung klinischer Studien dar. Vor diesem Hintergrund ist die Etablierung zuverlässiger Biomarker für eine Quantifizierung des Krankheitsverlaufs der Patienten, vor allem hinsichtlich ihrer individuellen Krankheitsaggressivität und -phase, von großer Bedeutung in der Forschung zu Amyotropher Lateralsklerose. Ziel der beiliegenden Publikation war es daher, einen krankheits-spezifischen Biomarker in der Magnetresonanztomographie zu validieren, welcher eine Stratifizierung von Patienten sowie das Monitoring ihrer Erkrankungsprogression ermöglicht.

Mithilfe des D50-Krankheitsprogressionsmodells wurde ein Instrument etabliert, das individuelle Erkrankungsverläufe bei Amyotropher Lateralsklerose und somit die klinische Heterogenität genauer beschreibt. Mithilfe des Modells lassen sich Patienten-spezifische Parameter berechnen und Patienten in unterschiedliche Phasen ihres Krankheitsverlaufes einteilen.

Die Korrelation der individuellen Parameter des D50-Modells mit Biomarkern der Bildgebung mittels Magnetresonanztomographie wurde bereits in vorhergehenden Arbeiten unserer Gruppe untersucht und validiert.

Zielstellung der vorliegenden Arbeit war es, krankheits-spezifische Marker der grauen Hirnsubstanz in T1-gewichteten Sequenzen zu identifizieren und diese auf Korrelationen mit Parametern des D50-Modells hin zu prüfen. Studien haben bereits nachweisen können, dass die Amyotrophe Lateralsklerose typischerweise mit einer Atrophie grauer Substanz einhergeht. Bisher fehlen jedoch zuverlässige Daten hinsichtlich der Veränderungen im Krankheitsverlauf und in Abhängigkeit von der Krankheitsaggressivität. Daher war die spezielle Fragestellung dieser Arbeit, ob die Dicke der kortikalen grauen Substanz (GM) sowie das Volumen subkortikaler Regionen abhängig sind vom Krankheitsstadium eines Patienten zum Zeitpunkt

der bildgebenden Untersuchung und ob Veränderungen der grauen Substanz zugleich unabhängig sind von der allgemeinen Krankheitsaggressivität. Um diese Fragestellung zu beantworten, wurden Magnetresonanzaufnahmen von insgesamt 100 Patienten mit Amyotropher Lateralsklerose und 72 gesunden Kontrollen untersucht. Sämtliche Aufnahmen wurden zunächst mithilfe der Surface-Based Morphometry analysiert, um spezifische Parameter der grauen Hirnsubstanz, einschließlich der kortikalen Dicke, zu berechnen. Zur Untersuchung von subkortikalen Veränderungen grauer Substanz wurden Hirnareale in verschiedene Regionen unterteilt und deren Volumen grauer Substanz analysiert. Die kortikale und subkortikale graue Substanz wurde im ersten Schritt zwischen Patienten und gesunden Kontrollen verglichen. Um Unterschiede innerhalb der Patientenkohorte zu analysieren, wurden die Patienten anschließend mit Hilfe des oben beschriebenen D50-Modells in Subgruppen unterteilt, abhängig von a) ihrer Erkrankungsphase im individuellen Krankheitsverlauf b) der Gesamtkrankheitsaggressivität. Neben den Gruppenvergleichen wurden zudem Magnetresonanz-Marker und Parameter des D50-Modells mittels Regressionsanalysen auf Korrelationen überprüft. Im Ergebnis zeigte sich eine Reduktion kortikalen und subkortikaler grauer Substanz Patienten im Vergleich zu gesunden Kontrollen. Weiterhin konnten die Untersuchungen zeigen, dass die Atrophie grauer Hirnsubstanz im Krankheitsverlauf fortschreitet und dass diese Veränderungen unabhängig sind von der individuellen Krankheitsaggressivität eines Patienten. Der Vergleich von Patienten in unterschiedlichen Erkrankungsphasen ergab eine verminderte kortikale Dicke bei Patienten in späteren Krankheitsphasen. Die Abnahme der kortikalen Dicke bestätigte sich in der multiplen Regressionsanalyse mit rD50, in welcher sich eine signifikante negative Korrelation zwischen kortikaler Dicke einiger Hirnareale und rD50 zeigte.
Dass die Abnahme kortikaler Dicke im Verlauf des Krankheitsprozesses unabhängig von der individuellen Gesamtaggressivität der Erkrankung zu sein scheint, zeigten Subgruppenvergleiche zwischen Patienten unterschiedlicher Krankheitsaggressivität und D50-Regressionsanalysen. Die Analysen der grauen Substanz in subkortikalen ROI zeigten ebenfalls eine Abnahme der grauen Substanz bei Patienten, welche im Verlauf der Krankheit zunimmt, aber keinen Zusammenhang mit der Gesamtkrankheitsaggressivität besitzt.

Zusammenfassend zeigte sich durch die Analysen der grauen Substanz in der Magnetresonanztomographiem dass die kortikale Dicke im Verlauf einer Amyotrophen Lateralsklerose abnimmt und ein Parameter ist, welcher die Veränderungen im Krankheitsverlauf unabhängig von der Krankheitsaggressivität widerspiegeln kann.

Zusammenfassung (Englisch)

The enclosed publication with the title "Cortical and subcortical grey matter atrophy in Amyotrophic Lateral Sclerosis correlates with measures of disease accumulation independent of disease aggressiveness" investigates the possibility of using structural magnetic resonance imaging examinations as a surrogate for clinical disease progression in amyotrophic lateral sclerosis. Amyotrophic lateral sclerosis is a neurodegenerative disease characterized by progressive muscular atrophy and motor function loss during disease progression. The strong heterogeneity of the disease with interindividual variability of spontaneous progression is a challenge for the therapeutic management of patients, but also for the conduct of clinical studies. Against this background, the establishment of reliable biomarkers to quantify the disease progression of patients, especially with respect to their individual disease aggressiveness and phase, is of great importance in amyotrophic lateral sclerosis research. The aim of the enclosed publication was to validate a disease-specific biomarker in magnetic resonance imaging which allows stratification of patients and monitoring their disease progression.

By the D50 disease progression model, an instrument was established that describes individual disease progression and thus clinical heterogeneity more accurately. With the help of this model, patient-specific parameters can be calculated, and patients can be divided into different phases of their disease progression.

The correlation of individual parameters of the D50 model with biomarkers of the magnetic resonance imaging has been investigated and validated in previous work by our group.

The aim of the present work was to identify disease-specific gray matter changes in T1-weighted magnetic resonance imaging sequences and to evaluate correlations with parameters of the D50 model. Studies have already demonstrated that amyotrophic lateral sclerosis is typically associated with atrophy of gray matter. However, reliable data regarding changes in the course of the disease and in relation to disease aggressiveness are missing. Therefore, the specific question of this study was whether cortical gray matter thickness and the volume of subcortical regions of interest depend on the disease stage of a patient at the time of the magnetic resonance imaging examination and whether changes in gray matter are at the same time independent of overall disease aggressiveness. To answer this question, magnetic resonance images of 100 ALS patients and 72 healthy controls were examined.

All images were first analyzed using surface-based morphometry, to calculate specific brain gray matter parameters, including cortical thickness. To investigate subcortical gray matter alterations, subcortical brain areas were subdivided into different regions and their grey

matter volume was analyzed. Both, cortical thickness, and subcortical gray matter volumes were compared between amyotrophic lateral sclerosis patients and healthy controls in the first step. To investigate differences in gray matter within the patient cohort, patients were subsequently divided into subgroups using the D50 model described above, depending on a) their disease phase in the individual course of the disease and b) their overall disease aggressiveness. In addition to the subgroup analyses, the magnetic resonance imaging markers, and parameters of the D50 model were tested for correlations using regression analyses. Results showed a reduction of cortical thickness as well as of subcortical gray matter volumes in amyotrophic lateral sclerosis patients compared to healthy controls. Furthermore, they showed that the atrophy of gray matter in the brain progresses during the course of the disease and that these changes are independent of individual disease aggressiveness. The comparison of patients in different stages of the disease showed a reduced cortical thickness in patients in later stages of disease.

The decrease in cortical thickness was confirmed in the multiple regression analysis with rD50 where a significant negative correlation between cortical thickness of some brain areas and rD50 was found.

The decrease in cortical thickness during the disease progression showed to be independent of the individual overall aggressiveness of the disease, this was the result of our subgroup comparisons between patients with high and low disease aggressiveness as well as in D50 regression analyses. The gray matter analyses in subcortical regions also showed a decrease in gray matter in amyotrophic lateral sclerosis patients, which increases during the disease, but has no correlation with overall disease aggressiveness. In summary, the analyses showed that gray matter decreases during amyotrophic lateral sclerosis and is a parameter that reflects changes in disease progression independently of the disease progression.

1 Introduction

The paper included in this dissertation is part of research in the field of Amyotrophic Lateral Sclerosis (ALS). ALS is a progressive neurodegenerative disease. In European population the incidence ranks from 2 to 3 cases per 100.000 (Volk et al. 2018), which is slightly higher than the worldwide incidence (Longinetti und Fang 2019). ALS is a motor neuron disease; however, many researchers discuss underlying pathophysiological processes which may involve different domains of the neuromuscular system (Mahoney et al. 2021, Masrori und Van Damme 2020). Most cases of ALS are not monogenetically inherited, but a familial accumulation of cases makes genetic dispositions likely and approximately 5-10% of patients suffer from familial ALS (Zou et al. 2017). Genetic studies discovered several candidate genetic variants associated with an increased risk to develop ALS, especially C9orf72, SOD1, TARDP and FUS (Masrori und Van Damme 2020, Goutman et al. 2022).

Patients with ALS suffer from progressive loss of motoric functions due to the degeneration of the first and second motor neuron. Depending on the ALS-subtype, muscles in different body regions are affected at different times in the disease course. There are different phenotypes of ALS and the various forms of disease show a highly heterogenous clinical course. Survival rates rank between a few months and more than four decades after the onset of first ALS symptoms (Chio et al. 2011). However, on average patients with ALS die approximately three years after the first diagnosis (Kiernan et al. 2011, Longinetti und Fang 2019, van Es et al. 2017).

Observations of the ALS disease spectrum show that besides pure motoneuronal involvement, many patients also suffer from frontotemporal dementia symptoms with variable severity. Therefore, it is recommended that the diagnostic workup of patients suspected to suffer from ALS should include neuropsychological assessments. The Edinburgh Cognitive and Behavioral ALS Screen (ECAS) was first introduced in 2013 (Abrahams et al. 2014) and is an established instrument to screen for (sub-)clinical neuropsychological impairments. The ECAS differentiates between ALS-specific and non-specific cognitive functions and is therefore more sensitive for ALS-specific dysfunctions and behavior changes compared to other cognitive screening instruments (such as FAB, MoCA) which are more generic (Lule et al. 2015).

Cognitive impairments in ALS have been found to be selectively associated with changes in subcortical grey matter (GM) (Bede et al. 2018, Machts et al. 2015, van der Burgh et al. 2020).

A study published in 2013 by Braak and colleagues suggested a theory of corticofugal spread of ALS-related neuropathology originating from motoneurons in the agranular motor

cortex, brainstem motor nuclei of cranial nerves V, VII, and X–XII, and spinal cord α-motoneurons (Braak et al. 2013). This theory which is, up to today, the most common hypothesis about the spread of ALS-pathology, indicates that the disease develops in 4 different stages, according to distribution patterns of the phosphorylated 43-kDA TAR DNA-binding protein (pTDP-43) in post-mortem neuronal tissue. However, naturally there is no in-vivo evidence of such a sequential spread of ALS-related pathology in the human brain. Some studies demonstrated a stage-wise involvement of neuronal fiber tracts via MRI (Kassubek et al. 2014, Gorges et al. 2018), however, they used a track-of-interest technique. Part of the motivation of the study presented here was to examine if a regional spread can be found in MRIs of patients suffering from ALS using a whole-brain approach.

To measure clinical disease burden, most clinical studies use the ALS Functional Rating Scale Revised (ALSFRS-R). The ALSFRS-R is a validated rating tool that measures the remaining motoric functions of a patient based on 12 different items. This questionnaire is therefore able to monitor the progression of disability and accumulation of disease burden (Cedarbaum et al. 1999). However, many studies suppose a linear decrease of the ALSFRS-R total score over time, while observations have shown that functional decline in ALS is typically curvy-linear (Gordon et al. 2010, Shaabi 2022). Another pitfall of the ALSFRS-R is that scorings of the items are quite susceptible to intra- and inter-rater variability (van der Burgh et al. 2020, Steinbach et al. 2021a). Further, most longitudinal studies use fixed time-intervals requiring ALSFRS-R ratings close to the time of MRI necessary. Such fixed follow-up intervals are problematic since they make longitudinal studies prone to selection bias because MRIs later in disease are less often possible for patients with bulbar-onset or patients with a fast disease progression. Other consequences are high drop-out rates, typical for ALS studies (de Albuquerque et al. 2017, van der Burgh et al. 2020).

Here arises the advantage of the D50-disease progression model which has been introduced and validated by our group. The D50-model describes the course of disease as a sigmoidal curve representing the accumulating loss of motoric functions from full health to functional loss. It is calculated by iterative fitting of regularly gathered ALSFRS-R scores from a patient. The D50 value itself is defined as the time taken (in months) to reach halved functionality, thus it represents a measure of a patient's overall disease aggressiveness. By normalizing a patient's D50 value onto 0.5 the relative D50 (rD50) parameter can be calculated. rD50 is an open-ended reference scale where 0 represents the symptom onset and 0.5 the point of halved functionality. The rD50 value thus represents the accumulation of disease, independent of disease aggressiveness. The model thus overcomes limitations of traditional

clinical metrics and enables robust correlations with parameters of structural MRI (and other biomarkers) (Steinbach et al. 2021a, Dreger et al. 2021). Another strength of the model is the possibility to calculate additional descriptors of disease such as the acute functional state and acute functional loss-rate (cFS/ cFL) at any given time point.

With use of the D50 model, pseudo-longitudinal studies with a greater number of patients are possible, including those with no available ALSFSR-R score close to MRI scanning. In most studies (not using the D50 model), these patients would have been excluded from further analyses resulting in a higher risk of selection bias. Additionally, the D50-model does not depend on strict time intervals for follow-ups which further reduces selection bias and drop-out rates.

By now, there are no reliable biomarkers that can specifically monitor the course of the ALS disease. The comparability of biomarkers from symptomatic patients (e.g. neuroimaging, blood-, liquor ,or skin probes etc.) is difficult due to highly heterogenous clinical courses which challenges monitoring of disease progression and clinical trials (Kiernan et al. 2021). Therefore, ALS-researchers worldwide try to identify and validate surrogates of the progression of the neurodegenerative process in ALS, i.e., the disease stage/accumulation. Desirably these biomarkers should be independent of background disease activity, such as speed of disease progression. In this context, there are joined international efforts to use MRI as a non-invasive technique for staging and therapeutic monitoring in ALS

. Namely, the Neuroimaging Society in Amyotrophic Lateral Sclerosis (NiSALS) (Turner et al. 2011) was founded to bundle up efforts in order to develop reliable neuroimaging-based biomarkers.

From a conservative point of view, there are only a few MRI changes known that directly mirror motoneuron degeneration in ALS (e.g. hyperintensities in T1-weighted images of the cortico-spinal tract) but they cannot be found in every patient and therefore cannot be used as diagnostic criteria for ALS (Turner et al. 2012, Turner et al. 2011, Turner und Verstraete 2015). However, since MRI is non-invasive and relatively easily accessible it is a promising tool for biomarkers that could be helpful in stratifying patients with ALS. There is a growing number of analyzing techniques for MRI-images that give detailed greater insight into (micro-)structures of the brain. For example, Voxel-based Morphometry (VBM) is used to identify global and regional atrophy of white and grey matter (WM and GM) by statistically measuring changes in signal-intensity of T1-weighted MRI images (hereafter referred to as “density”). Surface-based Morphometry (SBM) uses similar pre-processing routines but concentrates on the cortical architecture of GM.

Besides the pure volume, specific features of GM are its regional surface and thickness; both can be assessed by SBM in a semi-automatic manner (Jiao et al. 2010, Voets et al. 2008). Several ALS-studies demonstrated alterations in cerebral GM of ALS-patients by using VBM. However, VBM seems to be less accurate regarding the detection of upper motoneuron involvement and disease-related changes in ALS than SBM which is more sensitive for this purpose (Turner et al. 2012, Verstraete et al. 2015).

The D50-model described above has already been used in a variety of correlation analyses with neuroimaging and other biomarkers. The first study applying the D50 disease progression model to GM and WM pathologies as measured by MRI used VBM (Steinbach et al. 2020). There, it was demonstrated that widespread GM and WM changes can be found in ALS and, most important, that these alterations progressed with the ongoing course of the disease. The study was able to show structural changes in direct subgroup comparisons between patients in different phases of disease on a group-level, whereas VBM-regression analyses revealed no significant correlations between rD50 and GM density. A probable explanation are the limitations of the VBM method which does not offer specific measures of GM such as Cortical Thickness (CT) (Jiao et al. 2010, Pereira et al. 2012). Interestingly, in this study, the GM alterations that could be found had no association with disease aggressiveness, neither in subgroup comparisons nor in multiple regression analyses.

Therefore, the study presented here was conducted to identify robust correlations between GM alterations and the accumulation of disease (described by the D50 model) by using SBM. Aim of the enclosed publication was consequently to determine if integrity of cortical and subcortical GM can be used as a marker in ALS, specifically for the monitoring of the course of disease. Based on the knowledge of previous studies we hypothesized that SBM-derived metrics of GM integrity could reflect the ongoing accumulation of ALS pathology without being influenced by background disease activity. A biomarker fulfilling these criteria is of great interest for further ALS-research and could help to monitor the effectiveness of therapeutic interventions. Before our research, we thought what the main objectives of the work should be. A major challenge in ALS-research is the high clinical heterogeneity between patients. There is a great diversity regarding the chronological involvement of upper and lower motoneurons, body regions and speed of disease progression. Therefore, studies in ALS face the major challenge that patients with highly divergent forms of disease are included into the trials. Today, there are no validated biomarkers for either stratification or therapeutic management that could be used for more individualized and cohort-specific study designs. For this reason, our study aimed to investigate alterations in grey matter in ALS-patients and along disease progression

to evaluate whether CT can be used as a disease-specific surrogate to monitor the course of the disease independent of disease aggressiveness. Validation of cortical thickness as a biomarker reflecting disease accumulation would be highly useful for stratification of patients in upcoming study trials and could in addition serve as improved outcome measure.

With use of SBM, we wanted to investigate specific patterns of cortical thinning in ALS and correlations of these measures with disease accumulation and aggressiveness. Further, we used VBM to examine decrease of grey matter volume in subcortical regions of interest.

The enclosed published paper therefore addresses the following questions:

1. Are there alterations in cortical thickness between patients with ALS and healthy controls?
2. Are alterations of cortical thickness limited to distinct areas in the brain?
3. Does cortical thickness change along disease progression?
4. Is cortical thickness dependent on overall disease aggressiveness?
5. Are there voxel-wise correlations between cortical thickness and parameters of the D50 disease progression model?

2 Original published paper

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Cortical and subcortical grey matter atrophy in Amyotrophic Lateral Sclerosis correlates with measures of disease accumulation independent of disease aggressiveness

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ABSTRACT

There is a growing demand for reliable biomarkers to monitor disease progression in Amyotrophic Lateral Sclerosis (ALS) that also take the heterogeneity of ALS into account.

In this study, we explored the association between Magnetic Resonance Imaging (MRI)-derived measures of cortical thickness (CT) and subcortical grey matter (GM) volume with D50 model parameters.

T1-weighted MRI images of 72 Healthy Controls (HC) and 100 patients with ALS were analyzed using Surface-based Morphometry for cortical structures and Voxel-based Morphometry for subcortical Region-Of-Interest analyses using the Computational Anatomy Toolbox (CAT12). In Inter-group contrasts, these parameters were compared between patients and HC. Further, the D50 model was used to conduct subgroup-analyses, dividing patients by a) Phase of disease covered at the time of MRI-scan and b) individual overall disease aggressiveness. Finally, correlations between GM and D50 model-derived parameters were examined.

Inter-group analyses revealed ALS-related cortical thinning compared to HC located mainly in frontotemporal regions and a decrease in GM volume in the left hippocampus and amygdala. A comparison of patients in different phases showed further cortical and subcortical GM atrophy along with disease progression. Correspondingly, regression analyses identified negative correlations between cortical thickness and individual disease covered. However, there were no differences in CT and subcortical GM between patients with low and high disease aggressiveness.

By application of the D50 model, we identified correlations between cortical and subcortical GM atrophy and ALS-related functional disability, but not with disease aggressiveness. This qualifies CT and subcortical GM volume as biomarkers representing individual disease covered to monitor therapeutic interventions in ALS.

1. Introduction

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease and survival rates are short with patients succumbing three years after the onset of symptoms on average (Kiernan et al., 2011; Longinetti and Fang, 2019; van Es et al., 2017). There is marked heterogeneity in terms of different disease onsets, patterns of clinical spread of symptoms, and most of all vastly differing disease progression-speed

Abbreviations: ALS, Amyotrophic Lateral Sclerosis; ALSFRS-R, ALS Functional Rating Scale (Revised); CAT12, Computational Anatomy Toolbox; cFL, Calculated Functional Loss-rate; cFS, Calculated Functional State; CT, Cortical Thickness; D50, disease aggressiveness; GM, Grey Matter; HC, Healthy Controls; MRI, Magnetic Resonance Imaging; rD50, relative D50 (individual disease covered); PrCG, Precentral Gyrus; ROI, Region-Of-Interest; SBM, Surface-based Morphometry; SPM, Statistical Parametric Mapping; TIV, Total Intracranial Volume; VBM, Voxel-Based Morphometry; WM, White Matter.

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(Westeneng et al., 2018). This impairs the comparability of ALS cohorts and challenges monitoring disease progression in clinical trials (Kiernan et al., 2021). Therefore, reliable biomarkers are needed that allow continuous and objective assessment of ALS pathology and progression.

The opportunity of neuroimaging to support diagnostics, staging, and therapeutic monitoring in ALS, led to international joined efforts, notably the Neuroimaging Society in Amyotrophic Lateral Sclerosis (NiSALS) (Turner et al., 2011). Nevertheless, discrepancies remain between the measurements derived from Magnetic Resonance Imaging (MRI) and the individual ALS disease course (Steinbach et al., 2018; Verstraete et al., 2015). Many of these studies used the ALS Functional Rating Scale Revised (ALSFRS-R) or the disease duration as singularly assessed indicators of clinical disease burden. However, some could not reveal any correlation between these markers of disability and MRI-derived measures of Cortical Thickness (CT) analyses (de Albuquerque et al., 2017; Schuster et al., 2014), whilst others described correlations occurring in single cortical areas (d'Ambrosio et al., 2014; Kwan et al., 2012; Spinelli et al., 2020). Reasons for these inconsistencies could be attributed to 1) the heterogeneous biological basis of ALS, 2) the differences and limitations of the used imaging techniques and analyzes, and 3) the inaccuracies of clinical measures.

For the latter, the D50 disease progression model was developed to overcome such limitations of traditional clinical metrics and has already proven to facilitate robust correlations with values originating from structural MRI analyses as well as other biomarkers (Dreger et al., 2021; Magen et al., 2021; Steinbach et al., 2021a). Briefly, the D50 model describes overall disease aggressiveness as the time taken to reach halved functionality (D50) and further enables the calculation of individual disease covered (e.g., in distinct phases) and of acute descriptors of local disease activity. A strength of the D50 model in comparison to traditional disease metrics, e.g., the linearly approximated disease progression-rate, is that it takes into account the individual clinical course as a whole and reflects its typically curvilinear decline of disability more appropriately (Gordon et al., 2010; Thakore et al., 2018). It describes the accumulating loss of motoric functions over time as a sigmoidal curve from full health to functional loss.

Applying this model, we demonstrated that grey matter (GM) alterations, as assessed via Voxel-Based Morphometry (VBM) (Ashburner and Friston, 2000), are sequentially worsening and spreading into several brain regions with increasing disease covered in ALS (Steinbach et al., 2020; Steinbach et al., 2021b). In contrast, GM structures did not correlate with disease aggressiveness, as quantified by the D50-value. In principle, this qualifies assessments of GM structural integrity to serve as a marker of disease accumulation in ALS, independent of disease aggressiveness. However, on a continuous level, correlations between disease accumulation and VBM measures of GM structures were lacking, as suggested in other studies before (Agosta et al., 2007; Menke et al., 2014).

This might be partly explained by the limitations of the VBM method. It is susceptible to segmentation failures, thus resulting in less accurate classifications as compared to CT-based methods (Jiao et al., 2010; Pereira et al., 2012). Surface-based Morphometry (SBM) has the advantage to decompose cortical volume into thickness, gyrification, and surface area and is, therefore, more reliable for cortical topology (Turner et al., 2012). Several studies using SBM were able to reveal patterns of cortical atrophy in ALS (Schuster et al., 2013; Verstraete et al., 2010; Verstraete et al., 2012) and CT seemed to be more sensitive to upper motoneuron involvement and disease-related changes in ALS than VBM (Turner et al., 2012; Turner and Verstraete, 2015). Of note, GM changes in ALS disease are not limited to cortical or motoric structures (Bede et al., 2013; Westeneng et al., 2015).

In this study, we aimed to determine if and how the integrity of cortical and subcortical GM can be used as a neuroimaging biomarker in ALS. We hypothesized that decreasing CT and/or subcortical volume are a continuous, one-directional process during the course of the disease. The D50 disease progression model was applied here to probe such direct correlations of accumulated disease/disease covered with SBM metrics of GM integrity for the first time. We further postulated that these GM measures are independent of disease aggressiveness, thus exclusively reflecting the ongoing accumulation of ALS pathology. Having a suitable biomarker fulfilling these criteria of being specific for the amount of disease covered, whilst being independent of disease aggressiveness would be an essential step towards using MRI to monitor effectiveness of therapeutic interventions.

2. Methods

2.1. Subject identification

All patients were recruited from the center for neuromuscular and motoneuron diseases at Jena University Hospital (Jena, Germany). All participants signed written informed consent prior to the commencement of the study. All experiments were approved by the local Ethics Committee of the Friedrich Schiller University in Jena (3633–11/12) and conducted in accordance with the Declaration of Helsinki and its later amendments.

In first instance, 162 patients were identified from a prospectively recruited cohort study. All patients received MRI via the same harmonized protocol, acquired in a scanner used for clinical routine diagnostics. They all fulfilled the revised El Escorial criteria for definite, probable, or probable laboratory-supported ALS (Brooks et al., 2000). All patients were examined and diagnosed by a neurodegenerative specialist before the commencement of the study and in follow-up visits. Patients fulfilling the following criteria were excluded from this cohort of ALS patients: 1) juvenile ALS, 2) primary lateral sclerosis, 3) manifest dementia, or 4) other comorbidities that could affect motor performances. In a next step, we also excluded patients with cognitive deficits or unavailable/incomplete cognitive testing according to neuropsychological screening assessments. For patients enrolled before 2015, these were Mini-Mental State Examination 26 points (Creavin et al., 2016) and/or frontal assessment battery 13.5 points (Appollonio et al., 2005; Dubois et al., 2000). For patients enrolled after 2015 the Edinburgh Cognitive and Behavioral Amyotrophic Lateral Sclerosis Screen (ECAS) was used with German education and age-adjusted cutoffs (Abrahams et al., 2014; Lule et al., 2015).

Consequently, 110 patients with ALS could be identified for this study (for a CONSORT diagram please refer to Supplementary Fig. S1).

Healthy Controls (HC) were recruited from whole population and did not suffer from any morbidities affecting either cognitive or motoric functions.

2.2. Clinical characterization of patients

The D50 disease progression model was developed to provide a quantifiable characterization of the individual ALS disease course and thereby overcome disadvantages of traditional disease metrics such as noise or rater-variability (Bakker et al., 2020; Gordon et al., 2010). It describes the disease state transition as a sigmoidal curve and is calculated by iterative fitting of regularly gathered ALSFRS-R scores for each patient. Interested readers may also refer to Dreger et al. (2021) or Steinbach et al. (2021a). The D50-value itself is defined as the estimated time taken in months after onset of symptoms until a patient loses half of his motoric functions. Thus, D50 is a descriptor of the individual overall disease aggressiveness. A lower D50 value reflects a more aggressive form of the disease, meaning that it takes less time until halved functionality. For subgroup comparison of patients with different forms of disease, they were subdivided into having an overall low aggressive ($D50 > 30$ months) or high aggressive ($D50 \leq 30$ months) ALS.

Normalizing the D50-value onto 0.5 yields the relative D50 (rD50) which allows comparability of differing disease courses of patients. rD50 measures individual disease covered, independent of disease aggressiveness, where 0 represents the symptom onset and 0.5 equals the time

point of halved functionality. Based on rD50, the disease course can be divided into distinct phases: a) the early semi-stable Phase I (rD50 0.25), b) the early progressive Phase II (0.25 ≤ rD50 0.50), and c) the late progressive/stable Phases III/IV (rD50 ≥ 0.50). In this study patients were subdivided according to their individual rD50 at the time of MRI, as either being in Phase I (rD50 0.25) or in higher phases (0.25 ≤ rD50).

Besides, descriptors of local disease activity can be calculated for any given time point, namely the calculated Functional State (cFS) and the calculated Functional Loss-rate (cFL). The cFS uses the same scale as the ALSFRS-R score, the cFL measures the acute decay rate of points lost per month for any given point in time, e.g., at MRI. Thus, the model provides information about disease accumulation (rD50 and cFS) and the acute disease activity (cFL) at time points where no original ALSFRS-R was acquired which was particularly useful for patients in our cohort who were not scored with the ALSFRS-R close to MRI-scanning.

2.3. MRI processing

2.3.1. MRI acquisition and preprocessing

MRI-scans were performed with a 1.5 Tesla Siemens Sonata Scanner at Jena University Hospital with a FLASH 3D sequence acquiring 192 sagittal slices (repetition time = 15 ms, echo time = 5 ms, Flip Angle = 30°, FOV 240 mm * 256 mm, slice thickness 1 mm, pixel size 1 mm * 1 mm) using a standard 4-channel head coil. The original T1-weighted DICOM images were then converted into Nifti format using Dcm2Nii (MRIcroN).

All T1-weighted and FLAIR raw images (acquired in the same scanning session) underwent visual inspection for artifacts (done by RS). Preprocessing and analysis of images was conducted with the CAT12-Toolbox (version 12.7; https://dbm.neuro.uni-jena.de/cat/) as implemented in SPM12 (version v7771 for MRI/VBM data on Windows; Wellcome Trust Centre for Neuroimaging; https://fil.ion.ucl.ac.uk/spm/software/spm12/) on Matlab2020a Surface. The steps of preprocessing followed the CAT12 standard pipeline using default settings unless indicated otherwise.

The images were segmented into white matter (WM), grey matter (GM) and Cerebrospinal Fluid. Following, a second quality assurance was conducted using the "Check homogeneity function" in CAT12 and potential outliers were again visually inspected (done by ND and RS). Ten patients were excluded due to segmentation failure or insufficient image quality and finally the images of 100 patients with ALS could be used for further analyzes. The images of all 72 HC fulfilled the quality criteria for this study.

2.3.2. Cortical thickness

Surface parameters were extracted using the SBM functions in CAT12 ("Surface Tools > Extract Surface Parameters") which calculates quantitative measures of cortical GM such as Cortical Thickness (Frangou et al., 2022). An advantage of SBM is that accuracy of brain registration has proven to be higher than in VBM methods (Anticevic et al., 2008) and by inflation and spherical mapping blurred sulci can be brought to the surface (Dahnke et al., 2013). CT was estimated in one step using a project-based distance measure (Dahnke et al., 2013). Measures were resampled and smoothed with a 15 mm full-width at half maximum Gaussian Kernel as described before (Spalthoff et al., 2018). No absolute masking threshold was applied, as recommended in the CAT12 manual.

2.3.3. Regions-Of-Interest

Besides whole-brain vertex-wise analyses of surface parameters, Region-Of-Interest (ROI) analyses of subcortical GM structures were performed. To correct for different brain sizes, we estimated the Total Intracranial Volume (TIV) ("CAT12 > Statistical Analysis > Estimate TIV"), which was applied in all ROI-analyses as a nuisance covariate. The segmented GM images were smoothed with an 8 mm full-width at half maximum kernel (with SPM12 module "Smooth"). The 22 ROIs per hemisphere were defined based on the CoBra-Atlas (Entis et al., 2012; Park et al., 2014; Tullo et al., 2018; Winterburn et al., 2013). These consisted of bilateral thalamus, amygdala, hippocampus (with subfields CA1, CA2/3, CA4/dentate gyrus, stratum radiatum/ stratum lacunosum/stratum moleculare, and subiculum) globus pallidus, striatum, and the cerebellum (divided into 13 subregions per hemisphere).

2.3.4. Statistical analysis

The specification of 2nd-level models and further check for homogeneity and design orthogonality was done using full-factorial model designs for cross-sectional data ("Basic Module" function in CAT12). By checking design orthogonality, we assured that there were no considerable correlations between the different parameters of interest and calculated parameters TIV and CT in our analyses.

First, we tested for inter-group differences between patients and HC, using age and gender as nuisance covariates. Within the group of patients with ALS, subgroup contrasts were calculated for a) Phase I (early stable Phase: rD50 0.25) versus higher phases (0.25 ≤ rD50), and b) low disease aggressiveness (D50 ≥ 30 months) versus high disease aggressiveness (D50 30 months). Further, regression analyses were performed with the modeled parameters rD50, D50, cFL, and cFS. In analyses between subgroups of patients and regression contrasts, we applied disease onset (bulbar/spinal) and the congruent parameter to our parameter of interest as nuisance covariates. For example, for the comparison of rD50-derived phases, type of onset and D50 were considered covariates; or for the regression with cFL, onset-type and cFS were included in the design. TIV was additionally included as nuisance covariate in all ROI-analyses. For CT-analyses the significance level was set at p 0.001 and a cluster extent threshold at the number of expected vertices per cluster was applied (Woo et al., 2014). Analyzing subcortical ROI volumes, the significance level was set at p 0.05 Holm-Bonferroni corrected.

Statistical analyses of demographic and clinical data were performed using the SPSS® software program (IBM®, v27.0.0.0). Non-normal distribution of all demographic and clinical variables was confirmed using the Shapiro Wilks test. Metric variables are expressed as mean with standard deviation, skewed variables as median with interquartile-range and categorical variables as absolute numbers. Comparison of groupwise means was appropriately conducted either with a two-sample t-test, a Mann-Whitney-U test, or chi-square-test.

3. Results

3.1. Study cohort

Detailed clinical and demographic data for patients with ALS are given in Table 1. There were no significant differences in distribution of gender (ALS: 45 female; HC: 38 female; $p = 0.314$) or handedness (ALS: 93 right-handed; HC: 64 right-handed; $p = 0.803$) between patients and HC. Patients were significantly older than HC (ALS: 64.17 16.77; HC: 53.87 14.28; p 0.001). Patients in higher rD50-derived phases were older than those in Phase I (Phase I: 61.46 15.96; higher phases: 66.37 12.45; $p = 0.01$; for further information see Supplementary Table S1). There was no significant difference in age between patients with different levels of disease aggressiveness (for further information see Supplementary Table S2).

3.2. Comparison between ALS patients and HC

SBM revealed 15 clusters with significant cortical thinning in ALS as compared to HC. These clusters were located in multiple areas of the brain, predominantly in frontotemporal regions: bilateral superior frontal gyri, superior and medial temporal gyrus, left medial frontal gyrus, left temporal pole, and left precentral sulcus (p 0.001; Fig. 1A). There were no clusters with increased CT in ALS in comparison to HC.

The subcortical ROI-analyses also revealed ALS-related significant

Table 1
Demographic and clinical data for patients with ALS.

Characteristics	ALS, $n = 100$
Demographic	
Age at MRI [years] #	64.17 16.77 (32.75–81.41)
Gender [male/female] Θ	55/45
Handedness [left/right/unknown] Θ	93/6/1
Neurocognitive Screening	
ECAS	*for n = 68*
- ECAS total score [points] ⊞	152.76 21.39 (100–192)
- ALS-specific subscore ⊞	74.22 10.91 (46 – 96)
- ALS Non-specific subscore ⊞	78.54 10.63 (54 – 96)
FAB	*for n = 33*
- FAB Total score [points] ⊞	17.48 0.87 (15–18)
MMSE	*for n = 35*
- MMSE Total score [points] ⊞	29.29 1.02 (26–30)
Disease Metrics	
Symptom Duration [months] #	13 12 (4–59)
onset [bulbar/spinal] Θ	33/67
D50 [months] #	30.04 19.78 (6.04–96.20)
Low Aggressiveness (D50 ≥ 30) Θ	51
High Aggressiveness (D50 30) Θ	49
relative D50 (rD50) ⊞	0.26 0.12 (0.05–0.52)
Phase I at MRI (rD50 0.25) Θ	48
higher phases at MRI (0.25 ≤ rD50) Θ	52
- Phase II (0.25 ≤ rD50 ≤ 0.50)	49
- Phases III/IV (0.50 ≤ rD50	3
cFS [points] ⊞	38.80 6.03 (24.63–50.78)
cFL [points lost per month] ⊞	0.95 0.75 (0.11–4.02)

Abbreviations: ECAS Edinburgh Cognitive and Behavioral ALS Screen; FAB Frontal Assessment Battery; MMSE Mini-Mental State Exam; D50 overall disease aggressiveness; rD50 (relative D50) individual disease covered; cFS calculated Functional State; cFL calculated Functional Loss-rate.
Note: Continuous data are summarized for ⊞ as mean SD or for # as median interquartile range (each with the total range in brackets). For Θ categorial data, the number of cases (equals percentages) are given. Variables that are time point dependent refer to the day of MRI-acquisition; others depict constant characterization of patients' overall disease course.

decreases in GM volume in the left amygdala and left stratum of the hippocampus (p 0.05 Holm-Bonferroni corrected, Fig. 1B). As in the CT-analyses, there were no significant increases in GM volume in any ROI for patients with ALS in comparison to HC.

3.3. Grey-matter alterations in relation to disease accumulation

Patients in Phase I (rD50 0.25) presented with higher CT in three different clusters compared to patients in higher phases. These clusters were in the inferolateral regions of bilateral precentral gyrus (PrCG) corresponding to facial and bulbar mototopic parts with an additional cluster in the right angular gyrus (p 0.001; Fig. 2A). Adding age and gender as nuisance covariates to this phase comparison (in addition to type of onset and D50), still revealed the largest cluster in the right PrCG as significant (p 0.001; indicated with a blue circle in Fig. 2A).

There were no significant increases in CT for patients in higher phases compared to patients in Phase I.

The subcortical ROI-analyses showed decreased GM volume in CA4 /dentate gyrus of the left hippocampus for patients in higher phases, while no subcortical volume increases were identified (p 0.05 Holm-Bonferroni corrected, Fig. 2B).

Regression analyses revealed 20 clusters where decreased CT significantly correlated with higher rD50 (p 0.001; Fig. 3A). These were located in bilateral occipital gyri, left inferolateral postcentral gyrus, right medial temporal gyrus, inferolateral PrCG, supramarginal gyrus and temporal pole. Adding age and gender as covariates in the model (in addition to the type of onset and D50) still resulted in three right-hemispheric significant clusters (right PrCG, right supramarginal gyrus and right temporal pole). Regression analyses between subcortical GM volume and rD50 revealed negative correlations in bilateral thalamus, amygdala, Striatum and CA2_3, left inferior posterior cerebellum LVIIIA, CA4, stratum radiatum/ stratum lacunosum/stratum moleculare, and right inferior posterior cerebellum LX (p 0.05 Holm-Bonferroni corrected, Fig. 3B).

No positive correlations were found for the parameter rD50, neither in CT- nor in ROI-analyses.

Another parameter describing the accumulated disease progression is the cFS (here also calculated for the time point of MRI). The regression analysis between cFS and CT identified 10 clusters with a positive correlation. Most of these clusters were alike with those clusters identified to show negative correlations between rD50 and CT. We revealed that CT of left inferolateral postcentral gyrus and parasagittal superior frontal gyrus, as well as right inferoprecentral sulcus and parasagittal occipital correlated positively with the cFS.

We observed positive correlations between cFS and GM volume in our predefined subcortical ROIs, namely bilateral thalamus, striatum, superior posterior cerebellum (LVIIB), left inferior posterior cerebellum (LX), and in right inferior posterior cerebellum (LVIIA). There were no significant negative correlations for cFS with neither CT nor ROI volumes.

3.4. Grey matter alterations in relation to disease aggressiveness

Both, CT- and ROI-analyses did not reveal any differences if comparing patients with higher disease aggressiveness (D50 > 30) to those with lower disease aggressiveness (30 ≤ D50) or vice versa (at p 0.001; Fig. 2C and 2D).

Likewise, our analyses did not find any significant correlations in regression analyses with the parameter D50 (neither for CT-, nor subcortical ROI-analyses).

3.5. Grey matter alterations in relation to cFL

There were neither positive nor negative correlations between CT and the cFL calculated for the timepoint of MRI. In subcortical ROI-analyses, a significant positive correlation between GM volume and cFL was revealed in the right thalamus (p 0.05 Holm-Bonferroni corrected). Other ROIs showed neither positive nor negative correlations with the cFL.

4. Discussion

In this study we evaluated Cortical Thickness (CT) and subcortical GM volumes in the context of progressing ALS-pathology in a large cohort of well-characterized patients. We revealed several areas with significant cortical thinning and reductions of deep GM volumes in ALS patients as compared to HC (Fig. 1). Within the group of patients with ALS, we revealed that progressive decreases of CT and subcortical GM volume reductions correlate with accumulation of the ALS-disease. This was shown in subgroup-comparisons of patients in advanced phases with those in Phase I (Fig. 2); as well as in regression analyses with the parameters rD50 and cFS (Fig. 3). In contrast, measures of GM structural integrity were not associated with ALS disease aggressiveness, neither in subgroup, nor in direct regression analyses.

Our findings of widespread thinning of cortical GM in ALS patients (Fig. 1A) underscore pre-existing data reporting patterns of cortical atrophy in ALS (Agosta et al., 2012; Benbrika et al., 2021; Chen et al., 2018; Mezzapesa et al., 2013; Schuster et al., 2013; Verstraete et al., 2012). However, there are inconsistencies concerning findings, especially within the PrCG and some studies could not reveal any cortical thinning for the respective group of ALS patients (Cardenas-Blanco et al., 2016; Spinelli et al., 2020). We assume, that these differences can to a certain degree be attributed to the substantial heterogeneity in the ALS-populations investigated as well as methodological differences between

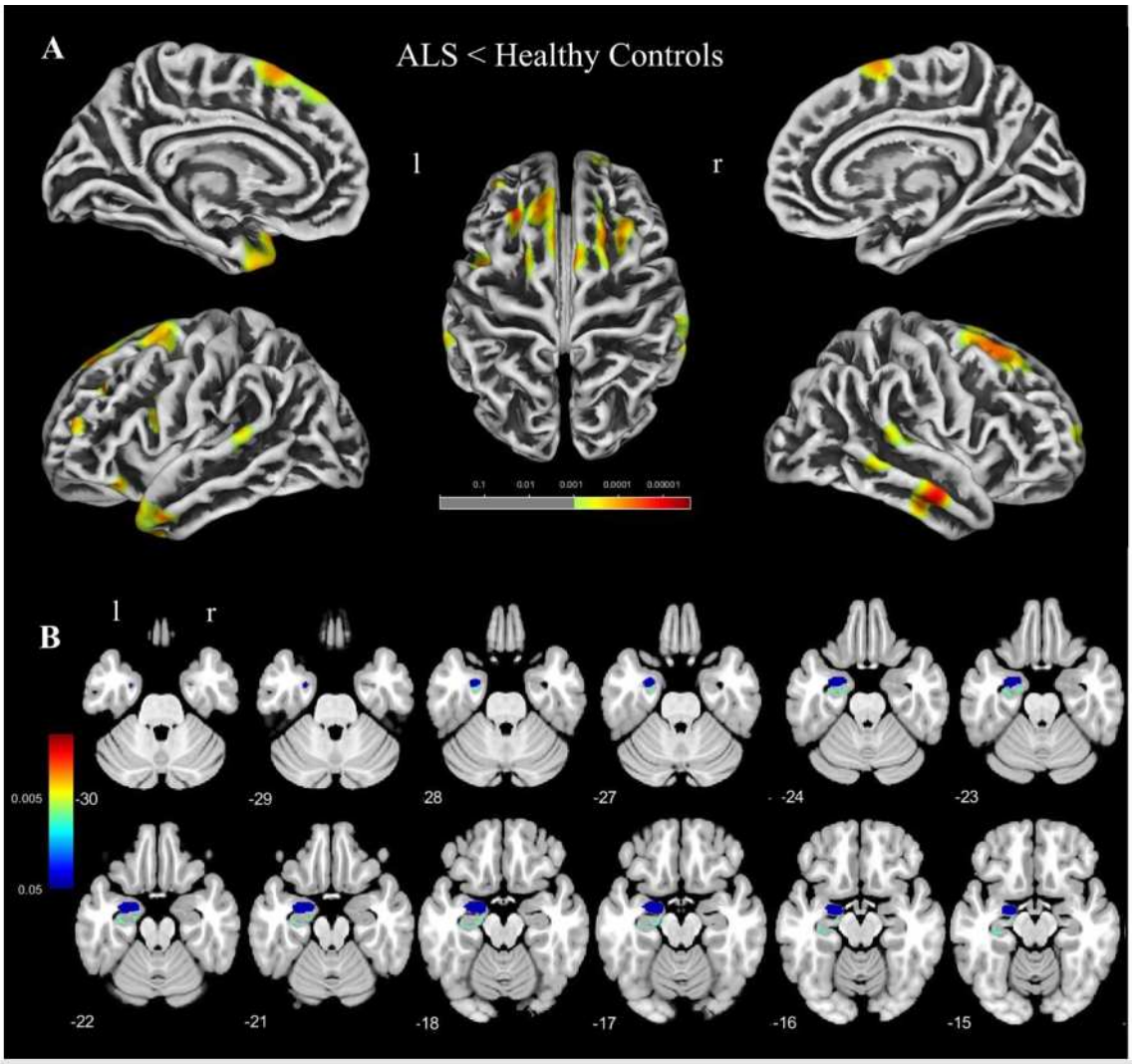

Fig. 1. Comparison of ALS-cohort with Healthy Controls.
a) Cross-sectional Cortical Thickness (CT)-analysis: A decrease in CT was observed mainly frontotemporal. Bilateral superior frontal gyrus, superior temporal gyrus, right medial temporal gyrus, and left temporal pole showed decreased CT in ALS-patients. (p 0.001; cluster extent threshold at expected number of vertices per cluster, nuisance covariates: age, gender)
b) Cross-sectional ROI-analysis. Decrease of GM volume was significant in left-hemispheric amygdala and stratum of the hippocampus. (p 0.05 Holm-Bonferroni corrected; nuisance covariates: age, gender, TIV)
Colorbars represent p-Values. *Abbreviations:* CT: Cortical Thickness; GM: Grey Matter; l: left hemisphere; r: right hemisphere; ROI: Region-Of-Interest; TIV: Total Intracranial Volume.

studies. More specifically, these may be differing degrees of ALS disease accumulation (Goyal et al., 2020; Spinelli et al., 2016), differing relations of bulbar and spinal involvement (Jin et al., 2018; Schuster et al., 2013; Steinbach et al., 2020), or the usage of 1.5T MRI-scans (Chu et al., 2017; Livshits et al., 2012; Sicotte et al., 2003), and the CAT12 algorithm (Seiger et al., 2018), just to name a few.

We observed decreased GM volumes in left amygdala and hippocampal ROIs in the case-control-comparison (Fig. 1B), which is in line with the existing literature (Finegan et al., 2020; Machts et al., 2015; van der Burgh et al., 2020; Westeneng et al., 2015). However, there are also discrepancies in the extent of subcortical structural involvement in ALS reported so far and some studies did not find any subcortical changes on a case-control level (Bede et al., 2018; Fu et al., 2021). Regarding the whole spectrum from pure-motoneuronal ALS to fronto-temporal dementia, previous studies suggest that selective vulnerability of subcortical grey-matter depends on the extend of cognitive and/or behavioural deficits, as well as the presence of ALS-causing genetic variants such as C9orf72 (Ahmed et al., 2021; Bede et al., 2018; Machts et al., 2015; van der Burgh et al., 2020). Although the ALS patients of the study reported here had cognitive screening tests within normal ranges, subclinical deficiencies and especially behavioural impairment cannot be excluded and further studies are needed to decipher the underlying interplay of factors potentially influencing deep GM pathology. Altogether, our study supports and extends the existing evidence about widespread cortical and subcortical GM involvement in ALS in the sense of a multisystemic neurodegenerative disease not being restricted to motoric areas.

Findings in our ALS-Subgroup comparisons and regression analyses revealed consistent correlations between CT and disease accumulation, independent of disease aggressiveness. Comparison of patients in different phases of disease (early stable Phase I versus higher phases) showed a systematic decrease of CT along with disease progression. Areas of lower CT associated with advanced disease phases were mainly located in bulbar parts of the PrCG, emphasized for the right hemisphere (Fig. 2A). This observation was additionally confirmed by regression analyses between individual rD50 and CT. With advanced disease

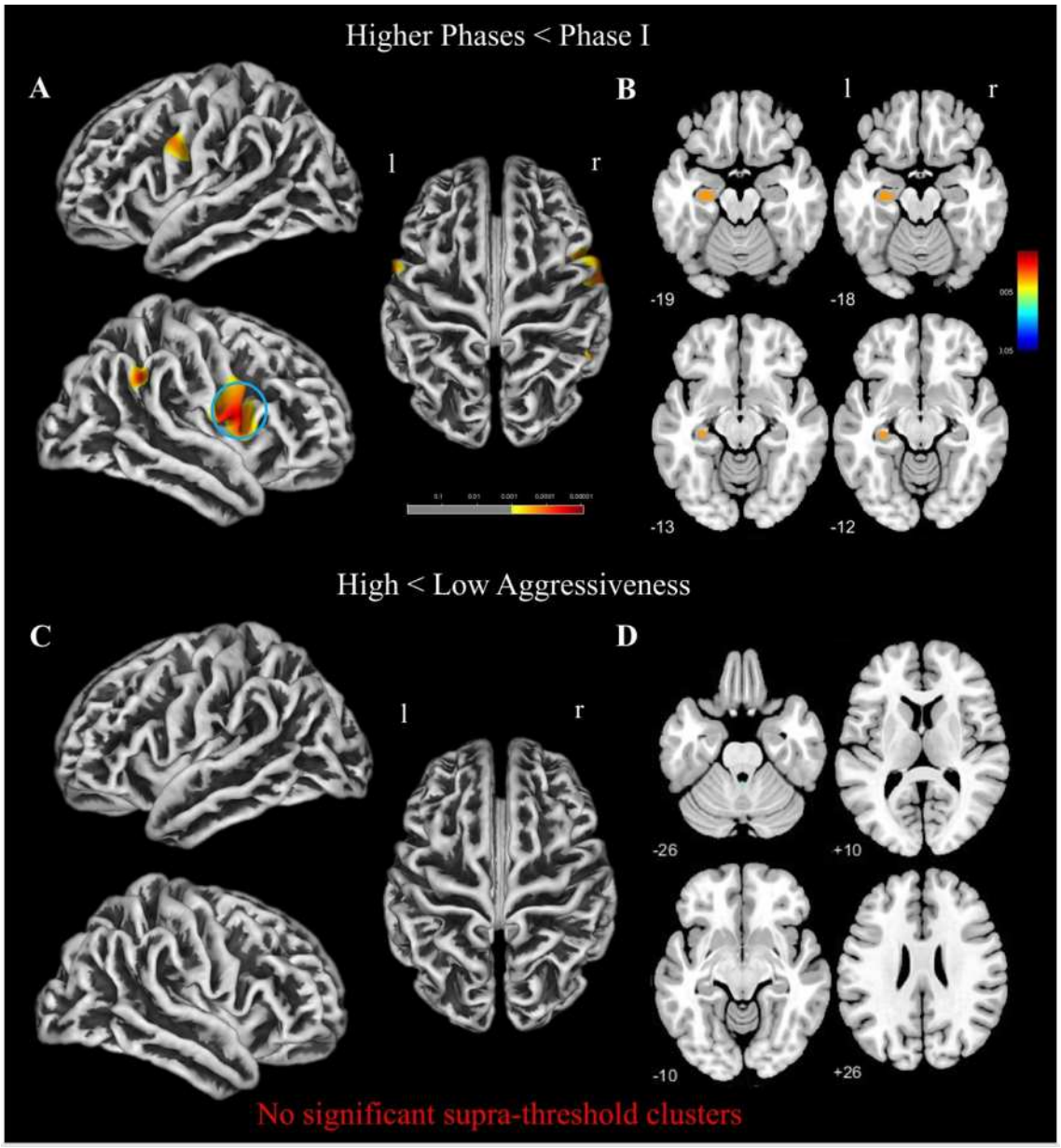

Fig. 2. Comparison of patients across disease phases.
a) Cortical Thickness (CT) subgroup-analysis of patients in disease Phase I (rD50 0.25, $n = 48$) versus higher phases (rD50 $\geq$ 0.25, $n = 52$) at the time of MRI acquisition: Decrease in CT in higher phases were observed in the bilateral inferolateral precentral gyrus and the right angular gyrus in contrast to Phase I. (p 0.001; cluster extent threshold at expected number of voxels per cluster; nuisance covariates: type of onset, D50). Blue circles represent clusters remaining significant after adding age and gender as nuisance covariates.
b) Subgroup ROI-analysis between patients in Phase I and higher phases at the time of MRI acquisition: Decrease in GM volume was identified in the CA4 of hippocampus (p 0.05 Holm-Bonferroni corrected; nuisance covariates: type of onset, D50, TIV)
There were no significant increases of CT or GM volume across disease phases.
Comparison across disease aggressiveness subgroups
c) CT subgroup analysis of patients with high (D50 30 months, $n = 49$) versus low (D50 $\geq$ 30 months, $n = 51$) overall disease aggressiveness revealed no CT increases or decreases. (p 0.001; cluster extent threshold at expected number of vertices per cluster; nuisance covariates: type of onset, rD50)
d) Subgroup ROI-analysis also revealed no significant differences between subgroups. (p 0.05 Holm-Bonferroni corrected; nuisance covariates: type of onset, rD50, TIV)
Colorbars represent p-Values. *Abbreviations:* CT: Cortical Thickness; GM: Grey Matter; l: left hemisphere; r: right hemisphere; ROI: Region-Of-Interest; TIV: Total Intracranial Volume.

covered (higher rD50), CT significantly decreased in right precentral sulcus, temporal pole, and supramarginal gyrus. Altogether, this suggests that declining CT is an ongoing process whilst the ALS disease progresses. There are previous studies aiming at finding associations between clinical disease progression and measures of structural GM integrity, however reporting controversial results. Some authors did not find any alterations in CT in the course of the disease (Cardenas-Blanco et al., 2016; de Albuquerque et al., 2017; Spinelli et al., 2020). In contrast, other longitudinal studies were able to identify specific regions with progressive decline of GM volume or CT over time (Bede and Hardiman, 2018; Benbrika et al., 2021; Kwan et al., 2012; van der Burgh et al., 2020). However, whilst some other studies found longitudinal

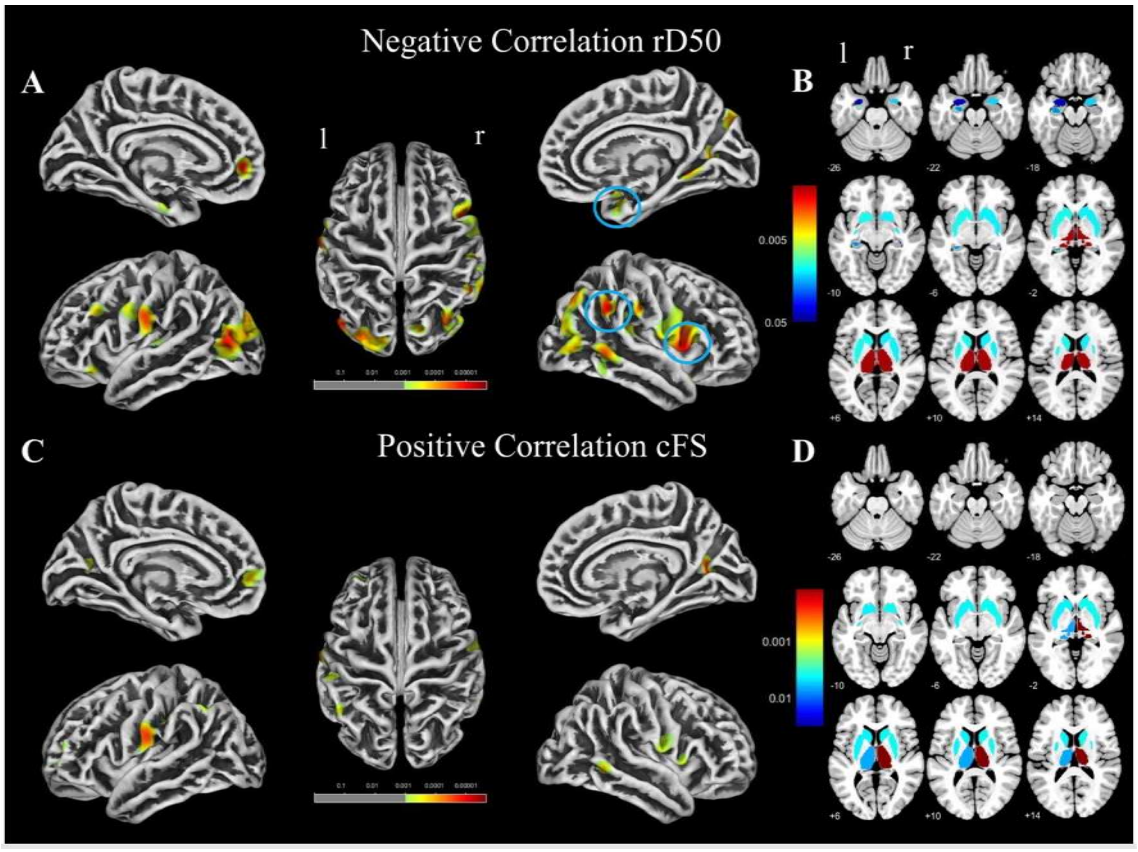

Fig. 3. Multiple Regression analyses with rD50.
a) CT decreases were inversely correlated with rD50 (with increasing disease covered) in bilateral inferolateral pre-, and postcentral sulcus, bilateral occipital gyri, and multiple frontal and temporal clusters. (p 0.001; cluster extent threshold at expected number of vertices per cluster; nuisance covariates: type of onset, D50). Blue circles represent cluster remaining significant after adding age and gender as nuisance covariates.
b) In subcortical ROI decrease of GM volume with increase of rD50 was identified in the bilateral thalamus, amygdala, Striatum and CA2_3, the left inferior posterior cerebellum LVIIIA, CA4, stratum and the right inferior posterior cerebellum LX. (p 0.05 Holm-Bonferroni corrected; nuisance covariates: type of onset, D50, TIV) Multiple Regression analyses with cFS
c) Positive correlations between cFS and CT were revealed in the bilateral inferolateral pre-, and postcentral gyrus, occipital gyri, right medial temporal and supramarginal gyrus and in smaller clusters in the frontal cortex. (p 0.001; cluster extent threshold at expected number of vertices per cluster; nuisance covariates: type of onset, cFL)
d) Increase of GM volume along with increase of cFS was significant in the bilateral thalamus, striatum, superior posterior LVIIB cerebellum, the left inferior posterior cerebellum LX and in the right inferior posterior cerebellum LVIIA. (p 0.05 Holm-Bonferroni corrected; nuisance covariates: type of onset, cFL, TIV) There were no significant positive correlations with rD50 or negative correlations with cFS observable.
Colorbars represent p-Values. *Abbreviations:* cFS: calculated Functional State; cFL: calculatedFunctional Loss-Rate; CT: Cortical Thickness; GM: Grey Matter; l: left hemisphere; r: right hemisphere; ROI: Region-Of-Interest; TIV: Total Intracranial Volume.

decreases of CT in GM regions, they were not able to correlate them to the decline of clinical disabilities as measured by the ALSFRS-R (de Albuquerque et al., 2017; Schuster et al., 2014). Another group investigating C9orf72 variant carriers found a weak correlation between longitudinal ALSFRS-R changes and the CT of bilateral PrCG but not for other cortical regions (Floeter et al., 2016).

The correlations between parameters of disease accumulation (i.e., rD50 and cFS) and CT-decreases reported in this study therefore again highlight the advantages of using the D50-model instead of the original ALSFRS-R scores susceptible to (intra- and inter-)rater-variability (Bakker et al., 2020; Steinbach et al., 2021a). A pitfall of longitudinal studies in ALS and therefore another advantage of the D50-model arises from the usage of fixed time-intervals in between MRI-scans. This leads to known difficulties in recruitment and sampling-bias due to the naturally high drop-out rates in ALS cohorts (e.g., poor longevity or inability to lie in a MRI scanner). The remaining patients in longitudinal MRI studies are often those with lower disease aggressiveness, longer survival, more spinal onset, and younger age at onset (de Albuquerque et al., 2017; van der Burgh et al., 2020). Therefore, results cannot reliably represent the ALS-population as a whole or lack effect size (de Albuquerque et al., 2017; Spinelli et al., 2020). We already suggested the possibility that the D50-model can approximate longitudinal results from a cross-sectional data set in a former study (Prell et al., 2020). Thus, we were able to principally circumvent restrictions of longitudinal studies, because the D50-model allows quantification of patients' individual disease course.

Here, we could examine relations between the loss of brain structure and the amount of clinical disease covered (rD50), independent of disease aggressiveness (corrected for D50). As such, found correlations represent progressive loss of GM structural integrity that are directly correlated with clinical disability-related functional impairment caused by the progressing ALS disease. This observation was further substantiated by the significant correlations between the cFS and CT in areas mainly overlapping with those significant in the regression between rD50 and CT. This is of note since cFS describes the local remaining rest-functionality for daily live abilities of a patient, here calculated for the time point of MRI, and is therefore the parameter that corresponds the most closely to the traditional ALSFRS-R value.

Altogether, we are confident to conclude that decreasing CT is a reliable biomarker reflecting progressive neurodegeneration in ALS, related to worsening of clinical disability. Further, the possible use of CT as a marker of disease progression is supported by the observation, that no inverse association could be found. Indeed, there was no increased GM in patients at advanced diseases phases, and accordingly neither positive correlations with rD50 nor negative correlations with cFS could be revealed. Hence, our data support the notion that atrophy of GM during the clinical course of the ALS disease is a constant, one-directional process.

Few studies so far examined potential associations between subcortical alterations and disease parameters of ALS disease progression. In a longitudinal analysis of subcortical GM in ALS, enlargement of ventricles and decreased GM volumes in hippocampal subfields were found in follow-up MRI but no significant longitudinal alterations in thalamus, caudatus, putamen, pallidum, hippocampus, amygdala or accumbens (Westeneng et al., 2015).

Menke et al. (2014) reported a longitudinal GM decline in subcortical areas, but they were not able to correlate clinical disability (ALSFRS-R total score) with GM integrity using VBM, neither in whole-brain nor in ROI-based analyses.

However, in our study we observed that loss of GM in subcortical regions is also associated with parameters of disease accumulation, similar to our CT-analyses. Subgroup comparison between patients in Phase I and higher phases showed a decline of GM volume limited to CA4/DG of the left hippocampus. In regression analyses with the parameter rD50, decreasing subcortical volumes could also be revealed in bilateral thalamus, amygdala, striatum, different regions in right and left hippocampus and areas in inferior posterior cerebellum – all these regions showing negative correlation between GM volume and rD50. Further, regression with cFS showed matching results with a positive correlation significant in mostly similar regions. Especially decreases in hippocampal subfields and thalamus along the progressing disease is in good accordance with previous findings of studies using longitudinal models in their research (Menke et al., 2014; Westeneng et al., 2015).

In this study we used a ROI-based approach for analyses in subcortical structures and were able to show consistent associations between subcortical decline of GM volume and clinical measures of disease accumulation. Our results regarding subcortical ROIs reflect, that ongoing atrophy of GM is not restricted to cortical structures but can equally be detected in hippocampal regions, thalamus, basal ganglia, and the cerebellum.

In contrast to the observed associations with parameters of disease accumulation, we could not find any relations between GM atrophy and disease aggressiveness. This was the case for subgroup-comparison of patients subdivided according to their individual D50-value as well as in direct regression analyses with the parameter D50. This underlines our prior observations that structural integrity of GM in ALS is independent of disease aggressiveness, using a whole-brain-based approach with VBM (Steinbach et al., 2020).

So far, some former studies used the Progression Rate (PR) as a parameter for disease progression-speed in ALS, calculated as the linear ALSFRS-R decline over time ((48 – ALSFRS-R) / disease duration). Using the PR, Menke et al. (2014) did not identify associations between PR and GM measures, which is in accordance with our findings. In another approach, studies examined associations between discovered longitudinal changes in neuroimaging measures of grey matter structures and the local, linearly approximated decline of the ALSFRS-R score over time, i.e. the ALSFRS-R slope. This also revealed inconsistent results in former studies: de Albuquerque et al. (2017) did not find any correlations between observed reductions of brainstem GM volume and clinical scores including the ALSFRS-R slope, but (Kwan et al., 2012) reported correlations between the decline of the ALSFRS-R score and loss of precentral GM volume. However, due to the known curvilinear decline of ALSFRS-R points over time, slopes calculated based on the ALSFRS-R score differ depending on defined time points used to calculate the ALSFRS-R slope (Gordon et al., 2010; Senda et al., 2017). The data of the study reported here strongly supports that disease aggressiveness in ALS does not influence the degree of structural loss in GM. In contrast, several previous studies underscore that WM rather than GM alterations are driving disease aggressiveness in ALS (Steinbach et al., 2021a). Our recent findings in this new work are now able to accentuate the likely independence between GM and disease aggressiveness. We could extend knowledge about missing associations between GM atrophy and disease aggressiveness onto the biomarker cortical thickness, described as having better accuracy than VBM-measures (Desai et al., 2005) and further to subcortical atrophy patterns.

Regarding the high associations between GM atrophy and parameters of disease accumulation (rD50 and cFS) after correcting for background disease activity and connecting those findings to the noticeable results that there are no correlations with disease aggressiveness (D50), it is reasonable to conclude that alterations in GM are robust markers of disease accumulation, independent of disease aggressiveness.

Our study has some limitations. Our data is monocentric, including only patients from one tertiary ALS center and validation of the neuroimaging results in independent cohorts is necessary. Moreover, the data cannot be generalized for the entire ALS population because of potential referral bias. Patients in tertiary ALS centers are often younger (thus having longer survival times) and show a relatively smaller proportion of bulbar-onset patients (Logroscino et al., 2018; Sorenson et al., 2007). In our study, the patients in higher phases of ALS were significantly older than those in Phase I. However, the D50-model allows better comparability of different patients and provides the ability to calculate parameters that represent the course of the disease as a whole for every given time point. This allowed us to perform a pseudolongitudinal study of the entire cohort of ALS patients in this MRI study. However, verification through real longitudinal data is desirable but naturally will depend on large, well-designed multi-center studies (Kalra et al., 2020; Steinbach et al., 2018). Besides, our patients were only assessed via neuropsychological screening tests and therefore subclinical cognitive deficits and/or behavioral impairment are possible. Further, genetic testing was not available for all patients, and we could not characterize or excluded patients based on genetic profiles. A statistical limitation of our study is that CT results are reported with a cluster extent threshold to minimize the probability of an inflation of Type I error, however a lower spatial specificity in larger clusters has to be taken into account if using this method. Future studies using a higher MRI field strength (e.g., 3T) are also needed to see, if this would influence the neuroimaging results, as would be methodological comparison studies (e.g., using FreeSurfer).

5. Conclusion

In this study, we analyzed cortical thickness and subcortical grey matter in relation to ALS disease progression. By application of the D50 model, we were able to examine associations of those neuroimaging markers with disease accumulation independent of disease aggressiveness and vice versa. This allowed quantification and comparability of the heterogeneous disease course of the patients with ALS and a well-defined big cohort could be included representing the population referred to our tertiary center. Thus, we were able to identify correlations between loss of grey matter structure, cortical and subcortical, and disease accumulation. Most important, independence of grey matter structural integrity was confirmed in relation to disease aggressiveness. Altogether, this highly qualifies the neuroimaging measures used in this study to be applicable as biomarkers of pure disease accumulation, independent of the background activity of disease. This is most relevant for upcoming clinical trials as well as clinical management in ALS since the success of therapeutic interventions relies on biomarkers robustly quantifying disease covered/accumulation.

CRediT authorship contribution statement

Nora Dieckmann: Software, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Annekathrin Roediger:** Investigation, Writing – review & editing. **Tino Prell:** Formal analysis, Writing – review & editing. **Simon Schuster:** Data curation, Writing – review & editing. **Meret Herdick:** Data curation, Writing – review & editing. **Thomas E. Mayer:** Investigation, Resources, Writing – review & editing. **Otto W. Witte:** Writing – review & editing, Funding acquisition. **Robert Steinbach:** Conceptualization, Methodology, Software, Validation, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Funding acquisition. **Julian Grosskreutz:** Conceptualization, Methodology, Investigation, Resources, Data curation, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nicl.2022.103162.

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3 Discussion

The enclosed paper was designated to examine if changes in GM of patients with ALS can be used as a surrogate marker of disease progression. MRI-datasets were therefore used in a pseudo-longitudinal approach to see if an ALS-specific pattern of gray matter atrophy can be found and if these changes correlate with parameters of the D50 model. All patients included were well characterized regarding their forms of disease and by D50 measures. Our Analyses showed significant correlations between the atrophy of cortical and subcortical GM and disease progression. In contrast, no associations between atrophy and overall disease aggressiveness could be found.

Before this study, there was a scientific consensus that a reduction of GM is inherent in ALS and patients have reduced cortical thickness in comparison to healthy controls. To determine whether cortical GM-atrophy could also be found in the dataset of this study, the first step was a comparison of GM between patients with ALS and healthy controls by SBM. Further, subcortical ROIs in the MRI-sequences of our patients were examined to assess alterations in GM volume.

In fact, our analyses showed regions of reduced cortical thickness mainly in the fronto-temporal brain regions in the cohort of ALS-patients. Atrophy in these locations is in line with comparable previous studies examining cortical thickness in ALS (Agosta et al. 2012, Benbrika et al. 2021, Chen et al. 2018, Mezzapesa et al. 2013, Schuster et al. 2013, Verstraete et al. 2012). However, in contrast to most of these studies, the data presented here revealed only a few alterations in the pre-central gyrus (PrCG). This may be explained by several reasons. At first, the heterogeneity of patients is characteristically inherent in ALS cohorts (Westeneng et al. 2018). Additionally, compared to most other studies, the cohort of patients in this paper was relatively big and included patients with different types of onset and different courses and phases of the disease. A former VBM-study comparing patients with bulbar and spinal onset showed that ALS-related changes in GM are more pronounced in bulbar-onset patients and they have different patterns of spread (Steinbach et al. 2021b). Demography of the patients in our study showed that 67% of patients had a spinal-onset and therefore bulbar-onset patients were less represented. Therefore, possibly most spinal-onset patients might have concealed alterations in the PrCG.

There is an internationally accepted neuro-pathological model suggesting a corticofugal spread of ALS-pathology (24). It was suggested before, that patterns of atrophy in the PrCG are inhomogeneously distributed between patients with spinal and bulbar onset and therefore cannot be seen examining a mixed cohort in comparison to HC (Steinbach et al. 2021b). atrophy

in frontal regions is probably more homogenous in-between patients with different disease-onset types and therefore can be more easily detected in case-control contrasts.

Another potential confounder of our study is the use of an MRI-scanner with a field strength of only 1.5 T. Studies comparing results of 1.5T and 3T (or higher) MRI-scans showed that the 1.5T technology is less sensitive in morphometric analyses of the human brain (Livshits et al. 2012, Sicotte et al. 2003). However, it is a strength of our study that we were able to use a big dataset of patients with MRI-scans acquired in a clinical context. Moreover our study design allowed us to include patients with MRI-scanning independent of an ALSFRS-R scoring close in time.

Additionally, we used the CAT12-Toolbox for analyses of cortical thickness. According to the existing literature, most of the cited studies used the FreeSurfer software instead. To the best of our knowledge, only Chen and colleagues (Chen et al. 2018) used CAT12 as well. There are studies comparing both software programs which show that results are well comparable (Seiger et al. 2018) but one cannot rule out the possibility that differences in results are due to the use of different software. We decided to use CAT12 due to different reasons: First, CAT12 enables the use of FLASH 3D-MRI-sequences whilst FreeSurfer is rather designed for analyzing MPRAGES. Second, in pre-processing procedures, FreeSurfer depends on a manual rework of segmentation while segmentation in CAT12 is completely automated and requires only visual control. Another major advantage of CAT12 is the noticeable reduction in processing times and the graphical interface that may be easier to use for researchers not familiar with the command line (Seiger et al. 2018).

Besides the fact that we found only a few alterations in the PrCG compared to other studies, our results regarding the reduction of cortical thickness in frontotemporal regions are in line with existing literature. Furthermore, the involvement of frontal brain areas in the pathology of ALS matches the assumption of a high overlap between classical ALS and frontotemporal dementia. It has been discussed in several studies if the degree of frontal cortical atrophy is linked to behavioral or cognitive dysfunctions, but results are not congruent. Some authors reported a pronounced atrophy in frontal brain regions of patients with cognitive, executive or behavioral disorders (Mak et al. 2014, Rajagopalan und Pioro 2015) but other studies could not reproduce such associations (Dalaker et al. 2010, Elamin et al. 2013).

By analyzing subcortical ROIs, we discovered a decrease of GM volume in the left amygdala and hippocampus of ALS patients compared to HC. These results correspond to the existing data of other authors (Finegan et al. 2020, van der Burgh et al. 2020, Westeneng et al. 2015, Machts et al. 2015). However, as in cortical thickness, there are discrepancies between

different studies regarding the extent of subcortical involvement and some studies did not find any alterations in comparisons with HC (Bede et al. 2018, Fu et al. 2021).

In summary, the results of the case-control comparisons of our study extend the existing evidence that ALS-pathology involves cortical and subcortical GM, thereby confirming that ALS must be considered a multi-systemic neurodegenerative disease that is not restricted to motoric brain areas.

As already indicated by previous studies, our work was able to reveal associations between disease accumulation on the one hand and CT and subcortical GM volume on the other hand in ALS-subgroup comparisons and multiple regression analyses. Both, the reduction of CT and subcortical GM volume, were independent of disease aggressiveness. A previous study using the D50 model with VBM already demonstrated that GM decreases along with disease progression (Steinbach et al. 2020). However, the methodology used in this previous study was not as sensitive as SBM and no significant GM reductions and no associations between GM-reductions and rD50 could be found in multiple regression analyses which had no significant results. These results indicate that reduction of CT is not only ALS-specific but an ongoing process along the course of the ALS disease. The assumption that CT continues to decrease with progression of disease has already been reported by other authors; however, reports about associations between clinical disease progression, measured by ALSFRS-R, and measures of GM have been controversial. The robust correlations between parameters of disease accumulation described with the D50 model (rD50, cFS) and GM-atrophy that we could reveal, highlight the advantages of this model in contrast to ALSFRS-R scores. Using the ALSFRS-R scores for multiple regression analyses has several disadvantages such as a high susceptibility to interrater-variability, the necessity of fixed time-intervals between MRI-scans and high drop-out rates in ALS cohorts in longitudinal studies (de Albuquerque et al. 2017, van der Burgh et al. 2020) – all of which are circumvented by the use of the D50 model.

Our study showed no inverse associations, meaning a higher rD50 or lower cFS showed no correlations with greater cortical thickness/ subcortical volume in regression analyses and patients in advanced disease stages had no clusters with higher CT/ subcortical GM-volume compared to those in phase 1. These missing inverse associations additionally support CT as a possible marker of disease progression and underline that cortical atrophy in ALS-pathology is a one-directional process.

Changes in subcortical GM of patients with ALS have, according to existing literature, not been studied intensively so far. The study presented here, therefore, provides important evidence of GM volume loss in some subcortical ROIs. Comparing patients in different phases

of disease, revealed that those in higher phases showed reduced GM volume in several subcortical ROIs. Loss of GM was also correlated with rD50/cFS parameters, representing the worsening whilst the ALS disease progresses.

If GM atrophy is used as a marker of disease accumulation, it is of importance that this measure is independent of other aspects of the disease, especially disease aggressiveness. This was confirmed in this study by subgroup comparisons between patients with high and low disease aggressiveness, and further by regression analyses with the parameter D50: neither of these showed any association with D50. This extends prior findings that the structural integrity of cortical and subcortical GM is independent of the underlying disease aggressiveness (Steinbach et al. 2020).

In conclusion, the study presented here showed high associations between GM atrophy and D50 model parameters describing disease accumulation (rD50, cFS) after correcting for background disease aggressiveness/activity (D50, cFL). Connecting these results with the missing associations between GM atrophy and underlying disease aggressiveness (described by D50), it is reasonable to assume that GM alterations could serve as markers of disease accumulation. However, this study has some limitations and further studies are needed to validate its results. For example, all patients in this study were recruited at a tertiary center in the University hospital and further analyses are necessary studying a large, independent, and possibly international cohort of patients. Further, this study used 1,5T MRI and upcoming studies using higher field strengths should be performed since they are more sensitive to GM-alterations (Chu et al. 2017, Livshits et al. 2012). At last, the D50-model enabled a pseudo-longitudinal study, but analyses of real longitudinal data could lead to further verification of the results of this study.

To conclude we can say that in ALS research, there is a need to identify reliable biomarkers that can monitor disease progression and thus evaluate the effectiveness of therapeutic interventions. This work was designated to evaluate whether cortical thickness and subcortical grey matter volume could serve as such an MRI-based biomarker. With use of a relatively big cohort and a pseudo-longitudinal approach, the study was able to prove that CT and subcortical GM volume decreases with ongoing disease and can therefore monitor disease progression, being at the same time independent of overall disease aggressiveness. Therefore, this work suggests CT as a possible parameter representing disease progression in ALS which could be used in clinical trials for cohort stratification or as an outcome measure.

Neuroimaging has been widely used in diagnostic workups of patients with (suspected) ALS, but biomarkers fulfilling the criteria to reliably monitor the disease progression without being influenced by other characteristics of disease are still missing.

Especially due to the high heterogeneity of patients and courses of disease, patients in neuroimaging studies must be well classified. The D50-disease progression model, which has been developed in Jena, proved to be a suitable model to characterize patients based on their individual form of disease and disease aggressiveness. By applying this model, it is possible to analyze correlations and associations between alterations in neuroimaging and clinical disease. The model enabled the implementation of a pseudo-longitudinal dataset and the characterization of a big cohort of patients based on their overall disease aggressiveness and phase of disease. Thereby, limitations of the ALSFRS-R, which is typically used as singular clinical measure, could be circumvented and the unbiased inclusion of a higher number of patients was possible.

This study proved the usability of the D50-model for correlation analyses between clinical disease and biomarkers in MRI and can, therefore, be recommended for further biomarker studies. It would be of interested if the correlations found in this study can be reproduced in studies using different software (e.g., FreeSurfer) and with MRI of higher field strength. Further, a validation of our results with real longitudinal data and in a multicentric, international cohort of patients would be desirable. These are important future steps to establish CT as a non-invasive biomarker for monitoring disease accumulation and/or the effectiveness of therapeutic interventions.

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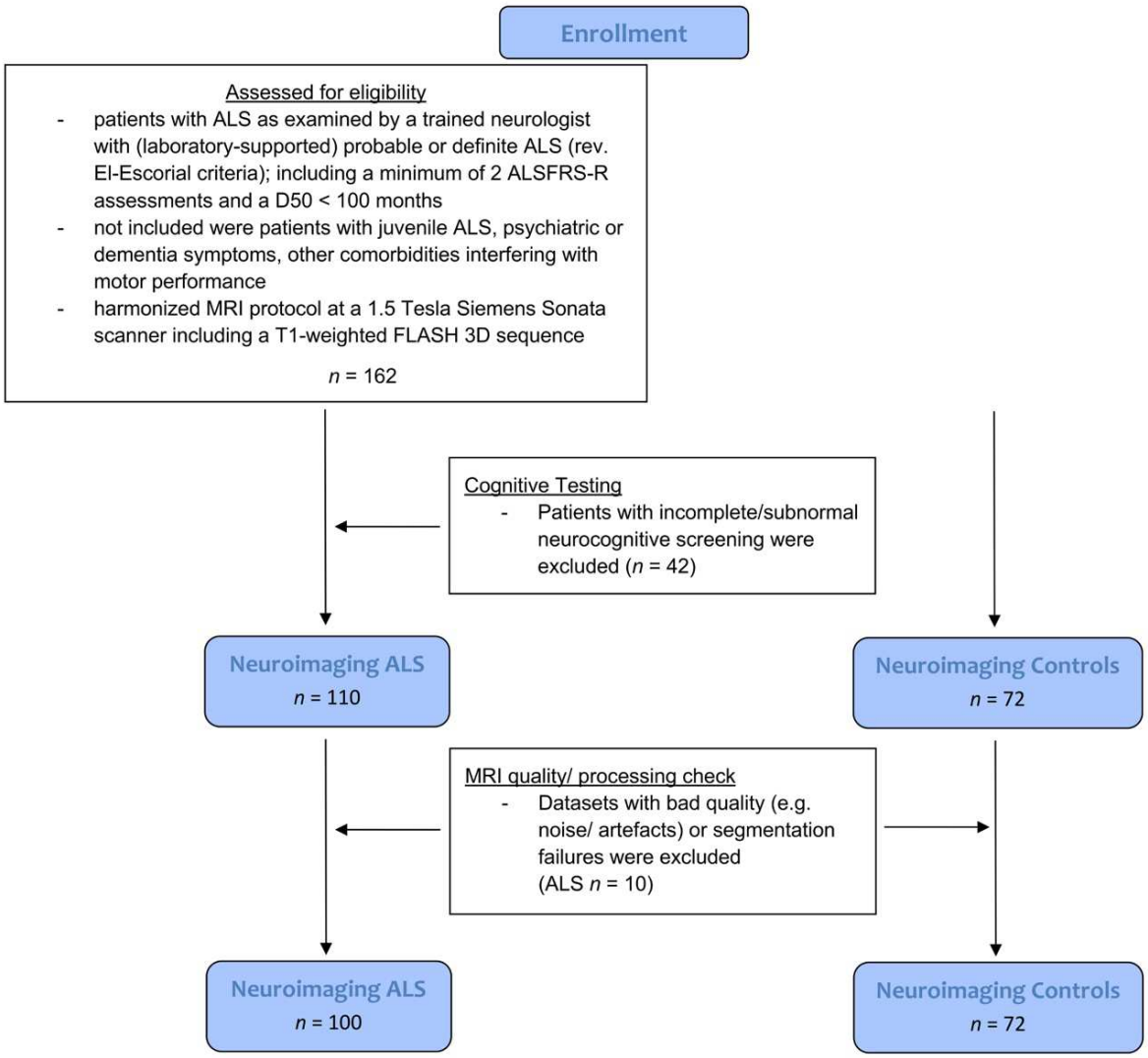
Appendix

A Supplementary material of the published paper

Supplementary Figure S1:



Supplementary Table S1:

ALS (*n* = 100)	**Phase I**	**Phase II & III/IV**	**p**
Demographics			
n	48	52	
	(48%)	(52%)	
age_test [years] #	61.46 ± 15.96	66.37 ± 12.45	0.01
	(32.75 - 29.41)	(40 - 81.41)	
gender [male/female] ◎	27/21	28/24	0.81
	56.3%/ 43.8%	53.8%/ 46.2%	
handedness [left/right/unknown] ◎	46/1/1	47/5/0	0.11
	95.8%/ 2.1%/ 2.1%	90.4%/ 9.6%/ 0.0%	
Traditional Disease Metrics			
symptom duration [months] #	9.50 ± 6	17.00 ± 14	< 0.001
	(4 - 23)	(5-59)	
onset [bulbar/spinal] ◎	17/31	16/36	0.62
	35.4%/ 64.6%	30.8%/ 69.2%	
D50 disease progression model parameters			
D50 [months] #	32.88 ± 19.74	23.30 ± 16.00	< 0.001
	(12.02 - 96.20)	(0.25 - 0.52)	
relative D50 [rD50] #	0.16 ± 0.09	0.34 ± 0.13	< 0.001
	(0.05 - 0.24)	(0.25 - 0.52)	
calculated functional state [points] #	44.41 ± 3.54	34.65 ± 7.69	< 0.001
	(38.20 - 50.78)	(24.63 - 42.87)	
calculated functional loss rate [points lost per month] #	0.50 ± 0.34	1.13 ± 0.72	< 0.001
	(0.11 - 1.71)	(0.40 - 4.02)	
# non-parametric Mann-Whitney-U-Test			
⊞ parametric t-statistic			
◎ chi-squared test			

***Abbreviations*: *D50* estimated time in months for an individual to lose 50% of functionality; rD50 (relative D50) individual disease covered; *cFS* calculated Functional State; *cFL* calculated Functional Loss-rate *Note*: Continuous data are summarized for # as median ± interquartile range (each with the total range in brackets). For Θ categorial data. the number of cases (equals percentages) is given. Variables that are time-point dependent refer to the day of MRI-acquisition; others depict constant characterization of patients' overall disease course.**

Supplementary Table S2:

ALS (*n* = 100)	**High Aggressiveness**	**Low Aggressiveness**	**p**
Demographics			
n	49	51	
	49%	51%	
age_test [years] #	65.33 ± 16.84	63.08 ± 14.00	0.15
	(32.75 - 81.41)	(37.75 - 79.41)	
gender [male/female] ⊙	26/23	29/22	0.56
handedness [left/right/unknown] ⊙	45/4/0	48/2/1	0.67
	91.8%/ 8.2%/ 0.0%	94.1%/ 3.9%/1.9%	
Traditional Disease Metrics			
symptom duration [months] #	0.88 ± 0.56	0.36 ± 0.21	< 0.001
	(4 - 27)	(5 - 59)	
onset [bulbar/spinal] ⊙	21/28	12/39	0.04
	42.9%/ 57.1%	23.5%/ 76.5%	
D50 disease progression model parameters			
D50 [months] #	20.88 ± 7.16	40.58 ± 13.72	< 0.001
	(6.04 - 29.58)	(30.04 - 96.20)	
relative D50 [rD50] ⊞	0.29 ± 0.12	0.22 ± 0.12	0.01
	(0.06 - 0.52)	(0.05 - 0.51)	
calculated functional state [points] #	37.95 ± 9.98	41.26 ± 7.83	< 0.001
	(24.63 - 45.77)	(24.98 - 50. 78)	
calculated functional loss rate [points lost per month] #	1.22 ± 0.74	0.50 ± 0.30	< 0.001
	(0.42 - 4.02)	(0.11 - 1.11)	
# non-parametric Mann-Whitney-U-Test			
⊞ parametric t-statistic			
⊙ chi-squared test			

***Abbreviations*: *D50* estimated time in months for an individual to lose 50% of functionality; rD50 (relative D50) individual disease covered; *cFS* calculated Functional State; *cFL* calculated Functional Loss-rate *Note*: Continuous data are summarized for ⊞ as mean ± SD and for # as median ± interquartile range (each with the total range in brackets). For Ⓜ categorial data. the number of cases (equals percentages) are given. Variables that are time-point dependent refer to the day of MRI-acquisition; others depict constant characterization of patients' overall disease course.**

B Declaration of honour

Hiermit erkläre ich, dass mir die geltende Promotionsordnung der Medizinischen Fakultät der Friedrich-Schiller-Universität bekannt ist,

ich die Dissertation selbst angefertigt habe, keine Textabschnitte eines Dritten oder eigener Prüfungsarbeiten ohne Kennzeichnung übernommen und alle von mir benutzten Hilfsmittel, persönlichen Mitteilungen und Quellen in meiner Arbeit angegeben habe,

mich folgende Personen bei der Auswahl und Auswertung des Materials sowie bei der Herstellung des Manuskripts unterstützt haben: Robert Steinbach, Tino Prell, Meret Herdick, Simon Schuster, Julian Großkreutz, Andrea Dieckmann.

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Jena, 23.12.2023, Nora Dieckmann

C Note of thanks

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