BACHELOR THESIS

Biotechnology

Symptomatic drug therapy impact on Amyotrophic Lateral Sclerosis survival

Author
Anabel Sáez Mas

External director
Fang Fang
Elisa Longinetti

Director URV
Dania García Sánchez

UNIVERSITAT ROVIRA i VIRGILI

Tarragona – 2018/19

“Be curious, and however difficult life may seem,

there is always something you can do, and succeed at.

It matters that you don’t just give up.”

Stephen Hawking

This thesis is based on the results obtained during curricular traineeships at the department of Medical Epidemiology and Biostatistics of the Karolinska Institutet of Stockholm (Sweden). It is enclosed in the research on neuroepidemiology under the guidelines and supervision of the senior researcher Fang Fang and the doctoral student Elisa Longinetti.

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1 ABBREVIATIONS

AD – Alzheimer's disease

ALS – Amyotrophic lateral sclerosis

ALSFRS-R – Amyotrophic lateral sclerosis functional rate scale (Revised)

BMAA – N-methylamino-L-alanine

BMI – Body mass index

CI – Confidence interval

FALS – Familial amyotrophic lateral sclerosis

HR – Hazard ratio

IV – Invasive ventilation

LPS – Lipopolysaccharide

MN – Motor neuron

MND – Motor neuron disease

MS – Multiple sclerosis

MV – Missing Values

NIV – Non-invasive ventilation

PD – Parkinson's Disease

PSMA - Progressive spinal muscle atrophy

PLD – Primary lateral sclerosis

SALS – Sporadic amyotrophic lateral sclerosis

SEALS – South-East England ALS

SOD1 – Superoxide dismutase 1

2 ABSTRACT

Background: Due to the heterogeneity of amyotrophic lateral sclerosis (ALS) in terms of clinical manifestations and trajectories, population-based registries are important to display a picture of the different clinical phenotypes and provide useful information of incidence, prevalence, and risk factors. Currently, the lack of knowledge about the molecular bases of ALS is an obstacle to develop disease-modifying therapies, so most of the management strategies of ALS are focused on improving the symptomatology of patients. Nonetheless, drug prescription for management of ALS symptoms is based on strategies followed to treat the same symptoms in other diseases, so their effect on ALS patient's survival specifically remains unknown.

Materials and methods: Swedish Motor Neuron Disease (MND) Quality Registry has been used to perform a survival analysis using a Cox proportional hazard regression to evaluate the impact of the different symptomatic medications prescribed to ALS patients, and to provide an epidemiological description of Swedish MND patients.

Results and discussion: Epidemiology traits of Swedish MND patients agree with most of European studies, except for sex prevalence, which is almost for both sexes in Sweden whereas to the rest of Europe is clearly higher in men. Data collection in the registry has several deficiencies, mainly in the register of treatments, so the results of analysis are not conclusive enough. However, our performed review of recent studies related with antibiotics, gut microbiota, and ALS points out that antibiotic prescription may have an impact on ALS survival through modification of microbiota population.

Conclusions: Quality of the Swedish MND registry should be improved to aid future research. Prescription of antibiotics that impact negatively on butyrate-producer bacteria may be related with a worse survival of ALS patients.

Keywords: Amyotrophic lateral sclerosis, Swedish Motor Neuron Disease Quality Registry, symptomatic treatment, gut microbiota, antibiotics.

3 INTRODUCTION

3.1 AMYOTROPHIC LATERAL SCLEROSIS

Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease (MND) characterized by a progressive degeneration of upper and lower motor neurons leading to increasing muscle weakness until paralysis.

With an estimated prevalence in Europe around 2–3 cases each 100.000 people, ALS is listed as a rare disease. Most of the recent epidemiological studies suggest an increased incidence in male than female. Despite the wide range of age of onset, most of the cases use to appear at median-advanced age, placing the peak of onset around 65 years. Site of symptoms onset, genetic background and nutritional status are some of the prognostic factors that can influence survival. Although about 5–10% of ALS patients live longer than 10 years, the majority of patients is no longer alive within four years from symptom onset (Bourke and Gibson 2010).

ALS can be classified according to its heritability (sporadic or familial), site of onset (bulbar or spinal-onset disease) or the level of certainty of diagnosis following El Escorial criteria (suspected, possible, probable or definite ALS) (Table 1).

ALS cases can be catalogued as sporadic (SALS) if no known relative has been previously diagnosed with ALS, or familial (FALS) if at least one family member has been diagnosed with ALS (10% of ALS cases). Although the average survival in both SALS and FALS cases seems quite equal, some genetic mutations associated to FALS cases, such as A4V mutation in superoxide dismutase 1 gene (*SOD1*), are linked with a faster progression while other mutations such as E21G in *SOD1* are related with longer survival (Chiò et al. 2009).

Clinical manifestations of ALS are summarized in Figure 1. Even though all patients achieve a similar clinical picture as the disease progresses, the order of emergence of symptoms is closely linked to the site of onset. The majority of patients have a spinal/limb onset and just 20% of patients have bulbar onset.

Depending on the rate of affection of upper and lower MN, the symptomatology varies among patients. Patients with spinal onset present first symptoms related to muscle affection of the limbs. In patients with spinal-onset, upper MN affection is closely linked with muscle weakness, hyperreflexia and spasticity (continuous contraction of skeletal muscles) while lower limb MN signs are linked with muscle atrophy, hyporeflexia and fasciculations.

Table 1. El Escorial diagnosis criteria.

El Escorial category	Clinical signs
Suspected ALS	LMN signs only
Possible ALS	UMN and LMN signs in only one region*, or UMN signs alone in two or more regions, or LMN signs rostral to UMN signs.
Probable ALS	UMN and LMN signs in two regions*, with some UMN signs rostral to LMN signs.
Definite ALS	UMN and LMN signs in three regions* of the body.

*Regions of the body: bulbar, cervical (neck, arm, hand, diaphragm, and cervical spinal cord-innervated muscles), thoracic (back and abdomen muscles), and lumbar (back abdomen, leg, foot and lumbosacral spinal cord-innervated muscles). **LMN** – Lower motor neurons; **UMN** – Upper motor neurons (Al-Chalabi et al. 2016)

Patients with bulbar-onset combine upper and lower MN signs in bulbar muscles (pharyngeal, oral and respiratory), which leads to alterations in the abilities of speech (dysarthria), swallow (dysphagia) and breathing (respiratory insufficiency/dyspnea). Therefore, due to early apparition of these symptomatology in bulbar onset patients, their prognosis is worse (Chiò et al. 2009).

About half of the patients with ALS show cognitive and behavioral impairments due to a frontotemporal dysfunction leading to frontotemporal dementia in 15% of the patients. The symptoms related to frontotemporal dysfunctions include depression, difficulty to take decisions and pseudobulbar affection (Tiryaki and Horak 2014).

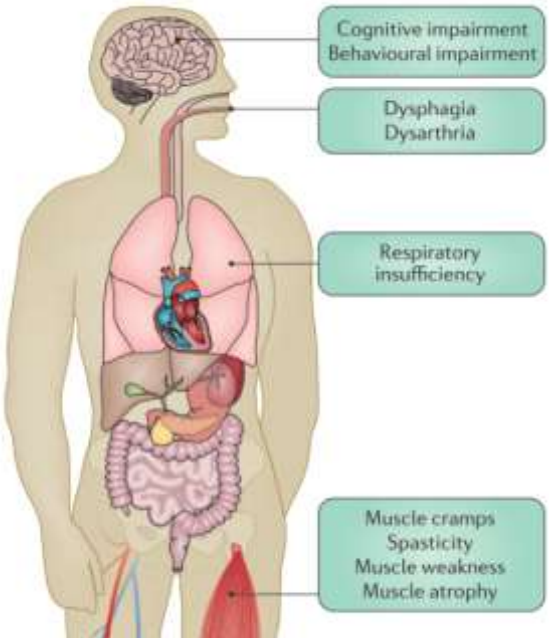

Figure 1. Clinical manifestations of ALS (Hardiman et al. 2017).

3.2 CAUSES OF AMYOTROPHIC LATERAL SCLEROSIS

Due to the rarity of ALS, basic research that allows the understanding of underlaying molecular mechanisms in ALS progresses slowly, so the causes of ALS are still poorly understood. However, the identification of some genetic and environmental factors related to increased risk of developing ALS provides some clues to decode its pathophysiology.

3.2.1 Genetic factors

More than 30 genes have been associated with the susceptibility to develop the disease (Table 2). Among all ALS cases, around 10 % has family history behind. Of these, 70% are linked with the mutations of four genes: *SOD1*, *C9orf72*, *TARDBP* and *FUS* (Hardiman et al. 2017).

SOD1 gene encodes for the Cu, Zn superoxide dismutase enzyme. Mutation of *SOD1* is present in the 20 % of familial cases of ALS (FALS), and can have both dominant and recessive inheritance, being the recessive the most common one. SOD1 is an antioxidant enzyme present in the outer mitochondrial membrane which catalyze the conversion of superoxide radicals to hydrogen peroxide and molecular oxygen (Kaur et al. 2016).

Hexanucleotide GGGGCC extension in *C9orf72* is by far the most common mutation, present in 40% of FALS cases. *C9orf72* gene codify for the C9orf72 protein, which is found in neurons cytoplasm acting as a guanine nucleotide exchange factor for small GTPases. Recent studies have shown that its main function is the regulation of autophagy process and endosomal traffic (Balendra and Isaacs 2018).

TARDBP gene encodes for TAR DNA-binding protein 43 (TDP43). The mutation is found in the 3% of FALS and around 1.5% of SALS cases. Even though the protein is ubiquitous in human tissues, large amounts have been localized in large motor neurons cytosol. With two RNA-recognition motifs and nuclear localization and export signal motifs in C-terminal extreme, the role of the protein is associated with the splicing and transport of mRNA as well as with the promotion of dendritic branching.

FUS gene codes for RNA- binding protein FUS. Mutations on FUS gene are found in 5% of FALS patients. Similarly to TDP43, FUS protein role is related to the RNA transcription, splicing and transport as well as with neuronal development processes.

With the ongoing MinE Consortium Project, the knowledge about the genetic factors that influence sporadic ALS is expected to increase, helping the overall understanding of the genetic background of the disease.

Table 2. Main genes implicated in amyotrophic lateral sclerosis (Hardiman et al. 2017)

Locus	Gene (protein)	Inheritance	Implicated disease mechanisms
ALS1	*SOD1* (superoxide dismutase 1)	AD or AR	Oxidative stress
ALS2	*ALS2* (alsin)	AR	Endosomal trafficking
ALS3	Unknown	AD	Unknown
ALS4	*SETX* (senataxin)	AD	RNA metabolism
ALS5	Unknown	AR	DNA damage repair and axon growth
ALS6	*FUS* (RNA-binding protein FUS)	AD or AR	RNA metabolism
ALS7	Unknown	AD	Unknown
ALS8	*VAPB* (vesicle-associated membrane protein-associated protein B/C)	AD	Endoplasmic reticulum stress
ALS9	*ANG* (angiogenin)	AD	RNA metabolism
ALS10	*TARDBP* (TAR DNA-binding protein 43)	AD	RNA metabolism
ALS11	*FIG4* (polyphosphoinositide phosphatase)	AD	Endosomal trafficking
ALS12	*OPTN* (optineurin)	AD or AR	Autophagy
ALS13	*ATXN2* (ataxin 2)	AD	RNA metabolism
ALS14	*VCP* (valosin-containing protein)	AD	Autophagy
ALS15	*UBQLN2* (ubiquilin-2)	XD	UPS and autophagy
ALS16	*SIGMAR1* (sigma non-opioid intracellular receptor 1)	AD	UPS and autophagy
ALS17	*CHMP2B* (charged multivesicular body protein 2B)	AD	Endosomal trafficking
ALS18	*PFN1* (profilin 1)	AD	Cytoskeleton
ALS19	*ERBB4* (receptor tyrosine-protein kinase erbB 4)	AD	Neuronal development
ALS20	*HNRNPA1* (heterogeneous nuclear ribonucleoprotein A1)	AD	RNA metabolism
ALS21	*MATR3* (matrin 3)	AD	RNA metabolism
ALS22	*TUBA4A* (tubulin α4A)	AD	Cytoskeleton
ALS-FTD1	*C9orf72* (guanine nucleotide exchange C9orf72)	AD	RNA metabolism and autophagy
ALS-FTD2	*CHCHD10* (coiled-coil-helix-coiled-coil-helix domain-containing 10)	AD	Mitochondrial maintenance
ALS-FTD3	*SQSTM1* (sequestosome 1)	AD	Autophagy
ALS-FTD4	*TBK1* (serine/threonine-protein kinase TBK1)	Unknown	Autophagy

AD, autosomal dominant; AR, autosomal recessive; UPS, ubiquitin–proteasome system; XD, X-linked dominant

3.2.2 Environmental factors

The information about the disease that can be extracted from clinical trials is quite limited since trials development is difficult due to the low incidence and because the studied cohorts are mainly focused on patients with better prognosis. Therefore, the data recorded in different registries are the most representative and complete source of information, taking into account all the different variants of the disease. Population-based registries are useful to display a picture of the different clinical phenotypes and provide information of incidence, prevalence, and risk factors.

In this sense, retrospective cohort studies based on these registers have been shown to be very helpful to establish a correlation between environmental factors and the developing of the illness and its progression (Hardiman et al. 2017).

Among all the environmental factors related to the disease (Table 3), smoking have been identified as a clear risk factors to develop ALS, and exposure to cyanotoxins and lead are likely other risk factors (Oskarsson, Horton, and Mitsumoto 2015).

Table 3. Environmental risk factors to develop ALS (Oskarsson et al. 2015).

Proposed Risk Factor	**Strength and Type of Evidence**	**Proposed Mechanism**
Smoking	Level A	Oxidative stress
US military service	Level B	Multiple
Lead	Level B	Neurotoxicity
Pesticides	Level B	Neurotoxicity
Physical activity	Level U	Physical fitness, early testosterone exposure
Head trauma	Level U	Direct neuronal injury
Electromagnetic radiation	Level U	Electromagnetic field
Low body mass index	Level U	Higher metabolism
Statin treatment	Level U	Altered lipid metabolism
BMAA	Level U	Neurotoxicity

BMMA: β-N-methylamino-L-alanine
Level A rating: Stablished risk factor; Level B rating: Probable risk factor (quite likely): Level U rating; It is unknown whether this is a risk factor or not.

3.2.3 Pathophysiology

Even though ALS has been classified as a neuromuscular disorder for many years, latest contributions in cognitive impairment suggest that its classification as a neurodegenerative disease is more accurate. Studies in genetic modified animals show some links between genetic and epigenetic factors with the phenotypic manifestations of the disease (Hardiman et al. 2017).

In a similar way as in other neurodegenerative diseases such as Parkinson's disease (PD) or Alzheimer's disease (AD), protein aggregation has been detected in motor neurons. Interestingly, despite the low frequency of mutations in TDP43 encoding gene, around 97 % of patients have cytoplasmic inclusions of TDP43 proteins. However, patients with *C9orf72* mutation have shown TDP43 aggregates outside the motor neurons. Even though TDP43 is the protein aggregate-constituent found in most cases, other protein aggregates have been detected, such as misfolded SOD1 in SOD1-related ALS (Deerlin et al. 2013). Therefore, progression of the disease may be associated with a prion-based propagation in the same way that it happens with Lewi's bodies or amyloid plaques in PD and AD, respectively.

Mutations in some ALS-related genes have shown to have impact in the protein homeostasis by disrupting the ubiquitin-proteasome system, chaperone proteins, and autophagy machinery (Urushitani et al. 2002). Decreased protein degradation, problems with protein folding and disruption of autophagy system may be the causes of the toxic aggregation of proteins in ALS.

Moreover, other mutations are related to genes involved in RNA metabolism that can lead to changes in gene transcription, pre-mRNA splicing, generation of microRNAs and DNA damage though the formation of R-loops (DNA-RNA hybrid structures) (Haeusler et al. 2016). The impairment observed in the DNA repair mechanism can aggravate the DNA damage.

Endosomal and vesicle transport is also dysregulated, interfering with the nucleus-cytoplasm transport. The disruption of a proper transport has been linked with the neurotoxicity and defects in neurotransmitters release (Devon et al. 2006).

The impairment of the excitatory amino acid transporter 2 (EAAT2), which is a glutamate reuptake transporter found in MN-associated astrocytes, has been reported in animal models and ALS patients. Due to the low buffering capacity and the increased permeability of motor neurons AMPA receptor, MN are more sensitive to glutamate-stimulated calcium entry. Therefore, inability to remove the glutamate from the synaptic space causes calcium-associated excitotoxicity in MN (Laslo et al. 2001).

Both ALS patients and mutant-SOD1 ALS mice models have shown to have oligodendrocyte degeneration. Due to its important role in metabolic axon support, oligodendrocyte impairment leads to neuron axonopathy. Ineffective mitochondria transport to axon region can also contribute to axonopathy. Mutant SOD1 can also form aggregates in mitochondrial inter-membrane space leading to disruption of protein transport with cytosol and respiratory chain (Ferraiuolo et al. 2016). Therefore, the loss of proper mitochondrial function increases the oxidative stress causing DNA damage.

Postmortem samples of patients with ALS and rodent models indicate inflammation damage in motor neurons. SOD1-transgenic mice studies suggest that the neurotoxic microglial phenotype (M1 phenotype) can be the cause of the neuroinflammation through the release of proinflammatory cytokines in latest stages of the disease (Liao et al. 2012). Therefore, most of ALS-related genes are involved in cellular processes such as autophagy, RNA metabolism, and endosomal traffic.

All exposed mechanism can trigger to an impairing of the axonal projections of the motor neurons. The axonal retraction in upper neurons causes the loss of control of the lower neurons while axonal retraction in lower motor neurons suppose their denervation with the muscle. Regardless the origin, the axonal retraction leads to a disruption of efferent transmission from central nervous system to effectors causing the muscular weakness and atrophy characteristic of the disease.

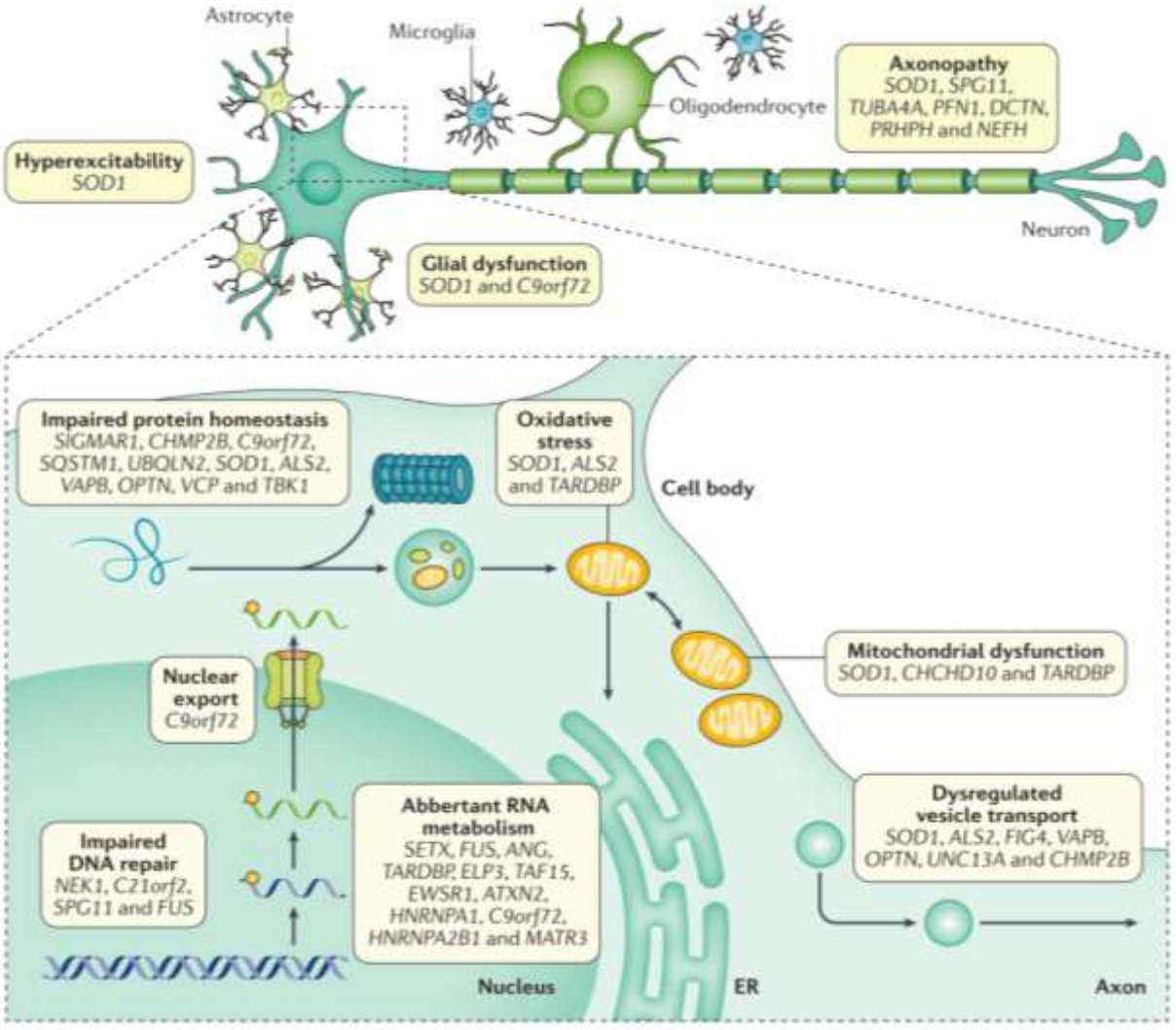

Figure 2. Pathophysiology of ALS (Hardiman et al. 2017).

3.2.4 Gut microbiota and neurodegeneration.

The term gut microbiota is defined as the microorganism population that inhabits the gastrointestinal tract, including different species from the *phylum* bacteria, archaea and fungi. Gut microbiota is involved in different roles, from fermentation of complexes carbohydrates to synthesis of vitamins B and K. The species that make up the diversity of the microbiota vary between individuals. There are many factors that influence its composition, including age, diet and exposure to antibiotics. The most common *phyla* of bacteria inhabiting in the human gut microbiota are *Firmicutes* and *Bacteroidetes.* Although previous definitions of microbiota highlighted its symbiotic role with the host, nowadays a distinction is made between the essential bacteria, which has a beneficial role to the host, and opportunistic bacteria, which is linked with negative effects in the host that can trigger different pathologies (Sarkar and Banerjee 2019). Main genus belonging to both groups are summarized in Table 4.

Table 4. Classification of bacterial genus according with their beneficial/opportunistic role in human gut microbiota (Roy Sarkar and Banerjee 2019).

Beneficial microbiota	**Opportunistic microbiota**
Lactobacteria	*Bacteroides*
Bifidobacterium	*Clostridia*
Enterococci	*Enterobacteria*
Propionobacteria	*Staphylococci*
Peptostreptococci	*Streptococci*

An imbalance that leads to increased population of opportunistic microorganism species is known as dysbiosis. It is linked with the pathogenesis of several disorders, including irritable bowel syndrome and colorectal cancer, among others. In latest years, many authors have reported the role of human gut microbiome in the development of different neurodegenerative diseases, including AD, PD, multiple sclerosis (MS), and ALS (Angelucci et al. 2019). The communication between gut microbiota and the brain has been named as “microbiota-gut-brain axis”, and it is carried out through microbe-derived neuroactive molecules that impact on different brain functions associated with many processes such as behavior, emotional state and stress reactivity.

A study in ALS mice model G93A SOD1 has reported a permeability disruption in gut and blood brain barrier, in the stage previous to the onset. Levels of ZO-1 protein, which is involved on

creating tight junctions in colon, were reduced in this model, increasing the permeability (Wu et al. 2015). The increased permeability can lead to increased levels in blood of bacterial components like lipopolysaccharide (LPS) and N-methylamino-L-alanine (BMAA), which have shown to have a role in ALS pathogenesis (Nguyen et al. 2004; Zhang et al. 2009)

BMAA is a neurotoxin produced by cyanobacteria. It is able to bind NMDA receptors as an antagonist of glutamate, causing neuroexcitatory effects that may be linked with the neuronal degeneration as described in 3.2.3 (Sarkar et al. 2019).

Increased levels of LPS in plasma triggers proinflammatory pathways in peripheral immune cells mediated by the binding to Toll-Like Receptor 4 (TLR4), which leads to the release of proinflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNFα). This pro-inflammatory environment is linked with the neuroinflammation reported in the pathophysiology of the disease (Zhang et al. 2009).

Microbiota dysbiosis with reduced levels of butyrate-producing bacteria has also been reported in studies in both transgenic G93A SOD1 mice model and ALS patients. Feces of mice model showed lower levels of the specie *Butyrivibrio fibrosolvens* while in ALS patient feces, the genus *Oscillibacter*, *Anaerostipes*, and *Lachnospira* were the most reduced ones (Sarkar et al. 2019).

Therefore, even though the role of gut microbiota in the disease is still poorly understood, the current evidences proves the existence of its relationship with the pathogenesis of the disease. Then, microbiota-based interventions could be raised as a promising therapeutic strategy to restore the eubiosis of ALS patients.

3.3 THERAPEUTIC STRATEGIES

The information of this section is extracted from Hardiman's review (Hardiman et al. 2017).

Due to the diversity of symptoms related with the disease, the management of ALS has to be carried out by different specialists including neurologists, pulmonologists, nutritionists, psychologists and rehabilitating physician, among others. The aim of the multidisciplinary approach is to increase the quality of life and prolongs the survival of ALS patients. The heterogeneity of this disease requires a personalized treatment, which combines both disease-modifying therapies and symptomatic treatments.

3.3.1 Disease-modifying therapies

The lack of knowledge of the molecular basis is the main obstacle to find an effective drug to modify the progression of the disease. Despite the research efforts to find drugs to slow the disease progression just two of them have shown to have some influence.

The first effective treatment identified was the Riluzole, a glutamate antagonist. Even though the mechanism of action is not completely clear, it is hypothesized to block the voltage-dependent sodium channels of presynaptic neurons modulating the calcium entry. Therefore, this drug is closely linked with the treatment of the suspected excitotoxicity of MN in the disease. Nowadays, Riluzole is prescribed to most of ALS patients. However, the extended survival related to Riluzole is just around three months, mainly among older patients with bulbar onset, and has not shown any impact on improving neither muscle strength nor quality of life.

Recent clinical trial has demonstrated the positive effects of Edaravone to attenuate patients deterioration. Edaravone is an antioxidant molecule (free radical scavenger) capable of crossing the blood-brain barrier. These conditions have been related with a neuroprotective effect on MN. However, slow progression effects of Edaravone are just proved in specific sector of patients, with early onset and fast progression. Therefore, the prescription of the drug for all ALS patients is still under discussion in Europe, whereas it is already approved in US and Japan.

3.3.2 Symptomatic treatments

Symptomatic management of ALS can be carried out with both pharmacological and non-pharmacological strategies, whose implantation has to be approved by each specialist doctor. The effects of the symptomatic treatments by randomized controlled trials in ALS patients has not been carried out, so their prescription is based on the management of the same symptoms in other diseases.

Spasticity is defined as the continuous contraction of skeletal muscles. Muscle relaxant drugs such as Baclofen and Tizanidine are prescribed in these cases. Muscle cramps have also been reported by around 25% of ALS patients, especially in those with spinal-onset. At the present time a phase II randomized trial is being carried out to assess the effects of Mexiletine treatment in ALS patients.

Sialorrhea is the increased production of saliva. Although most of the patients will present sialorrhea during the course of ALS, bulbar-onset ALS patients are affected by it at an early stage. The management of sialorrhea is based on anticholinergic drugs such as atropine, hyoscine and amitriptyline.

Patients commonly complain about nociceptive and neuropathic pain. Opioids and cannabis can be used to treat the nociceptive pain while tricyclic antidepressants are reserved for neuropathic pain. Tricyclic and other antidepressants are also used to treat depression episodes related with the mood alteration caused by ALS.

In addition to the depression, other mood alterations such as pseudobulbar affect are reported in the half of the patients. Pseudobulbar affection is characterized of uncontrollable episodes for crying or laughing and is treated with quinidine sold under prescription as Nuedexta®.

Problems with swallow capacity (dysphagia) are reported by two-thirds of bulbar-onset patients within two years of symptoms onset. For dysphagia, there is no pharmacological treatment. Consistence of the diet has to be decreased to help swallowing and avoid choking. As the problem progresses, enteral nutrition option by a gastrostomy tube has to be considered.

Dysarthria problems occur among more than 80% of the ALS patients, and can progress into a complete inability to speak. Speech therapy can be useful to slow the progression of dysarthria, but assisted speech devices, such as brain-computer interfaces, have to be considered in the last stages.

Despite the heterogeneity of all the aforementioned symptoms, the majority of ALS patients die because of respiratory insufficiency. In the advanced stages of the disease, muscle weakness affects the respiratory system, which hinders the ability to breathe properly (dyspnea). Acetylcysteine can be prescribed to reduce inflammation and mucus in the respiratory tract, improving respiratory skills. However, with increased respiratory weakness, invasive (IV) or non-invasive ventilation (NIV) should be applied to avoid respiratory failure and subsequent death.

Because the impact of most of the symptomatic treatments described above have not been evaluated on ALS patients, the effects of these drugs on the progression of the disease remains unknown.

As mentioned before, performance of clinical trials is complicated because of the low incidence of the disease. Additionally, clinical trials are often not inclusive of all the heterogeneous spectrum of ALS patients. Therefore, observational nationwide studies are the best tool to provide a first approach of the effects of symptomatic drugs in the progression of the disease, including all kind of ALS patients.

Then, based on the results obtained in the observational studies, clinical trials could be better designed by defining an adequate inclusion criteria, according to stratification of patients based on its pathophysiological traits, as it has been done with the Edaravone trial. In this way, the effects of medication on different types of ALS can be appreciated, avoiding them to be masked by misclassification issues.

4 INTEREST, HYPOTHESIS AND OBJECTIVES

At the present time, the lack of knowledge of the molecular bases of ALS is an obstacle to the development of disease-modifying therapies to slow down, stop or reverse the progression of the disease. Therefore, most of the management strategies of ALS are focused on improving the symptoms and face the new challenges that arise during the disease progression.

Drugs prescribed for the treatment of ALS symptoms are based on strategies followed to treat the same symptoms in other diseases, but their effect on ALS patient's survival specifically has not been tested. Therefore, in order to enlarger the life expectancy of ALS patients, it must be verified if the use of symptomatic medications has positive, negative, or neutral impact on their survival.

Consequently, the hypothesis of our work has been to demonstrate that early use of medication against ALS symptoms, in addition to symptoms management, could also have an impact on the survival of ALS patients.

Accordingly, our global goal has been:

- Identify the association between early prescription of medication against symptoms of ALS patients and their survival rates.

Secondary objectives:

- To provide an epidemiological profile of the Swedish MND patients, and a list of most prescribed medications for symptomatic treatment.
- Detect possible deficiencies in data collection and propose different suggestions to improve the quality of the registry.
- Design a suitable statistical study to assess the survival of ALS patients exposed to different medications in early stages of the disease.
- Based on the results of the analysis, hypothesize the underlying mechanisms that can lead to the positive or negative impact on ALS survival.

5 MATERIALS AND METHODS

We conducted a cohort study using data from the Swedish MND Quality Registry to determine the influence of different symptomatic treatments on ALS survival.

5.1 DATA SOURCE: THE SWEDISH MOTOR NEURON DISEASE QUALITY REGISTRY.

The Swedish Motor Neuron Disease Quality Registry is described in details elsewhere (Longinetti et al. 2018).

In brief, the Swedish MND Quality Registry was launched in Stockholm in 2015, following a retrospective study of the Stockholm area during 2013-2014. Since 2015, the registry collects the clinical data of patients diagnosed with MND, obtained during follow-up visits, which are programmed approximately every 12 weeks.

The registry includes patients diagnosed with definite, probable, possible or suspected ALS, based on El Escorial criteria (Table 1), progressive spinal muscle atrophy (PSMA), primary lateral sclerosis (PLS) and motor neuron disease (MND). Patients whose diagnosis changes during the progression of the disease to non MND diagnoses are removed from the MND Quality Registry.

A committee of senior ALS doctors from every university hospitals in Sweden were in charge of choosing variables collected in the registry, exceeding one hundred of them.

Among all variables contained in the registry, there are some mandatory variables that must be collected in a minimal dataset after each visit (Table 5). These mandatory variables are essential to provide a complete profile of the patient conditions, including the degree of progress of the disease and their emotional state.

Some variables can also be reported by the patients through the patient own report portal (PER). Patients receive a reminder to complete the questionnaires two weeks before the programed visit, so they can fill them out without time pressure. The collected data is imported to the MND registry once it is checked by the doctor or nurse in charge during the follow-up visits.

Table 5. Minimal dataset create at each clinic visit or contact, every 12 weeks (Longinetti et al. 2018).

Mandatory variables	Registered as
Motor dysfunction and progression pattern	ALSFRS-R (scale)
Bulbar dysfunction symptoms	Clinical signs, four questions
BMI	Weight and height
Anxiety and depression	HAD (scale)
Cognitive decline, emotional incontinence	Clinical signs, MoCA (scale)
Quality of Life	EQ-5D, LISAT-11
Hypoventilation symptoms	Clinical signs, four questions, lab, FVC%
Pain	VAS scale 0-10
New or ended treatments	Medical treatments, PEG, NIV/IV

ALSFRS-R: ALS functional rating scale (revised); BMI: Body mass index; EQ-5D: EuroQol five dimensions; HAD: Hospital anxiety and depression scale; IV: Invasive ventilation; LISAT-11: Life Satisfaction questionnaire; MoCA: Montreal cognitive assessment scale; NIV: Non-Invasive Ventilation; PEG: Percutaneous endoscopic gastronomy; VAS: Visual analog scale.

5.2 STUDY DESIGN

The aim of this study is to assess whether the exposure of the patients to symptomatic medications has an impact on the survival of ALS patients or not. To achieve this aim, we conducted a cohort study of ALS patients. Individuals diagnosed with ALS were identified using the Swedish MND quality registry, as of the 10th of April, 2019. Due to several problems related with the large amount of missing data, we restricted the study population to patients belonging to the Stockholm region whose data were more complete as compared to the other regions. We followed patients over time from ALS diagnosis to death, start of IV or last update of the register (10th of April of 2019), whichever came first. We considered IV placement as an outcome alternative to death because of the loss of autonomy.

Assessment of early exposure to symptomatic medication

Data of symptomatic medication was collected from the Swedish MND Quality Registry. In the registry, type, date of start, and date of end of each prescribed treatment is recorded. Due to the characteristic heterogeneity in terms of symptoms as well as the wide range of life expectancy, protective or harmful effects of symptomatic medication in highly advanced stages of the disease can be masked. We therefore decided to focus on patients exposed to the medication if this was prescribed during the early stages of the disease. We considered as early exposed patients that were exposed to the treatment within three months before or after the diagnosis date. Hence, the patients who are exposed after this period are considered non-exposed, together with the patients that have never been exposed.

Nonetheless, due to the average time from symptoms onset to diagnose is around a year and average survival of patients from symptoms onset is around 2-3 years, especially with patients with bulbar onset, three months after diagnosis can be already a quite advanced stage of the disease. For this reason, we adjusted the model for time from ALS diagnose which was the underling time-scale.

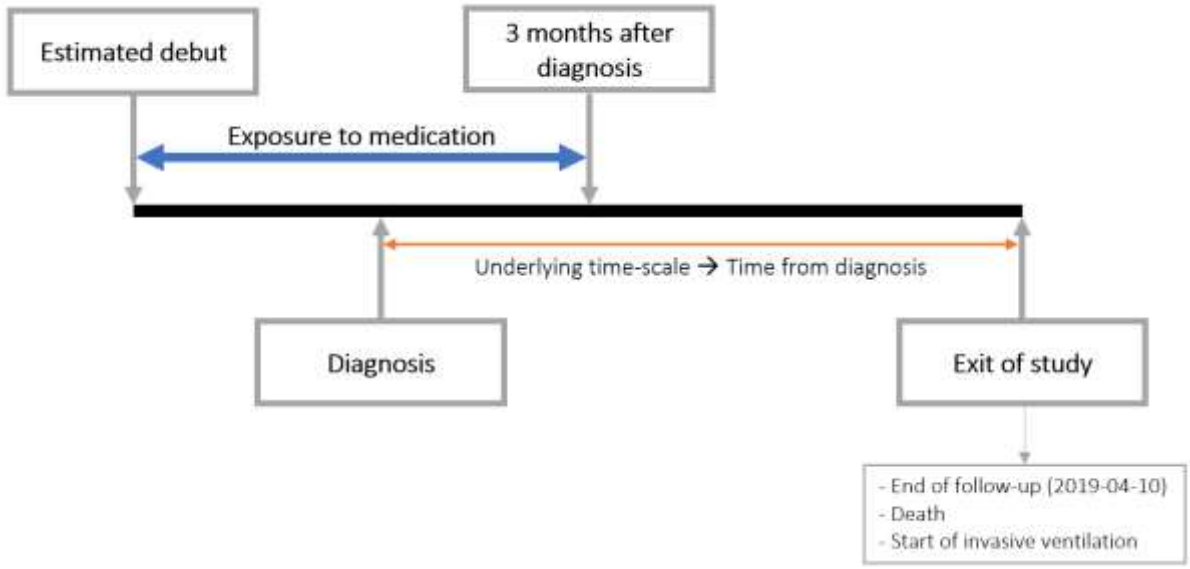

Figure 3. Schematic representation of the study design.

Statistical analysis.

In order to assess the association of early exposure to symptomatic medications and the risk of death or IV use, we fitted a Cox proportional hazard regression analysis to derive the hazard ratios (HRs) and their corresponding 95% confidence intervals (CIs). As we used time since diagnosis as the underlying time scale all the models were automatically adjusted for it. The assumption of hazard proportionality of the model was tested using Schoenfeld residuals.

To assess the independent effect of early exposure to symptomatic medications on survival we adjusted the model for the covariates: Sex (men vs women), age at diagnosis (continuous variable), site of symptoms onset (bulbar vs non-bulbar), diagnostic delay (continuous variable, in moths), body mass index (BMI) around diagnosis (continuous variable), revised ALS functional rate scale (ALSFRS-R) score around diagnosis (continuous variable) and progression rate (continuous variable).

Male gender, elder ages, bulbar onset, greater diagnoses delay, lower BMI, lower ALSFRS-R score and bigger progression rates are known to be associated with a worse disease prognosis.

Sex and site of onset are already provided as variables in the Swedish MND registry. Symptoms onset has been re-categorized to discern between two categories, bulbar and non-bulbar. Non-bulbar onset includes spinal, thoracic or dementia sites of onset. Age at diagnosis has been calculated by subtracting the date of birth to the date of diagnose, and expressed as a continuous variable in years. Diagnostic delay has been calculated by subtracting the date of estimated debut to the date of diagnose, and expressed a continuous variable in months. Several measurements of BMI and ALSFRS-R score are recorded for each patient, as they are variables that should be inserted in the minimal dataset at every follow-up visit. Because not all patients have a record of BMI and ALSFRS-R score at exactly the same time after diagnosis, it was considered suitable if the record belonged to an interval of three months before or after diagnose. The progression rate of the disease is a widespread measure to determine the prognosis. It is calculated form as (48 - ALSFRS-R score)/disease duration in months (Poesen et al. 2017). In our case, as we want to calculate the progression rate around diagnosis time, the ALSFRS-R score used is the one around diagnosis and the disease duration is equivalent to the time from symptoms onset to diagnosis (diagnosis delay). The progression rate could be categorized in three levels (slow, intermediate and fast) according with the distribution of all disease progression rate values. Disease progression is considered slow if the progression rate is situated below the 25 percentile, intermediate progression if it is distributed between 25 and 75 percentile and fast progression if the progression rate is found over the 75 percentile.

Missing data of the covariates BMI around diagnosis and ALSFRS-R score were estimated by multiple imputation using the Gaussian normal regression imputation method for continuous variables. Assigned values to missing data were assigned following the distribution of the other observations based on the predictor variables sex, site of onset, age at diagnosis and diagnose delay.

We considered statistically significant associations with a 2-sided p value ≤0.05. HRs and its CIs were plotted in a forest plot using the Excel 2016 software. All statistical analyses were performed with the statistical software for data science STATA 15.

6 RESULTS AND DISCUSSION

6.1 SWEDISH MOTOR NEURON DISEASE QUALITY REGISTRY – DESCRIPTIVE EPIDEMIOLOGY.

The Swedish MND quality registry is distributed in 19 excel files written in Swedish. The summary of reported information in each file is shown in Table 11 (annexes). As of the date of analysis, 10th of April of 2019, it contains a total of 969 patients visited at different hospitals across Sweden. Around 40% of these patients died during follow-up (N=404, 41.7%) and around 5% were on IV (N=32, 4.6%). The disease characteristics are displayed in Table 6.

Table 6. Characteristics of patients in Swedish motor neuron disease quality registry.

Categorical variables		
	N (Total N = 969)	%
Sex		
Female	437	45.1
Male	532	54.9
Diagnose		
ALS	647	66.8
MND	209	21.6
PLS	58	6.0
PSMA	55	5.7
Age at symptom onset (years)		
19-45	93	9.6
46-55	138	14.3
56-65	229	23.6
66-75	237	24.5
76-90	73	7.5
NA	199	20.5
Site of symptoms onset		
Bulbar	145	15.0
Spinal	294	30.3
Other [a]	80	8.3
NA	450	46.4
Cognitive impairment		
No	94	9.7
Yes – dementia	1	0.1
Yes – mild cognitive impairment	35	3.6
NA	839	86.8
Ever-use of NIV		
Yes	164	16.9
No/unknown	805	83.1
Ever-use of IV		
Yes	32	4.6

No/unknown	937	95.4
Ever-use of PEG		
Yes	195	20.1
No – other nutritional intervention[c]	95	9.8
No/unknown	679	70.1
Death during the follow-up		
Yes	404	41.7
No	565	58.3
Ever-exposed to medication		
Riluzole	649	67.0
Antidepressants	107	11.0
Acetylcysteine	39	4.0
Anticholinergics	69	7.1
Baclofen	35	3.6
Nuedexta ®	32	3.3

Continuous variables

	Median (IQR)	N (%)
Age at symptoms onset (years)	63 (53-69)	770 (79.5)
Age at diagnosis (years)	65 (55-71)	841 (68.8)
Diagnostic delay (months)	13 (8-23)	746 (77.0)
Survival after diagnose (months)	18 (10-32.5)	380 (39.2)
ALSFRS-R score around diagnosis [b]	39 (34-43)	207 (21.4)
BMI around diagnosis [b]		
Female	22.4 (20.3-25.6)	131 (13.5)
Male	24.3 (22.0-26.9)	151 (15.6)

[a] *Thoracic site of onset or dementia symptoms.*
[b] *Around diagnosis is considered between three months before and three months after diagnosis.*
[c] *Radiologically Inserted Gastronomy or special diet.*
ALS (Amyotrophic lateral sclerosis); MND (Motor neuron disease); PLS (Primary lateral sclerosis); PSMA (Progressive spinal muscle atrophy); IQR (Interquartile range); IV (Invasive ventilation); NIV (Non-invasive ventilation); NA (Non-available); BMI (Body mass index); ALSFRS-R (Amyotrophic lateral sclerosis functional rating scale-Revised; PEG (Percutaneous endoscopic gastronomy)

The most common diagnosis in the registry was ALS (N = 647, 66.8%), followed by MND (N=209, 21.6%), PLS (N=58, 6.0%) and PSMA (N=55, 5.7%).

The incidence of MND is slightly higher in men (N=532, 54.9%) than in women (N=437, 45.1%). This observation contrasts with the epidemiology studies performed in other countries of Europe, which report a clearly higher incidence in males, achieving around 50% increased ratio (Alonso et al. 2009). As other authors have pointed before, the similar incidence between both sexes found in Swedish MND patients may be related with sociological issues of the country (Longinetti et al. 2018). The increased gender equality achieved in Sweden allowed women to have a lifestyle traditionally associated to men, exposing them also to well known risk factors

such as smoking, physical exercise and occupational and environmental exposures (Ingre et al. 2015).

Even though almost half of record for site of symptom onset are missing, the incidence of spinal onset is twice more common (N=294, 30.3%) than bulbar onset (N=145, 15.0%). The two-fold increased incidence of spinal onset in comparison with bulbar onset is also reported in the patients belonging to the South-East England ALS (SEALS) register, but hypothesis to explain the incidence differences has never been raised yet (Hardiman et al. 2017).

The mean age at onset and the diagnostic delay of MND Swedish patients is consistent with data from the SEALS register, being in both around 63 years and 13 months, respectively.

The life expectancy of Swedish patients , which is 31 months (13 months of diagnosis delay + 18 months of survival after diagnosis) is consistent with the study conducted SEALS registry, which reports a life expectancy around 30 months from the symptoms onset.

Cognitive status was recorded for a total of 130 patients (13.4%), according to the MoCA scale (Chartier et al. 2015), a quarter of them shown mild signs of cognitive impairment (N=36, 27.7%), and only one patient was diagnosed with dementia. A large scale study in SALS patients performed in 2005 in Texas reported that about half of the patients had some degree of cognitive impairment, with 30% presenting mild symptoms and 20% diagnosed as dementia (Ringholz et al. 2005). According with these observations, cognitive impairment prevalence in Swedish MND patients is less than expected. These marked differences between both prevalence studies may be due to the cognitive assessment criteria of dementia used in both cases was not the same, leading to a misclassification problem, or due to the high percentage of unreported data in Swedish registry (N=839, 86,8%).

Regarding to the different therapies provided to patients, one-fifth of the patients have been treated with percutaneous endoscopic gastronomy (PEG) (N= 195, 20.1%), one-sixth NIV (N=164, 16.9%) and just one-twenty IV (N=32, 4.6%). The most prescribed medication in the Swedish registry was Riluzole (N=649, 67.0%), followed by antidepressants (N=107, 11.0%), anticholinergics (N=69, 7.1%), acetylcysteine (N=39, 4.0%), Baclofen (N35, 3.6%) and Nuedexta® (N=32, 3.3%). As there is no available information about prescribed medication in

other registries, prescription tendency of other countries cannot be compared with our results.

In contrast with the other files of the MND registry, the ones that contain the information regarding to therapies, including medication, nutrition (gastronomy) and ventilation, only have records if the patients have started any therapy related with these items. Therefore, it can lead to a problem of misclassification, as there is no way to discern weather the patients are not taking the treatment or they are taking the treatment but it is not recorded (missing values). The fact that the Riluzole prescription has fallen from the 85% reported in the update of January 20th, 2017 to around the 65% in this last update suspects the existence of a deficiency in treatment registration instead of lack of prescription.

As the Swedish MND quality registry was launched in Stockholm area at 2015 it can be thought that the quality of the data collection is not equal in all regions. Therefore, in order to verify the homogeneity of the quality of registered records, the presence of entries of most relevant variables and therapies where analyzed according to the different regions in Sweden (Table 7).

The patients from Stockholm area represent the vast majority of entries in Swedish MND registry (N = 390, 40.3%), followed by Uppsala-Örebro (N=148, 15.3%), Västsvenska (N=135, 13.9%), Södra (N=122, 12.6%), Norra (N=104, 10.7%) and Sydöstra (N=70, 3.2%).

Surprisingly, it was detected a high amount of missing values (MV) in the whole registry. With the exception of sex (0% MV), and the dates of diagnosis and estimated debut (< 25% MV), all other variables contain large percentages of MV (from 20.3% to 60.7% of MV) a. When the data is split by the different regions of Sweden, it can be appreciated that whereas Stockholm region contains around the 90% of records of each variable (except for the ALSFRS-R score, which contains 66.2% of records), the information is quite poor in the other regions. The lack of these basic variables impedes the proper inclusion of the patients in retrospective studies.

Moreover, when the information of the medication was divided by the different regions it was proved that around the 70% of the medication records were registered in Stockholm area while the other regions poorly contribute to the total amount of records or are practically inexistent, as is the case of Norra region. Therefore, it can be inferred that the low number of

cases is not due to a low prescription but a deficiency in recording system in the hospitals that do not belong to the Stockholm region. Due to this fact, the number of patients ever exposed to symptomatic medications is poorer than expected, which will have an impact in the statistic power of our study.

To fix the problem of lack of information about medication prescription and increase the quality of the registry, it has been suggested to perform a linkage of the Swedish MND quality registry with the Swedish Prescribed Drug Register (Lester 2009). The Swedish Prescribed Drug Register contains information about the patient identifiers and the drug utilization and expenditures in whole Sweden from 2005. This linkage would be very useful to show associations between drugs and MND not just in terms of survival, when drugs are prescribed to the patients because of the disease, but also to identify associations between drug prescription and risk of developing a MND.

However, as it is not possible to get access to the register in short term, the study was carried out with the data of the Swedish MND quality registry anyway, despite the low statistical power. Once the two databases are linked, the study could be repeated using the same design as the one proposed in this report and other medications that are not registered in the Swedish MND quality registry, as antibiotics, can also be tested.

Table 7. Geographical distribution of data collected Swedish Motor Neuron Disease Quality registry.

Area	Sweden		Swedish region					
			Norra	Stockholms region	Sydöstra	Södra	Uppsala-Örebro	Västsvenska
Number of patients (%)	969 (100)		104 (10.7)	390 (40.3)	70 (3.2)	122(12.6)	148(15.3)	135(13.9)
Variable	**Registered records**	**Missing values (MV)**	**Registered observations among patients in each region N (%)**					
Sex	969 (100)	0 (0)	104 (100)	390 (100)	70 (100)	122 (100)	148 (100)	135 (100)
Site of symptoms onset	519 (53.6)	450 (46.4)	19 (18.3)	368 (94.4)	11 (15.7)	28 (23.0)	39 (26.4)	54 (40.0)
Diagnose date	843 (87.0)	126 (13.0)	94 (90.4)	388 (99.5)	65 (92.9)	100 (82.0)	119 (80.4)	77 (57.0)
Estimated debut date	772 (79.7)	197 (20.3)	66 (63.5)	383 (98.2)	59 (84.3)	84 (68.9)	120 (81.1)	60 (44.4)
ALSFRS-R score	596 (61.5)	373 (38.5)	31 (29.8)	258 (66.2)	18 (25.7)	43 (35.2)	9 (6.1)	14 (10.4)
BMI	381 (39.3)	588 (60.7)	35 (33.7)	342 (87.7)	14 (20.0)	76 (62.3)	72 (48.6)	49 (36.3)
Medication	**Registered records**	**MV /No prescription**	**Registered observations among total registered records**					
Antidepressants	107	862	0 (0.0)	85 (79.4)	5 (4.7)	3 (2.8)	3 (2.8)	11 (10.3)
Acetylcysteine	39	930	2 (5.1)	27 (69.2)	5 (12.8)	0 (0.0)	0 (0.0)	5 (12.8)
Anticholinergics	69	900	0 (0.0)	50 (72.5)	4 (5.8)	3 (4.3)	6 (8.7)	6 (8.7)
Baclofen	35	934	0 (0.0)	27 (77.1)	1 (2.9)	1 (2.9)	2 (5.7)	4 (11.4)
Nuedexta®	32	937	1 (3.1)	26 (81.3)	0 (0.0)	1 (3.1)	3 (9.4)	1 (3.1)

6.2 SYMPTOMATIC MEDICATION AND ALS SURVIVAL.

Following the observations of the section 6.1, in order to obtain as most accurate results as possible, the study was performed using only the patient records from Stockholm region in the Swedish MND registry. Among all patients, we focused on patients with a diagnosis of ALS. During the data cleaning, we excluded three patients with data incongruences, two of them with a diagnosis date previous to the estimated onset date and one of them with a death date previous to the one of diagnosis. An additional patient was not included in the Survival because the IV (corresponding to the date of exit from this study) was placed prior to the date of ALS diagnosis (date of entry in this study). Therefore, the complete cohort for the study includes 322 ALS patients from Stockholm region (Figure 4). The relevant patient characteristics for the study, including covariates and outcome-associated variables, are shown in Table 8.

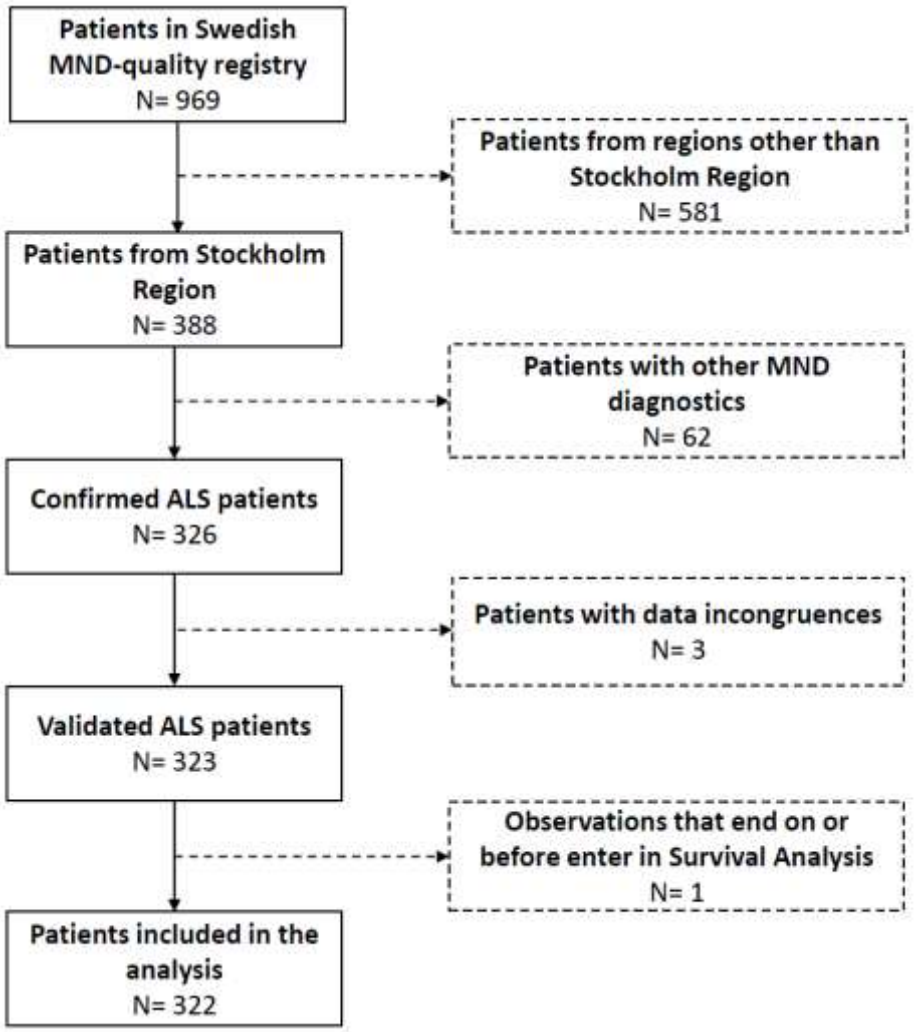

Figure 4. Flow chart of patients included in survival analysis after data cleaning.

Table 8. Characteristics of ALS patients from Stockholm region in Motor Neuron Disease quality registry associated with covariates and outcome variables used in the analysis.

Categorical variables		
	N	%
Sex		
Female	162	50.3
Male	160	49.7
Site of symptoms onset		
Bulbar	97	30.1
Non-bulbar	182	56.5
Other [a]	34	10.6
NA	9	2.8
Progression rate (categorized)		
Slow	40	12.4
Intermediate	81	25.2
Fast	35	10.9
NA	166	51.5
Death during follow-up		
Yes	163	50.6
No	159	49.4
Ever-use of invasive ventilation		
Yes	22	6.8
No/Unknown	300	93.2
Ever-exposed to medication		
Riluzole	279	86.6
Antidepressants	76	23.6
Acetylcysteine	23	7.1
Anticholinergics	45	14.0
Baclofen	14	4.3
Nuedexta®	25	7.8
Continuous variables		
	Median (IQR)	N (%)
Age at diagnosis (years)	65.5 (56-71)	322 (100)
Diagnostic delay (months)	12 (8-21)	321 (99.7)
BMI around diagnosis [b]	23.4 (21.1-26.7)	157 (48.8)
ALSFRS-R score around diagnosis [b]	38 (33-43)	156 (48.4)

[a] *Thoracic site of onset or dementia symptoms.*
[b] *Around diagnosis is considered between three months before andthreemonths after diagnosis.*
ALSFRS-R (Amyotrophic lateral sclerosis functional rating scale-Revised; BMI (Body mass index); IQR (Interquartile range); NA (Non-available).

The HR and its respective CIs for all symptomatic medications have been displayed in Table 9. Due to the availability of data related with the disease-modifying medication Riluzole, it has also been included in the analysis as a way to verify the accuracy of our study by comparing with previous survival studies made with Riluzole.

Although the percentages of patients ever-exposed to symptomatic medication were already quite low, the patients exposed up to three months after being diagnosed are even lower, only exceeding the 5% of total patients in the case of antidepressants.

The Cox model has been run without being adjusted (model 0) and adjusted for the covariates (model 1-7). In adjusted models, covariates were added one by one to the previous model in the order: sex (model 1), site of onset (model 2), age at diagnosis (model 3), diagnostic delay (model 4), BMI around diagnosis (model 5), ALSFRS-R score around diagnosis (model 6) and progression rate around diagnosis (model 7). Model seven is the full model adjusted for all the covariates.

Table 9. Associations of early exposure to medications with the risk of invasive ventilation or death.

Medication	Patients exposed N (%)	Model 0[a]	Model 1[a]	Model 2[a]	Model 3[a]	Model 4[a]	Model 5[a]	Model 6[a]	Model 7[a]
Antidepressants	42 (13.0%)	1.35 (0.89-2.04)	1.34 (0.89-2.03)	1.28 (0.85-1.94)	1.05 (0.69-1.58)	1.13 (0.75-1.71)	1.14 (0.75-1.73)	1.19 (0.79-1.82)	1.16 (0.76-1.78)
Acetylcysteine	8 (2.5 %)	0.75 (0.19-3.03)	0.74 (0.18-3.00)	0.61 (0.15-2.47)	0.49 (0.12-2.01)	0.81 (0.20-3.39)	0.84 (0.20-3.48)	0.78 (0.19-3.25)	0.82 (0.20-3.44)
Anticholinergics	16 (4.9 %)	2.38 (1.28-4.43)*	2.36 (1.26-4.39)*	1.44 (0.75-2.78)	1.16 (0.60-2.24)	1.14 (0.59-2.20)	1.14 (0.59-2.20)	1.16 (0.59-2.24)	1.12 (0.58-2.17)
Baclofen	5 (1.5 %)	0.99 (0.31-3.11)	0.96 (0.30-3.02)	0.93 (0.29-2.92)	0.89 (0.28-2.86)	0.86 (0.27-2.75)	0.87 (0.27-2.79)	0.85 (0.27-2.71)	0.80 (0.25-2.55)
Nuedexta®	15 (4.6 %)	0.88 (0.43-1.80)	0.88 (0.43-1.78)	0.83 (0.40-1.68)	0.75 (0.37-1.52)	0.70 (0.34-1.42)	0.69 (0.34-1.41)	0.69 (0.34-1.41)	0.66 (0.32-1.35)
Riluzole	267 (82.9%)	1.49 (1.00-2.24)*	1.50 (1.00-2.25)*	1.21 (0.87-1.99)	1.11 (0.74-1.68)	0.97 (0.64-1.46)	0.96 (0.63-1.46)	0.96 (0.64-1.45)	0.82 (0.54-1.28)

[a] Model: Exposure to medication within three months after diagnostic; Model 1: Adjusted for sex (men vs women); Model 2: Adjusted for sex and site of onset (bulbar vs non-bulbar); Model 3: Adjusted for sex, site of onset and age at diagnosis (continuous variable); Model 4: Adjusted for sex, site of onset, age at diagnosis and diagnostic delay (continuous variable, in months); Model 5: Adjusted for sex, site of onset, age at diagnosis, diagnostic delay and BMI around diagnosis; Model 6: Adjusted for sex, site of onset, age at diagnosis, diagnostic delay, BMI around diagnosis and ALSFRS-R score around diagnosis (continuous variable); Model 7: Adjusted for sex, site of onset, age at diagnosis, diagnostic delay, BMI around diagnosis, ALSFRS-R score around diagnosis and progression rate (continuous variable).
All models are automatically adjusted for time since diagnosis as the underlying time-scale.
*Statistically significant results ($p < 0.05$)
Assumption of proportional hazards tested using Schoenfeld residuals.
n/a – not applicable (there are not enough information for the model to calculate HR)

The only statistically significant differences are found in the case of anticholinergics, when they are not adjusted or adjusted by sex, showing around 2.5 fold increased risk of death or IV. Nonetheless, once it is adjusted by site of onset this association disappears, which makes sense if we take into consideration that patients with bulbar onset starts to have sialorrhea

problems before the patients with spinal onset, as well as a decreased life expectancy. Riluzole also seems to increase the risk around a 50% when it is not adjusted or adjusted only for sex, in the same way that anticholinergics do. Nevertheless, that negative effect on survival disappear when adjusted for all covariates, showing around the 20% of protective role against death or IV start. Although this result is not statistically significant it shows a protective tendency, as illustrated in Figure 6. Previous research on the protective effect of Riluzole on survival among ALS patients showed a similar reduction of approximately 20-30% of death risk (Rooney et al. 2013).

Due to the width of the CIs (Figures 5 and 6) caution is needed when interpreting the association of early exposure to symptomatic medications and risk of death or IV. Bibliography-based proposal of underlying mechanism of antibiotics in ALS pathophysiology.

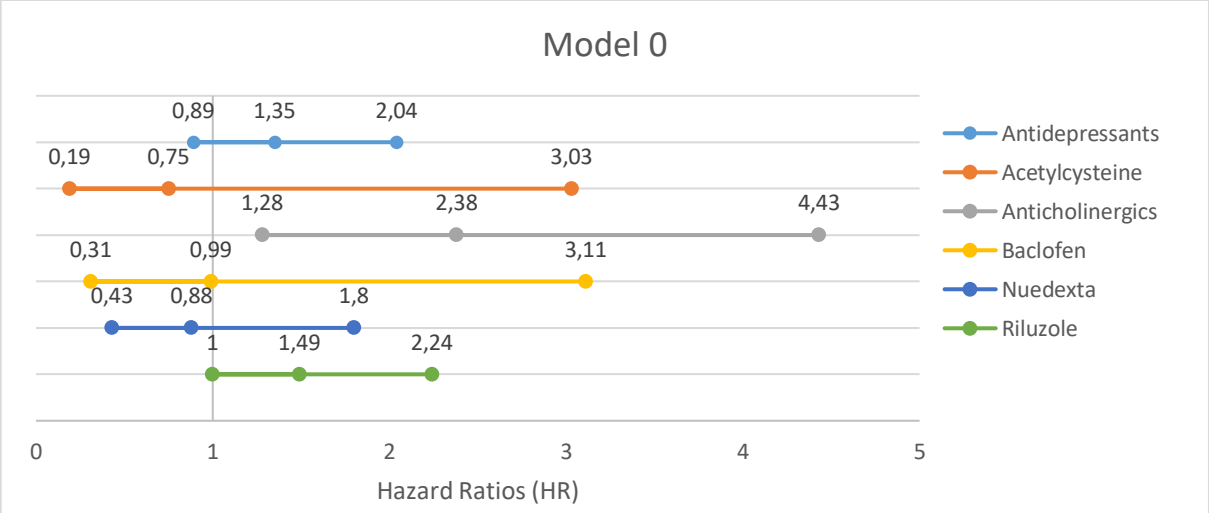

Figure 5. Forest plot with HRs and CIs to each medication.

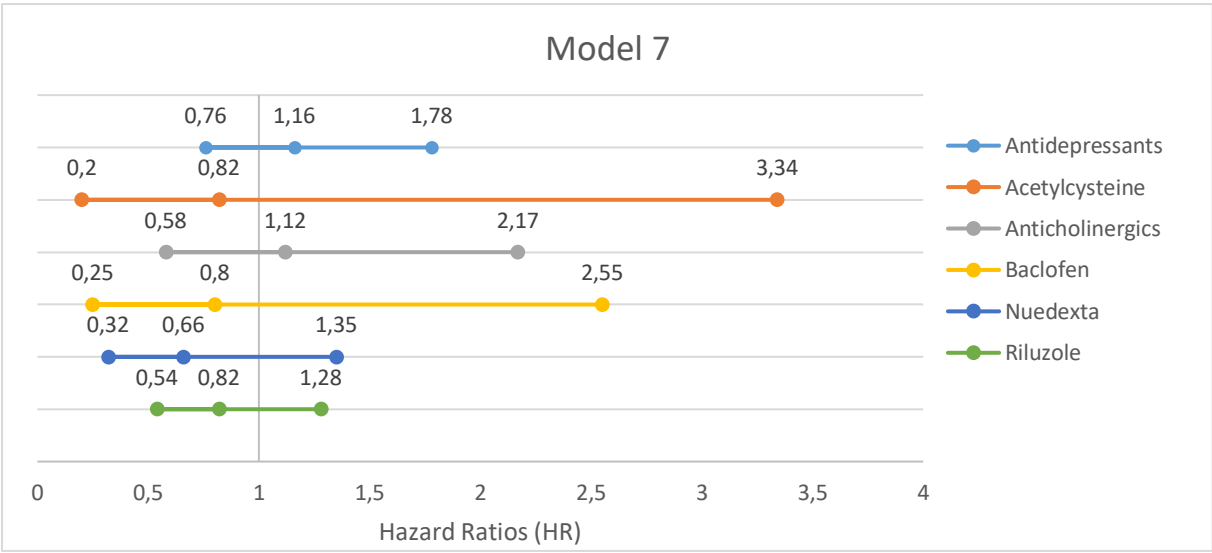

Figure 6. Forest plot with HRs and CIs to each medication once the model is adjusted by all covariates.

6.3 BIBLIOGRAPHY-BASED PROPOSAL OF UNDERLYING MECHANISM OF ANTIBIOTICS IN ALS PATHOPHYSIOLOGY.

Due to the lack of information of antibiotic prescription in the Swedish MND Quality Registry, the study of antibiotic impact on ALS survival could not be performed as originally planned. Even though it was tried to check the impact of other medications that were included in the registry on ALS survival, the results do not show even a tendency of beneficial or harmful effects so it is not possible to propose the underlying mechanism associated with the effects of these medication on survival. However, as it has been mentioned in section 3.2.4, there is a growing evidence that gut microbiota dysbiosis may have a role in ALS pathogenesis. Therefore, the use of antibiotics, which are prescribed to ALS patients mostly when they start to use IV and NIV, may have an impact in gut microbiota population and, consequently, have an impact in ALS survival. Then, a proposal of underlying mechanism of antibiotics in ALS pathophysiology is going to be proposed in this section, based on a review of recent studies related with microbiota and antibiotics in ALS.

Although antibiotic prescription is normally used to manage infections of specific pathogenic bacteria, they may target other species of the gut microbiota composition reducing its biodiversity. In this sense, broad-spectrum antibiotics are the most damaging ones.

During the development of the disease, mostly in the latest stages, when respiratory problems appears, ALS patients are prescribed with antibiotics to avoid complications related with the use of IV and NIV. In this case, the use of antibiotics is focused on a prevention measure related with a symptom of the disease. However, as it has been mentioned before, the antibiotic intake does not just affect the targeted bacteria, but also the ones present in the gut microbiota. Therefore, this symptomatic medication may have an impact on the patient's survival through modification of gut population by antibiotic prescription.

A study that analyzed fecal material of transgenic ALS mice model G3 SOD1 has shown a decreased number of butyrate-producer bacterial genus *Butyrivibrio* and *Peptostreptococcus* (Fang 2016). In other study, G3 SOD1 transgenic mice that were fed with 2% butyrate water had an onset delay in comparison with control mice (Zhang et al. 2017). The fact that butyrate supply caused an onset delay suggests that the lack of butyrate-producer species and,

consequently, the decrease of butyrate metabolite in plasma is associated with the development of the disease. *Oscillibacter*, *Anaerostipes,* and *Lachnospira* are some of the bacteria species that are described to be reduced in fecal human ALS samples. Therefore, it is worth to think that antibiotics that decrease butyrate-producer bacteria population will have a negative impact on the survival, whereas those who eliminate other species that compete with them for adhering to intestinal mucosa or epithelium may have a positive impact on the patients survival by increasing the possibilities of enhance the population of butyrate-producing bacteria.

Recently, in a study of antibiotics use on Swedish ALS patients, it has been demonstrated that exposure to antibiotics is a risk factor to develop the disease (Sun et al. 2019). The authors stratified exposure to antibiotics in four groups; 0, 1, 2-3, or ≥4 prescriptions and reported higher risk of ALS with increasing antibiotic prescriptions, specially 1 year before diagnosis. Additionally, they studied primary and secondary exposure to the different individual antibiotics, including tetracycline (J01AA), penicillin with extended spectrum (J01CA), beta-lactamase sensitive penicillin (J01CE), cephalosporin (J01DB and J01DC), Trimethoprim (J01EA and J01EE), Macrolides (J01FA), and Fluoroquinolanes (J01MA). Conclusions of this part of the study showed that beta-lactamase sensitive penicillin presented higher correlation with enhanced risk, increasing specially with more than two prescriptions.

The beta-lactamase sensitive penicillin is a type of penicillin antibiotics which is susceptible to the action of the beta-lactamase enzymes produced by bacteria. Penicillin antibiotics belong to the group of beta-lactam antibiotics, that contains a beta-lactam ring in their structure. The action of beta-lactamase is able to break through hydrolysis that ring, providing resistance to the bacteria. Therefore, bacteria that produces beta-lactamase are not susceptible to this antibiotic. Mechanism of action of the penicillin consist in inactivate the transpeptidase enzyme, disrupting the biosynthesis of the bacterial cell-wall. Therefore, the penicillin is mostly effective against gram positive bacteria, as they have the peptidoglycan cell-wall exposed in the last layer of bacterial covers.

Among all butyrate-producing bacteria, the major species/groups isolated from the human colon are displayed in Table 10 (Louis and Flint 2009). As it can be appreciated in Table 10, all these butyrate-producer bacteria belong to the phylum Firmicutes, and has gram-positive cell-

wall. Therefore, the relationship between consumption of penicillin and an increased risk of developing ALS may be due to the impact of that antibiotic on the butyrate-producer bacteria, which leads to a decrease of butyrate metabolite.

Table 10. Summarize of butyrate-producer bacterial species/groups isolated from human colon (Louis and Flint 2009).

Species/groups	Phylum	Cell-wall type
Eubacterium rectale	Firmicutes	Gram-positive
Eubacterium ramulus	Firmicutes	Gram-positive
Eubacterium cylindroides	Firmicutes	Gram-positive
Roseburia intestinalis	Firmicutes	Gram-positive
Roseburia faecis	Firmicutes	Gram-positive
Roseburia hominis	Firmicutes	Gram-positive
Roseburia inulinivorans	Firmicutes	Gram-positive
Butyrivibrio fibrisolvens	Firmicutes	Gram-positive
Anaerostipes caccae	Firmicutes	Gram-variable
Coprococcus catus GD/7	Firmicutes	Gram-positive
Coprococcus eutactus L2-50	Firmicutes	Gram-positive
Coprococcus comes A2-232	Firmicutes	Gram-positive

In another study, gut microbiota of ALS patients was compared with healthy population through a high-throughput sequencing. Results showed decreased ratio *Firmicutes/Bacteroidetes*, with an increased population of harmful bacteria of the genus *Dorea* and decreased population of beneficial bacteria of the genus *Oscillibacter, Anaerostipes, and Lachnospiraceae* being the two last ones butyrate-producer bacterial genus (Louis and Flint 2009; Zhang et al. 2018). These results also agree with our hypothesis that links the decrease of butyrate-producer bacteria with the increased risk of ALS.

Therefore, it could be expected that use of penicillin and other types of antibiotics that has a negative impact on the butyrate-producer bacteria has a bad impact on the survival of ALS patients. A study with a similar design to the one performed for other symptomatic medications could be useful to provide more information about the relationship between antibiotics and ALS survival. When it is known which bacterial species or derived metabolism products are linked with a beneficial or harmful effect, gut microbiota interventions may be considered to restore the eubiosis, trough fecal transplants or prescription of antibiotics, prebiotics and/or probiotics.

7 CONCLUSIONS

1. Regarding to Swedish MND quality registry, some deficiencies have been detected during the design of the models that limit the quality of the obtained results and its veracity. Therefore, we suggest to improve the quality of the registry as following:

- Clinicians and healthcare providers should be encouraged to properly register all the mandatory variables in the Swedish MND quality registry to properly aid future research studies.
- A linkage between Swedish MND quality registry and Swedish Prescribed Drug Register should be performed to obtain more accurate information about medication prescribed to ALS patients and, therefore, increase the quality of the registry.

2. Although no molecular underlying mechanisms that associate prescription of symptomatic medication with ALS survival could have been hypothesized based on the performed statistical analysis, some hypothesis can be extracted based on the bibliographic research regarding to antibiotics:

- Prescription of antibiotics as symptomatic treatment to avoid respiratory complications may have an impact on ALS patients survival through alteration of gut microbiota population.
- Antibiotics that target butyrate-producing bacteria (broad-spectrum, gram positive or narrower spectrum ones) may be associated with worse ALS prognosis
- Microbiota-modifying interventions based on antibiotic prescription may increase ALS survival by decreasing competitive exclusion of butyrate-producing bacteria with other damaging species susceptible to the prescribed antibiotic.

8 FUTURE APPLICATIONS

The fact that the performed analysis has not provided any useful information to determine if the symptomatic therapies have an impact on the survival of ALS patients has not been a problem of the study design itself, but a problem related to the lack of data in the Swedish MND Quality Registry. Therefore, the same statistical model could be run in the future to provide more accurate information, not just about the impact of antidepressants, anticholinergics, acetylcysteine, Baclofen or Nuedexta®, but also other prescribed drugs, such as antibiotics.

At it has been explained in sections 3.2.4 and 6.3, gut microbiota is linked with the pathophysiology of the disease. Therefore, therapies that has influence in the microbiota composition, such as antibiotic usage, could modify through molecular underlying mechanisms the progression of the disease and, consequently, have an impact on the survival of ALS patients.

9 SELF-ASSESSMENT

Although the biotechnology bachelor has different subjects to provide wide knowledge about cellular and molecular bases of biology (i.e. Cell biology), applications (i.e. bioreactors engineering), and tools to perform critical interpretations of scientific research (i.e. molecular microbial biotechnology), the biostatistical knowledge remains pushed into the background.

Since the heyday of omics sciences, the amount of biological data has increased exponentially, so basic knowledge of classical statistics is not enough to satisfy the requirements that these amounts of data need to be properly processed and interpreted. Due to I perceived as a limitation for a scientific career, I decided to expand my knowledge in biostatistics being part of the Medical Epidemiology and Biostatistics department of the Karolinska Institutet.

Although the planned investigation was about the impact of antibiotic use in ALS patients, several problems with the registry did not allow to obtain the required data for the study, so the topic of the thesis had to be changed. The original idea was to provide the knowledge in microbiology acquired during the biotechnology bachelor to suggest an underlying mechanism linked with the role of gut microbiome to explain the statistical results obtained.

With this topic, I wanted to achieve two main objectives: 1. Learn about the design, execution, and interpretation of multivariate biostatistics with large amounts of data, using a software of data management based on programming language (STATA in this case); 2. Provide my biotechnologist point of view to this research.

Therefore, in order to keep my both main objectives we decided to perform the statistical analysis with other medications (although the data quality was poor) and provide an epidemiology description of Swedish patients to increase the skills in the software use and then provide my biotechnological input with a bibliographic based discussion of the original topic.

All in all, although the original idea could not be performed as planned (which use to happen a lot in scientific research world, so it is also good to experience) I think the unforeseen has been handled well enough to achieve the goals so I am satisfied with the final result of the thesis.

10 PERSONAL ACKNOWLEDGMENTS

I would like to show my gratitude to:

Elisa Longinetti, for being the person that has always been on the front line with me. Thank you, Elisa, for teaching me so many things, for helping me whenever I needed it and for doing all this with patience and a kind smile. I really wish you the best, with your doctoral thesis and with everything because you really deserve it.

Fang Fang, for the trust she placed in me to help her in this research and all the facilities to stay in Stockholm. Thank you, Fangfang, for given me that chance despite being a bachelor student, it is been a pleasure to be part of your team during these months.

Dania Garcia for being my bachelor thesis tutor. Thank you, Dania, for guiding me to give the proper shape to this thesis and all the good and reassuring advices.

Andrea Estradera and **Iratxe Saiz,** for their invaluable moral support from Spain during my entire internship. Thank you, girls, for all the strength you have sent me when I needed it the most. This thesis couldn't have been possible without you… I love you both so much! <3

Ailema Gonzalez, for being the best office partner I could have had. Thank you for all the good advices you have given me, in scientific and personal life. I would wish you good luck with your doctoral thesis, but you don't need it… You have earned all the good things that happen to you. It is been a pleasure for me to stay by your side these four moths!

All the **workmates of MEB** for the nice work environment. Thank you very much for making this experience more enjoyable and enriching, I have learnt a lot from you all!

All the **friends** who have given me moral support during these four months in Sweden. Thank you very much, not just for being a good source of inspiration and escape route on many occasions, but for making me see how important it is to be surrounded by people like you.

Last, but not least, my **family**, especially to my father and stepmother. Thank you for being my daily motivation to start and keep working on this research, and for being such an amazing example of strength in the face of adversity.

11 BIBLIOGRAPHY

Al-Chalabi, A., Hardiman, O., Kiernan, MC., Chiò, A., Rix-Brooks, B., and Van Den Berg, LH. 2016. "Amyotrophic Lateral Sclerosis: Moving towards a New Classification System." The Lancet Neurology 15(11):1182–94.

Alonso, A., Logroscino, G., Jick, SS., and Hernán, MA. 2009. "Incidence and Lifetime Risk of Motor Neuron Disease in the United Kingdom: A Population-Based Study." *European Journal of Neurology* 16(6):745–51.

Angelucci, F., Cechova, K., Amlerova, J., and Hort, J. 2019. "Antibiotics, Gut Microbiota, and Alzheimer's Disease." *Journal of Neuroinflammation* 16(1):108.

Balendra, R., and Isaacs, AM. 2018. "C9orf72-Mediated ALS and FTD: Multiple Pathways to Disease." *Nature Reviews Neurology* 14(9):544–58.

Bourke, SC. and Gibson, GJ. 2010. "Amyotrophic Lateral Sclerosis." *Non-Invasive Ventilation and Weaning: Principles and Practice* 390(10107):351–60.

Chartier, M., Crouch, PC., Tullis, V., Catella, S., Frawley, E., Filanosky, C., and Wong, JK. 2015. "The Montreal Cognitive Assessment." *Journal of the International Association of Providers of AIDS Care (JIAPAC)*, *14*(3), 197–201.

Chiò, A., Logroscino, G., Hardiman, O., Swingler, R., Mitchell, D., Beghi, E., and Traynor, BG. "Prognostic Factors in ALS: A Critical Review." *Amyotrophic Lateral Sclerosis* 10(5–6):310–23.

Deerlin, V., Leverenz, JB., Bekris, LM., Bird, D., Yuan, W., Elman, LB., Clay, D., Wood, M., Chen-plotkin, AS., Martinez-lage, M., Mccluskey, L., Grossman, M., and Neumann, M. 2013. "TARDBP Mutations in Amyotrophic Lateral Sclerosis with TDP-43 Neuropathology: A Genetic and Histopathological Analysis." *Lancet Neurology* 7(5):409–16.

Devon, RS., Orban, PC., Gerrow, K., Barbieri, MA., Schwab, C., Cao, LP., Helm, JR., Bissada, N., Cruz-Aguado, R., Davidson, TL., Witmer, J., Metzler, M., Lam, CK., Tetzlaff, W., Simpson, EM., McCaffery, JM., El-Husseini, AE., Leavitt, BR., and Hayden, MR. 2006. "Als2-Deficient Mice Exhibit Disturbances in Endosome Trafficking Associated with Motor Behavioral Abnormalities." *Proceedings of the National Academy of Sciences* 103(25):9595–9600.

Fang, X. 2016. "Potential Role of Gut Microbiota and Tissue Barriers in Parkinson's Disease and Amyotrophic Lateral Sclerosis." *International Journal of Neuroscience* 126(9):771–76.

Ferraiuolo, L., Meyer, K., Sherwood, TW., Vick, J., Likhite, S., Frakes, A., Miranda, CJ., Braun, L., Heath, PR., Pineda, R. Beattie, CE., Shaw, PJ., Askwith, CC., McTigue, D., and Kaspar, BK. 2016. "Oligodendrocytes Contribute to Motor Neuron Death in ALS via SOD1-Dependent Mechanism." *Proceedings of the National Academy of Sciences* 113(42):E6496–6505.

Haeusler, AR., Donnelly, CJ. and Rothstein, JD.2016. "The Expanding Biology of the C9orf72 Nucleotide Repeat Expansion in Neurodegenerative Disease." *Nature Reviews Neuroscience* 17(6):383–95.

Hardiman, O., Al-Chalabi, A., Brayne, C., Beghi, E., Van Den Berg, LH., Chio, A., Martin, S., Logroscino, G., and Rooney, J. 2017. “The Changing Picture of Amyotrophic Lateral Sclerosis: Lessons from European Registers.” *Journal of Neurology, Neurosurgery and Psychiatry* 88(7):557–63.

Hardiman, O, Al-Chalabi, A., Chio, A., Corr, EM., Logroscino, G., Robberecht, W., Shaw, PJ., Simmons, Z., and Van Den Berg, LH. 2017. “Amyotrophic Lateral Sclerosis.” *Nature Reviews Disease Primers* 3:17071.

Ingre, C., Roos, PM., Pierl, F., Kamel, F., and Fang, F.. 2015. “Risk Factors for Amyotrophic Lateral Sclerosis.” *Clinical Epidemiology* 7:181–93.

Kaur, SJ., McKeown, SR., and Rashid, S. 2016. “Mutant SOD1 Mediated Pathogenesis of Amyotrophic Lateral Sclerosis.” *Gene* 577(2):109–18.

Laslo, P., Lipski, J., Nicholson, LFB., Miles, GB., and Funk, GD. 2001. “GluR2 AMPA Receptor Subunit Expression in Motoneurons at Low and High Risk for Degeneration in Amyotrophic Lateral Sclerosis.” *Experimental Neurology* 169(2):461–71.

Lester, D. 2009. “The new Swedish Prescribed Drug Register—Opportunities for pharmacoepidemiological research and experience from the first six months.” *Clinical Neuropsychiatry*, *6*(5), 188–191.

Liao, B., Zhao, W., Beers, DR., Henkel, JS., and Apple, SH. 2012. “Transformation from a Neuroprotective to a Neurotoxic Microglial Phenotype in a Mouse Model of ALS.” *Experimental Neurology* 237(1):147–152.

Longinetti, E., Ronnevi, LO., Zachau, A., Samuelsson, K., Ingre, C., Andersen, PM., Hillert, J., Kierkegaard, M., Fang, F., Press, R. and Regodón, A. 2018. “The Swedish Motor Neuron Disease Quality Registry.” *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration* 19(7–8):528–37.

Louis, P., and Flint, HJ. 2009. “Diversity, Metabolism and Microbial Ecology of Butyrate-Producing Bacteria from the Human Large Intestine.” *FEMS Microbiology Letters* 294(1):1–8.

Nguyen, MD., Aigle, TD., Julien, JP., and Rivest, S. 2004. “Exacerbation of Motor Neuron Disease by Chronic Stimulation of Innate Immunity in a Mouse Model of Amyotrophic Lateral Sclerosis.” 24(6):1340–49.

Oskarsson, B., Horton, DK., and Mitsumoto, H. 2015. “Potential Environmental Factors in Amyotrophic Lateral Sclerosis.” *Neurologic Clinics* 33(4):877–88.

Poesen, K., Schaepdryver, M., Stubendorff, B., Gille, B., Muckova, P., Wendler, S., Damme, P. Van. 2017. "Neurofilament markers for ALS correlate with extent of upper and lower motor neuron disease." *Neurology*, *88*(24), 2302–2309.

Ringholz, GM., Appel, SH., Bradshaw, M., Cooke, NA., Mosnik, DM., and Schulz, PE. 2005. “Prevalence and Patterns of Cognitive Impairment in Sporadic ALS.” *Neurology* 65(4):586–590

5p.

Rooney, J., Byrne, S. Heverin, M., Corr, B. Elamin, M., Staines, A., Goldacre, B., and Hardiman, O. 2013. “Survival Analysis of Irish Amyotrophic Lateral Sclerosis Patients Diagnosed from 1995-2010.” *PLoS ONE* 8(9).

Sakar, SR., and Banerjee, S. 2019. “Gut Microbiota in Neurodegenerative Disorders.” *Journal of Neuroimmunology* 328:98–104.

Sun, J., Zhan, Y., Mariosa, D., Larsson, H., Almqvist, C., Ingre, C., Zagai, U., Pawitan, Y., and Fang, F. 2019. “Antibiotics Use and Risk of Amyotrophic Lateral Sclerosis in Sweden.” *European Journal of Neurology* ene.13986.

Tiryaki, E., and Horak, HA. 2014. “ALS and Other Motor Neuron Diseases.” *CONTINUUM Lifelong Learning in Neurology* 20(5):1185–1207.

Urushitani, M., Kurisu, J., Tsukita, K., and Takahashi, R. 2002. “Proteasomal Inhibition by Misfolded Mutant Superoxide Dismutase 1 Induces Selective Motor Neuron Death in Familial Amyotrophic Lateral Sclerosis.” *Journal of Neurochemistry* 83(5):1030–42.

Wu, S., Yi, J., Zhang, YG., Zhou, J., and Sun, J. 2015. “Leaky Intestine and Impaired Microbiome in an Amyotrophic Lateral Sclerosis Mouse Model.” *Physiological Reports* 3(4):1–10.

Zhang, J, Song, L., Wang, Y., Liu, C., Zhang, L., Zhu, S., Liu, S., and Duan, L. 2018. “Beneficial Effect of Butyrate-producing Lachnospiraceae on Stress-induced Visceral Hypersensitivity in Rats.” *Journal of Gastroenterology and Hepatology* jgh.14536.

Zhang, R., Miller, RG., Gascon, R., Champion, S., Katz, J., Lancero, M., Narvaez, A., Honrada, R., Ruvalcaba, D., and McGrath, MS. 2009. “Circulating Endotoxin and Systemic Immune Activation in Sporadic Amyotrophic Lateral Sclerosis (sALS).” *Journal of Neuroimmunology* 206(415):121–24.

Zhang, YG., Wu, S., Yi, J., Xia, Y., Jin, D., Zhou, J., and Sun, J. 2017. “Target Intestinal Microbiota to Alleviate Disease Progression in Amyotrophic Lateral Sclerosis.” *Clinical Therapeutics* 39(2):322–36.

12 ANNEXES

Table 11. Summary of reported information in Swedish MND quality registry.

EXCEL FILE	REPORTED INFORMATION
ADF	Includes the overall score, the individual answers to different questions related with anxiety and depression and the date of data collection.
ALSFRS-R	Contains the data regarding to the ALSFRS-R scale. It includes the individual grading (0-4) to each of the 12 evaluated parameters (salivation, dyspnea, manuscript ability, etc.), the final score (0-48) and the date of data collection.
TREATMENTS	Includes the prescribed medication, start and last day of therapy, treatment time, therapy end cause and comments.
BMI	Includes the height, weight, BMI and the date of data collection.
DIAGNOSE	Includes diagnose date, estimated debut date, diagnose (ALS, PSMA, MND…)
SELF-REPORTED MEDICATION	Includes the own medication and comments of the patients (this document do not follow any standard rule to report the medication)
EQ-5D	The EQ-5D score is a measure of the global health condition. Includes the total score and the individual score of the variables pain/discomfort, anxiety/depression, mobility, usual activities and hygiene/self-care
GASTRONOMY	Includes the information regarding to the nutritional intervention of patients: nutrition method (ex. PEG), if referral or not to dietician, start and end of ALS diet and comments of the doctor.
HAD	The HAD is the hospital anxiety and depression scale. It includes the overall score (0-42), the anxiety scale (0-21), the depression scale (0-21), the individual answers to all the questions and the date of data collection.
CONTACT	Includes variables related with the need of personal assistance, such as dementia, urine incontinence, quality of sleep, emotional incontinence value, among others.
LABORATORY	Contains the values of several blood biomarkers (CRP, albumin, etc), genetic mutations (SOD1, FUS, C9orf72, TAU) and other clinical examinations (sensory neurography, muscle biopsy, etc)
LISAT	LISAT is the Life Satisfaction scale. It includes the overall score (0-66), the individual answers to all the questions and the date of data collection.
PATIENT	It includes diverse data of patient, including medical history (hemorrhage, head trauma, Parkinson, fractures…), familiar medical considerations, physic activity, profession, etc.
MOCA	MoCA is the Montreal cognitive assessment score, used to assess frontal cognitive impairments. It includes the overall score (0-66), the individual answers to all the questions and the date of data collection.
X-RAY	Includes information of x-ray test (pathologic/normal) in skull, MRT brain, MRT back, comments of radiologist and the date of data creation.
PAIN	Includes the VAS scale of pain (0-10), pain location, comments of both doctor and patients and the date of data collection.
VENTILATION	Contains all data regarding to the respiratory problems, including the spirometry values, use of invasive or non-invasive ventilation, tracheostomy interventions, and their corresponding dates of start.
EAT-10	EAT-10 it's a rate scale to assess the swallow abilities of the patients. It includes the overall score (0-10), the individual answers to all the questions and the date of data collection.
OTHER SYMPTOMS	Collects symptoms non-related with MND and the reported date (it is almost empty, just two entries)