Longitudinal Characterization of the FTD-ALS Spectrum

The Harvard community has made this article openly available. Please share how this access benefits you. Your story matters

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Citable link</td>
<td><a href="http://nrs.harvard.edu/urn-3:HUL.InstRepos:17613739">http://nrs.harvard.edu/urn-3:HUL.InstRepos:17613739</a></td>
</tr>
<tr>
<td>Terms of Use</td>
<td>This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at <a href="http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA">http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA</a></td>
</tr>
</tbody>
</table>
# TABLE OF CONTENTS

1. Abstract .................................................. 3  
2. Background, Significance, Innovation .................. 5  
3. Hypothesis and Specific Aims ............................ 9  
4. Experimental Design:  
   a. Study Design ......................................... 10  
   b. Study Population ..................................... 11  
5. Data Collection .......................................... 11  
6. Data Analysis .......................................... 12  
7. Current Study Status .................................. 13  
8. Additional Research and Activities .................... 14  
9. Conclusions ............................................ 18  
10. Acknowledgements ..................................... 20  
11. References ............................................ 21
1. ABSTRACT

Neurodegenerative diseases represent a remarkable unmet medical need, where the lack of a clear understanding of the underlying mechanisms, together with the clinical heterogeneity and overlap, significantly challenge biomarkers and therapy development.

Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD) are heterogeneous neurodegenerative diseases that can occur within the same person, despite presenting divergent clinical core features: a neuromuscular disease, ALS, and a dementing syndrome, FTD, affecting behavior and language. Based on known pathological overlap \(^1\) and the recent discovery of shared genetic basis \(^2\), \(^3\), the characterization of the ALS-FTD disease continuum with comprehensive clinical assessments and quantitative imaging measures over time has the potential to highlight patterns of disease progression and specific brain system vulnerabilities, leading to biomarkers development in these relatively rapidly progressing diseases. We propose to build, characterize and compare concurrent FTD-ALS, ALS and FTD cohorts with a prospective observational cohort study performed at a single institution, Massachusetts General Hospital (MGH), within the MGH FTD and ALS Units. Building on the clinical and research expertise of the MGH ALS and FTD Units, we are including measures of disease and disease progression specific to both ALS and FTD, such as: structured clinical interview of participant and informant, detailed neurological physical examination, quantitative strength and respiratory function assessments, functional measures, clinical questionnaires, comprehensive neuropsychological testing (evaluating cognition, language and behavior) and brain MRI on 3 Tesla scans. Study procedures are performed every 6 months up to one year. We plan to perform quantitative cortical thickness and sub-cortical structures analysis, evaluation of white matter anatomical and functional integrity, correlations between anatomical and clinical measures, both
within patient and inter-patient populations. To date, there are only 3 studies that evaluated the full FTD-ALS spectrum cross-sectionally and there are no longitudinal studies or quantitative cortical thickness evaluations across the entire FTD-ALS continuum. Our longitudinal comprehensive and specialized assessments will lead to the development of a unique dataset evaluating the full FTD-ALS spectrum with standardized measures in a multidisciplinary approach. This will provide the basis for further FTD-ALS research and it will contribute to the development of quantitative biomarkers of disease and disease progression across clinically heterogeneous diseases, impacting both the clinical and research practices.

ALS clinical care and clinical trials efficiency can also be remarkably improved by a better understanding of the ALS clinical heterogeneity and of the clinical relevance of changes in the ALS functional rating scale-revised (ALSFRS-R), the functional assessment used routinely in both the ALS clinical and research settings. We studied ALS heterogeneity to ascertain predictors of disease progression and survival to improve prognostication in the clinics and stratification in clinical trials. In addition, we explored the clinical meaningfulness of changes in the ALSFRS-R and its functional sub-domains to facilitate the interpretation of score changes with disease progression and during the assessment of treatment response.
2. BACKGROUND, SIGNIFICANCE AND INNOVATION

Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD) are devastating neurodegenerative diseases sharing many common features. Pathologically, they share TAR DNA-binding protein (TDP-43) pathology\(^1\), genetically, the hexanucleotide repeat expansion in the non-coding region of C9ORF72\(^2, 3\), and, clinically, the relatively young age of symptoms onset and lack of effective disease modifying therapies.

FTD is a common cause of presenile dementia that can present a wide range of symptoms, including behavioral symptoms (behavioral variant, bvFTD), language symptoms (primary progressive aphasia, PPA, semantic variant or progressive non-fluent aphasia, PNFA) or a combination of both\(^8\). FTD is often clinically associated with motor impairment as well, with overlap in some cases with Progressive Supranuclear Palsy (PSP), Corticobasal Degeneration (CBD) or ALS. Approximately 12-14\% of FTD patients present concomitant motor neuron disease\(^9, 10\). FTD patients who develop motor neuron disease or full-blown ALS exhibit a shorter survival than FTD patients who do not. Pathologically, FTD is most often associated with either tau or TDP-43 pathology.

ALS is a progressive fatal neuromuscular disease characterized by progressive degeneration of motor neurons, resulting in loss of motor function associated to voluntary muscle weakness and death 3 to 5 years after symptoms onset\(^11\). Patients suffering from ALS can also experience variable degrees of cognitive and/or behavioral changes\(^12-14\): \(\geq 40\%\) ALS incident cases present cognitive impairment, predominantly executive dysfunction\(^15\); approximately 20\% ALS patients show moderate-severe behavioral changes\(^16\), predominantly apathy and
disinhibition (17). 14% of incident cases (15) or 5-15% of ALS patients meet the diagnostic
criteria for FTD (12, 13). Patients with ALS and comorbid FTD exhibit a shorter survival than
patients with isolated disease (18, 19), with a median survival of 2-3 years from symptom onset
(20).

Due to the known overlap at different levels, FTD and ALS are most likely part of a clinical-
pathologic continuum, rather than two separate diseases (21). The overarching goals of this
project are to characterize the FTD-ALS continuum and to investigate how FTD-ALS differs
from isolated ALS and isolated FTD over time. Specifically, we propose to study clinical and
imaging biomarkers (MRI, Diffusion Tensor Imaging, DTI, resting state functional MRI, fMRI).
We are also obtaining blood for future studies on biofluid biomarkers. Although increasing effort
is being devoted to identifying biofluid and imaging biomarkers for use in clinical trials for both
ALS and FTD, a limited number of evaluations have combined FTD and ALS patients in a single
study. Our innovative project consisting of longitudinal clinical and imaging evaluations will
provide data on disease progression and evolution of the neurodegenerative process underlying
the FTD-ALS spectrum. This will constitute a solid foundation for further research on FTD-ALS
and hopefully improve the understanding of the FTD-ALS clinico-pathologic entity. The results
from the proposed studies will enable the development of more accurate diagnostic criteria,
which may translate into early diagnosis and improvement in patient care. Additionally,
longitudinal systematic assessment of changes in structural imaging will define quantitative MRI
biomarkers of disease progression within the FTD-ALS spectrum that will be critically important
to the assessment of the efficacy of disease-modifying therapies.
Currently, clinical assessment of ALS and FTD patients is typically limited to either an evaluation primarily using neuromuscular instruments or a neuropsychological evaluation, depending on clinical presentation. Disease progression in ALS and its response to intervention are measured with serial assessments of motor function using the Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R) \(^7\) and of respiratory function using Forced Vital Capacity (FVC). Changes in patients with FTD are measured using formal neuropsychological testing, structured interviews and caregivers’ feedback \(^22\). Survival remains an important clinical outcome measure in these populations.

As a result, we lack information about the full spectrum of the natural history of these conditions as well as biomarkers to help us measure their progression. This hinders early diagnosis since current diagnostic criteria for both conditions are not sufficiently sensitive in the early stage of the disease \(^23\, 24\). Early diagnosis is important for identifying patients eligible to participate in clinical trials aimed at finding new effective therapies \(^23\, 25\).

To date, MRI has identified areas of atrophy in ALS in the primary motor cortex \(^26\, 29\) and with variable involvement of extra-motor cortices \(^26\, 28\). A number of studies, including Dr. Dickerson’s work in the FTD Unit at MGH, have identified specific brain regions of atrophy in FTD \(^30\, 32\). Specifically, the behavioral variant shows predominant atrophy in the frontal lobe \(^30\, 31\), PNFA in the left peri-sylvian area, and the semantic variant in the left anterior temporal lobe \(^32\).

A few studies have evaluated ALS and FTD-ALS \(^33\, 37\) and found that, compared to ALS, FTD-ALS was associated with prominent atrophy in the fronto-temporal regions.
In addition, within patients with pathologically confirmed Frontotemporal Lobar Degeneration–Ubiquitin (FTLD-U), those with evidence of motor neuron disease had a more localized frontal lobe atrophy compared to those with isolated FTLD-U, who showed a more widespread pattern of atrophy involving the frontal, temporal and parietal lobes. Only three studies have examined FTD, ALS and FTD-ALS in the same analysis. The first evaluated brain gray and white matter changes in ALS, ALS-FTD and bvFTD with a volume based analysis approach, voxel based morphometry (VBM), and diffusion tensor imaging (DTI). It demonstrated that: (a) changes in the ALS group involved mainly the motor cortex and the anterior cingulate with their relative white matter tracts; (b) behavioral FTD was characterized by extensive prefrontal cortex changes and FTD-ALS had additional temporal lobe cortex and white matter involvement when compared to ALS; and (c) anterior cingulate and motor cortex with their relative white matter tracts showed atrophy in both FTD and ALS. This study only evaluated the behavioral variant FTD of the FTD continuum and did not perform correlations with cognitive-behavioral measures.

The second study evaluated semantic symptoms across ALS-FTD and their anatomical substrates utilizing VBM. It confirmed the presence of semantic deficits in ALS and FTD-ALS which were associated with predominant involvement of the anterior temporal lobe. Only the FTD semantic variant was included in this analysis.

The third focused on changes occurring in the cerebellum and their correlations with cognitive neuropsychiatric and motor symptoms across the ALS-FTD continuum. It showed that specific areas of the cerebellum were atrophied across the spectrum and correlated with the
symptoms considered. VBM was utilized for the imaging analysis and only the bvFTD subjects were enrolled.

There have been no studies applying quantitative cortical thickness approaches across the entire ALS-FTD spectrum. In addition, there have been no longitudinal imaging studies involving these clinical groups collectively.

Given the pathologic overlap between ALS and some forms of FTD (TDP-43 inclusions), we see an opportunity to design disease-modifying therapies targeted toward this molecule and other molecules in the putative cascade. In the future TDP-43 targeted therapeutic interventions, measuring progressive changes in MRI imaging characteristics would provide a unique outcome measure to assess response to treatment in these clinically diverse patient populations.

3. HYPOTHESIS AND SPECIFIC AIMS

**Hypothesis 1:** Given the faster disease progression in patients with FTD-ALS, we hypothesize that these patients are affected by a more aggressive underlying neurodegenerative process, which could be indicated by progressively more extensive and more pronounced cortical thinning, DTI changes and disruption of resting state functional connectivity than in patients with isolated ALS and isolated FTD.

**Specific aim 1:** To develop a longitudinal cohort of FTD-ALS patients who are assessed and followed comprehensively using standardized instruments measuring cognitive, affective and motor symptoms and signs.
Specific aim 2: To assess whether progressive cortical thinning, loss of white matter integrity and changes in functional connectivity detected by MRI are more pronounced in patients affected by FTD-ALS than in patients with isolated ALS and isolated FTD.

Hypothesis 2: We hypothesize that changes over time in specific brain regions and in the integrity of specific white matter tracts and in functional connectivity will be associated with longitudinal changes in specific clinical features, thus supporting the clinical validity of these MRI biomarkers.

Specific aim 3: To assess the clinical and disease progression correlates of longitudinal changes in imaging measures in ALS, FTD and FTD-ALS patients.

4. Experimental Design:

a. Study Design

This is a prospective observational cohort study performed at a single institution Massachusetts General Hospital (MGH) within the MGH FTD and ALS Units. Three groups of patients are evaluated and compared to healthy control subjects: patients with isolated ALS (assessed by El Escorial Criteria) [39]; patients with isolated FTD (assessed by FTD consensus diagnostic criteria) [40]; and patients with ALS and comorbid FTD [14]. After providing informed consent, all study participants undergo evaluations, including a complete clinical history, neurological physical examination, Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) [7], Forced Vital Capacity (FVC), quantitative strength measurements with hand held dynamometry (HHD), grip strength, ALS Cognitive Behavioral Screen (ALS CBS), formal neuropsychological testing, blood draw, brain MRI on a 3 Tesla (3T) scan. Complete assessments are repeated after six months, up to one year.
b. Study Population

Given the relative rarity of patients affected by FTD-ALS, if possible, healthy control subjects as well as patients with isolated ALS or FTD are matched demographically and according to education level and disease duration to FTD-ALS patients.

Individuals of both genders, older than 18 years and fluent in English are eligible for the study. Patients with prior history of cerebral injury (e.g., stroke), epilepsy, psychiatric disorders or other neurodegenerative diseases or with contraindications to MRI, including compromised respiratory function (FVC ≤ 50%), are excluded.

5. Data Collection

MRI data are collected using the standard common protocol of the 3T Bay 4 MGH/MIT/HMS Athinoula Martinos Center for Biomedical Imaging. Measures of disease progression including ALSFRS-R, FVC and formal neuropsychological testing are also performed. Brain regions are examined quantitatively by a cortical surface-based reconstruction associated with analysis of cortical thickness, following previously validated protocols [32, 41]. DTI analysis is also performed following previously validated protocols involving voxelwise acquisition and comparison of fractional anisotropy maps and tract-based spatial statistics [4, 42]. Additionally, analysis of spontaneous fluctuations in the blood oxygen level-dependent (BOLD) signal of resting state fMRI is conducted [43]. Potential confounding variables are age and sex. Educational level, ethnic group, language barrier, mood disorders, nocturnal hypoventilation, learning from prior testing and medications could affect the performance of study subjects in the...
cognitive behavioral testing. Information about these variables are obtained through the subject and informant interviews.

6. **Data Analysis**

On the basis of prior imaging studies involving the patient populations of interest \(^{4, 28, 32-34}\), cortical gray matter regions selected for morphometric analysis include: precentral and postcentral gyri; superior, middle and inferior frontal gyri; orbitofrontal cortex; cingulate and paracingulate gyri; insula; supramarginal gyrus; superior, middle and inferior temporal lobe; and frontal and temporal poles \(^{4, 28}\). The connectivity of these regions within large-scale circuits will be assessed using resting-state functional MRI connectivity as well as the integrity of white matter tracts connecting these regions, including corticospinal tracts, corpus callosum, interhemispheric commissure and association white matter tracts.

The study will use T-test and analysis of covariance, correcting for age, gender and educational level to compare continuous outcome measures of the study populations related to serial ALSFRS-R, FVC, formal neuropsychological testing, cortical thickness and DTI and functional connectivity changes of the various cortical areas considered. The possible correlation between progressive cortical thickness and DTI and functional connectivity changes and measures of disease progression (serial ALSFRS-R, FVC, formal neuropsychological testing) will be assessed with correlation analysis.

Using data from FTD-ALS epidemiology and from a longitudinal assessment of gray matter atrophy in ALS \(^{44}\), we determined that with 20 ALS or FTD patients and 15 ALS-FTD patients we would have 80% power to detect a difference with an effect size of 0.74 at a one-sided
p=0.10 level. This is because we consider this as a hypothesis generating pilot study. With 35 total patients in the paired analysis, we would have an 80% chance of detecting a correlation coefficient of 0.34 with changes in clinical measures of progression such as ALSFRS-R, FVC and neuropsychological testing.

7. Current Study Status

After obtaining additional funding for imaging costs and Partners IRB approval, the enrollment was opened to eligible patients who were seen within the MGH ALS and FTD Units and were interested in the study. To date, we enrolled a total of 42 subjects, including 19 ALS, 15 FTD (6 semantic PPA, 2 agramatic PPA, 7 bvFTD), 7 FTD-ALS patients and 1 control. 4 out of 19 ALS patients showed some degree of cognitive impairment and had Clinical Dementia Rating, CDR>0, denominated ALS-MCI. FTD patients have not been showing signs of motor weakness. 19 participants completed the 6 months follow-up visit: 10 ALS subjects, 2 ALS-MCI, 3 semantic PPA and 4 bvFTD.

6 participants completed the 1 year visit: 2 ALS subjects, 2 semantic PPA, 2 bvFTD.

The study continues to be open to enrollment and participants from all the cohorts are continuing being scheduled for follow up visits as per study protocol.

Preliminary cross-sectional imaging and clinical data analysis is currently in process.

In summary, aim 1 of the study was satisfied, while Aim 2 and 3 are now in progress.
8. Additional Research and Activities

Given the multidisciplinary nature and learning opportunity offered by this project, I had the
privileged position of helping bridging the ALS and FTD teams expertise and efforts aimed at
providing coordinated and comprehensive care to FTD-ALS patients.

To improve detection of possible underlying cognitive and behavioral symptoms in ALS
patients, I facilitated the introduction of the ALS Cognitive Behavioral Screen (ALS-CBS) in the MGH ALS Unit. To standardize screening delivery and recording, I provide teaching
sessions for nursing staff and research coordinators. To optimize the capture of the screening
results, I worked with the IT Department to develop a specific note template with drop-down
menus, which is now part of the MGH electronic medical record system.

I have also been gaining a precious experience as a clinical investigator while being involved in
clinical trials in ALS and ALS biomarkers projects run within the Neurological Clinical
Research Institute (NCRI) at MGH. In addition to the exploration of the scientifically intriguing
overlap of ALS and FTD, I am also particularly interested in discerning ALS heterogeneity,
predictors of disease progression and survival as well as the clinical importance of changes in
ALS outcome measures such as the ALSFRS-R (ALS Functional Rating Scale-Revised). These projects have potential practical implications in both the research and clinical settings,
where they can positively impact prognostication, patient and families counseling and patient
stratification in clinical trials, enhancing their efficiency.

Through the presentation of these projects to national and international ALS meetings, I had the
opportunity to meet experts in the field and establish precious contacts that have led to the
invitation to participate as a speaker in national and international conferences and may result in
important collaborations.
Oral Presentations:

2014 ALS Research Group Meeting

Presented: “Imaging in ALS-FTD”

Mall of America, Minnesota, USA, on September 18th 2014

Northeast ALS Consortium (NEALS) National Annual Meeting

Presented: “Clinically Meaningful Change on the ALSFRS-R”

Clearwater Beach, Florida, USA, on October 2nd 2013

Selected Poster Presentations:

Elena Ratti, James Berry, Mark Vangel, Eric Macklin, David Schoenfeld, Merit Cudkowicz. “Progression to Clinically Meaningful Changes in ALSFRS-R Bulbar and Fine Motor Domains is Faster in Bulbar Onset and in Limb Onset Amyotrophic Lateral Sclerosis Patients Respectively”

Presented at the Northeast ALS Consortium (NEALS) Annual Meeting in Clearwater, Florida, on October 23rd 2014

Will be presented at the 67th Annual Meeting of the American Academy of Neurology, Washington, DC, USA, on April 22nd 2015

Elena Ratti, James Berry, Nazem Atassi, Amy Shui, Douglas Hayden, David Schoenfeld, Hong Yu, Robin Conwit, Jeremy Shefner, Merit
Cudkowicz and Ceftriaxone Study Site Investigators. “Predictors of Outcome in Amyotrophic Lateral Sclerosis.”

Presented at the 66th Annual Meeting of the American Academy of Neurology, Philadelphia, PA, USA, on April 29th 2014

Elena Ratti, James Berry, Stacie Hudgens, Merit Cudkowicz, Douglas Kerr and Northeast ALS Consortium. “Clinically Meaningful Change on the ALSFRS-R”

Presented at the Motor Neuron Disease Symposium, Milan, Italy, on December 7th 2013

Elena Ratti, Nazem Atassi, Daniela Grasso, Robert Lawson, Christina Dheel, Matthew Jaffa, Robert Bowser, Merit Cudkowicz, James Berry and Northeast ALS Consortium. “Biofluids bio-repository as a study tool of the Amyotrophic Lateral Sclerosis phenotypic spectrum.”

Presented at the “Biomarkers in Brain Disease: Challenges and Opportunities” Meeting (Wellcome Trust) in Cambridge, UK, on February 4th 2013

Presented at the North East ALS Consortium (NEALS) Annual Meeting in Clearwater, Florida, on October 3rd 2013
In collaboration with Dr. Cudkowicz and Dr. Berry, I prepared a chapter on motor neuron diseases that has been published in the online Scientific American Medicine (Decker Intellectual Properties):

**Elena Ratti, Merit E. Cudkowicz, James D. Berry. Neurology. Motor Neuron Diseases.**


Given my clinical and research interest in ALS-FTD, I am an active member of the ALS-FTD taskforce in the multicenter North East ALS Consortium (NEALS) and I am part of the team of a multicenter collaboration that aims at studying cognitive and behavioral changes in ALS patients and testing behavioral interventions, which are absolutely novel in the field of ALS. This project: “Relationship of cognitive-behavioral symptoms in ALS to caregiver burden and development of a pilot randomized controlled behavioral intervention to improve caregiver burden” aims at developing a novel behavioral intervention to educate and alleviate caregiver’s burden. The project was the focus of a grant proposal submitted by our team at MGH in collaboration with the ALS clinic team at Drexel University College of Medicine and it was recently awarded funding from the ALS Association (ALSA) as part of the ALSA clinical management program.
ALS, FTD and particularly FTD-ALS are relatively rare neurodegenerative diseases that are characterized by a remarkable progressive disability, relatively fast disease progression and shortened survival. These characteristics significantly challenge critical steps of research in these fields: recruitment can be affected by the limited availability of subjects which can be further limited due to disease severity, while retention and longitudinal evaluations can be influenced by disease progression and inability to tolerate study procedures, such as imaging and neuropsychological testing.

Despite these challenges we have been able to be close to the completion of enrollment in a relatively short period of time and we feel that our study has the real potential to provide valid contribution to this challenging field of research were the medical need is remarkable.

It is also important to consider that, in addition to multimodal imaging approaches assessing both structure and function, multidisciplinary efforts and close collaborations are necessary to progress our knowledge in neurodegenerative diseases and catalyze biomarkers and drug development. In addition, to allow standardized and comprehensive assessments of patients with overlap syndromes, it is vital to ensure alignment of assessments and sharing specialized expertise across different neurodegenerative disease clinics and research projects. This can have a remarkable impact on the routine clinical care, guiding comprehensive clinical assessments to address needs and provide comprehensive patient counseling.

I feel extremely privileged to have the opportunity to help joining the efforts of different teams and disciplines and learn from such rich environments. I am also fortunate to have experienced many different research approaches, from the main FTD-ALS clinical and imaging characterization to my participation in clinical trials and biomarkers and outcome measures

9. Conclusions
research. These diverse exposures will help me translating different disciplines and type of research in a common major team effort aimed at better understanding and comprehensively caring for patients with ALS and FTD.
Acknowledgements

My sincere gratitude goes to the patients and their families, who, despite the challenges associated with the disease and its progression, are continuing to participate in the study, offering their generous support and contributions to progress our knowledge.

I would like to express special thanks to my mentors, Dr. Merit Cudkowicz and Dr. Bradford Dickerson, for sharing their unique expertise and advice and for their support in many projects.

In addition, I would like to acknowledge the entire study teams across the MGH ALS and FTD Units, the Neurological Clinical Research Institute (NCRI) at MGH and the Massachusetts Alzheimer’s Disease Research Center (ADRC). Their wonderful team effort joining multidisciplinary expertise not only made this study possible, but it also offered an invaluable learning experience.

I am also grateful to my thesis committee advisors, Dr. Darin Dougherty and Dr. Catherine Lomen-Hoerth, for their precious advice and guidance for the success of this study.

I would also like to acknowledge the MPCTI Program for having offered me a large breath of approaches and exposed me to unique expertise.

An acknowledgement to my study funding sources: R25NS065743, the National Institute of Neurological Disorders and Stroke (NINDS) R25 Research Training Award Program and the Harvard NeuroDiscovery Center/NCRI/Massachusetts ADRC Award covering imaging and patient visits costs.

Lastly, I am extremely grateful to my friends, geographically close or far, and to my wonderful family that, despite the geographical distance, has been enormously supportive in this adventure.

Elena Ratti
11. REFERENCES


