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AMYOTROPHIC LATERAL SCLEROSIS AND MULTIPLE SCLEROSIS ASSOCIATED NEUROINFLAMMATION - NATIONWIDE EPIDEMIOLOGICAL STUDIES ON ETIOLOGY, COMORBIDITIES, AND TREATMENT

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Amyotrophic lateral sclerosis and multiple sclerosis associated neuroinflammation – nationwide epidemiological studies on etiology, comorbidities, and treatment

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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Mamma, ho scritto ogni parola pensando a te.

Mi manchi tanto.

ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a relatively rare but fatal neurodegenerative disease characterized by progressive muscle paralysis, due to loss of upper and lower motor neurons. Signs of neuroinflammation have been reported in ALS, however it is still unknown whether neuroinflammation is a cause or a consequence of the motor neuron dysfunction. Neuroinflammation may also be one of the mechanisms underlying the overlap between ALS and other neurological, neuromuscular, and psychiatric disorders. Among neuromuscular disorders, the most studied disorder in association with ALS is multiple sclerosis (MS). MS is a complex disease of the central nervous system (CNS) characterized by chronic inflammation, demyelination, and neurodegeneration. A link between MS and psychiatric disorders has also been proposed. In this thesis we explored the associations of different correlates of neuroinflammation, including physical and cognitive fitness in early life (Study I), neurodegenerative and psychiatric disorders (Study II), neuromuscular diseases (Study III), depression and antidepressants use (Study IV) with the risks of either ALS or MS.

In **Study I**, we investigated the association between physical and cognitive fitness in young adulthood with future risk of ALS. The study population included 1,838,376 Swedish men aged 17-20. Information on physical fitness, body mass index (BMI), intelligence quotient (IQ), and stress resilience was retrieved from the Swedish Conscription Register between 1968 and 2010. Information on subsequent ALS diagnosis was retrieved from the Swedish Patient Register. There were 439 incident cases of ALS during follow-up. Higher physical fitness was associated with an increased risk of ALS before age 45, while higher BMI tended to be associated with a lower risk of ALS at all ages. Higher IQ was associated with an increased risk of ALS at age 56 and onwards, whereas higher stress resilience was associated with a lower risk of ALS at age 55 and earlier. These results indicate that physical fitness, BMI, IQ, and stress resilience in young adulthood are associated with the development of early onset ALS.

In **Study II**, we examined the risk of neurodegenerative and psychiatric disorders among individuals with ALS, compared to individuals free of the disease. The study population included 3,648 individuals with ALS and 36,480 age-, sex-, and county-of-birth-matched controls from the general population. In addition, we estimated the risk of neurodegenerative and psychiatric diseases among the relatives of ALS patients, compared to the relatives of the controls, to assess the potential contribution of familial factors. Individuals with previous neurodegenerative or psychiatric diseases had an increased risk of ALS, compared to individuals who did not have these diseases. After diagnosis, ALS patients had increased risks of neurodegenerative or psychiatric diseases, compared to individuals free of ALS. First-degree relatives of individuals with ALS had higher risk of neurodegenerative diseases compared to relatives of controls. Children of ALS patients had higher risk of psychiatric disorders, compared to children of controls. The increased risk of neurodegenerative disorders among relatives of ALS patients may be attributable to the overlapping etiopathogenesis of different neurodegenerative diseases. The increased risk of psychiatric disorders among ALS patients

and their children may be due to non-motor symptoms of ALS or a severe stress response after the diagnosis of ALS.

In **Study III**, we aimed at validating the co-occurrence of ALS and other neuromuscular diseases, investigating the temporal relationship between the diagnoses and clinical characteristics of patients with both ALS and other neuromuscular diseases. Using information from the Swedish Patient Register, we identified all patients diagnosed with ALS in Sweden between 1991 and 2014, who had also a concurrent MS, myasthenia gravis (MG), inflammatory polyneuropathies (IP), or dermatopolymyositis (DMPM). The group included 263 patients. We validated medical records for 92% of these patients to confirm the overlap. Then, we compared patients with a confirmed overlap (N=28) with an independent sample of patients with only ALS (N=271). Among the patients with a validated overlap, 12 had a confirmed diagnosis of MS, nine a confirmed diagnosis of MG, four a confirmed diagnosis of IP, and three a confirmed diagnosis of DMPM. Seventy-nine percent of the patients with a confirmed overlap had these diagnoses prior to ALS. Compared to patients with only ALS, patients with a confirmed overlap were older at symptoms onset, had higher prevalence of bulbar onset, but used riluzole and non-invasive ventilation less frequently. These results show that neuroinflammation around the motor unit might trigger ALS in a small subgroup of patients.

In **Study IV**, we estimated the risk of depression and antidepressants prescription among patients with relapsing-remitting MS (RRMS) and explored the bi-directional relationship between different disease modulatory therapies (DMTs) and depression or antidepressants use. We included 4,867 patients with RRMS who were diagnosed in Sweden between January 2005 and September 2018 and did not have a diagnosis of depression or antidepressants prescription before initiation of their first DMT. We compared the risk of depression (defined by diagnosis or antidepressants prescription) in relation to different DMTs, using interferons as the reference and examined DMT discontinuation or relapse in relation to depression. RRMS patients treated with rituximab had a decreased risk of depression, compared to patients treated with interferons. No differences were found for other DMTs examined (dimethyl fumarate, fingolimod, and natalizumab), compared to interferons. Depression was not associated with the risk of DMT discontinuation or MS relapse. These results provide evidence for a lower risk of depression among RRMS patients treated with rituximab, compared to interferons.

In conclusion, results from the studies included in this thesis support the notion that neuroinflammation might precede the onset of ALS. In addition, different neurodegenerative diseases might share common disease mechanisms including neuroinflammation. The reduced risk of depression in relation to treatment with rituximab among patients with RRMS also suggests that neuroinflammation might underlie the link between psychiatric disorders and neurodegenerative diseases because rituximab may modulate both the MS- and depression-related inflammation.

LIST OF SCIENTIFIC PAPERS

[*] Shared last authorship.

I. **Longinetti E**, Mariosa D, Larsson H, Almqvist C, Lichtenstein P, Ye W, Fang F. Physical and cognitive fitness in young adulthood and risk of amyotrophic lateral sclerosis at an early age. *European Journal of eurology*. 2017; 24(1):137-142.

II. **Longinetti E**, Mariosa D, Larsson H, Ye W, Ingre C, Almqvist C, Lichtenstein P, Piehl F, Fang F. Neurodegenerative and psychiatric diseases among families with amyotrophic lateral sclerosis. *Neurology.* 2017; 89(6):578-585.

III. **Longinetti E**, Sveinsson O, Press R, Ye W, Ingre C, Piehl F[*] and Fang F[*]. Concurrence of amyotrophic lateral sclerosis and multiple sclerosis, myasthenia gravis, inflammatory polyneuropathies, and dermatopolymyositis: a nationwide identification of patients and comparison with patients with amyotrophic lateral sclerosis only. *Manuscript Submitted.*

IV. **Longinetti E**, Frisell T, Fang F[*] and Piehl F[*]. Depression and antidepressants prescription in relapsing-remitting multiple sclerosis. *Manuscript.*

CONTENTS

LIST OF ABBREVIATIONS

AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
ALSFRS-R	Revised version of the ALS functional rating scale
ATC	Anatomical therapeutic chemical
BMI	Body mass index
C9orf72	Chromosome 9 open reading frame 72
CI	Confidence interval
CIDP	Chronic inflammatory demyelinating polyneuropathy
CNS	Central nervous system
DMPM	Dermatopolymyositis
DMT	Disease modulatory therapy
DNA	Deoxyribonucleic acid
EDSS	Expanded disability status scale
FDA	Food and drug administration
FTD	Frontotemporal dementia
FUS	Fused in sarcoma
GBS	Guillain-Barré syndrome
HR	Hazard ratio
ICD	International classification of disease
IP	Inflammatory polyneuropathies
IQ	Intelligence quotient
MG	Myasthenia gravis
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
MSIS	MS impact scale
MSSS	MS severity score
OR	Odds ratio
PD	Parkinson's disease
PPMS	Primary-progressive MS

PRMS	Progressive-relapsing MS
RHR	Resting heart rate
RNA	Ribonucleic acid
RRMS	Relapsing-remitting MS
SD	Standard deviation
SES	Socioeconomic status
SNRI	Serotonin–norepinephrine reuptake inhibitors
SOD1	Superoxide dismutase 1
SPMS	Secondary-progressive MS
SSRI	Selective serotonin reuptake inhibitor
TCA	Tricyclic antidepressant
TDP43	TAR DNA-binding protein 43
WMAX	Maximum working capacity

1 INTRODUCTION

The term “neuroinflammation” refers to the process that includes the inflammatory responses within the central nervous system (CNS). Neuroinflammation is a pathological component of several neurodegenerative diseases.

Amyotrophic lateral sclerosis (ALS) is a relatively rare neurodegenerative disease, which usually has its onset between age 40 and 70. In ALS, a progressive loss of upper and lower motor neurons leads to paralysis and wasting of the muscles, causing progressive disability and, ultimately, death, often due to respiratory failure. Different signs of neuroinflammation have been observed in association with ALS, but the causal relationship between them remains unknown. Because neuroinflammation has been reported in association with other neurodegenerative diseases, and with neurological and psychiatric disorders generally, neuroinflammation may also be one of the mechanisms that explain the overlap between ALS and these diseases. Multiple sclerosis (MS) is the most studied neuromuscular disease in association with ALS.

MS is a complex diseases of the CNS characterized by inflammation, demyelination, and neurodegeneration. In most patients, MS is diagnosed after the onset of clinical symptoms, which can be partially or totally reversible (relapsing-remitting MS, RRMS). There is however great variation in symptoms presentation and disease course. Currently, there is no available medication that can cure MS, but several treatment options are available to reduce symptoms severity and relapse frequency. Similar to ALS, the risk of psychiatric disorders has also been reported to be increased among MS patients.

In this thesis, we explored the relationship between different correlates of neuroinflammation with the risks of ALS (Studies I, II, and III) and MS (Study IV).

2 ALS BACKGROUND

2.1 ALS DISEASE CHARACHTERISTICS

ALS is a relatively rare neurodegenerative disease. Individuals with this disease experience progressive loss of upper and lower motor neurons. The mean age of ALS onset is 65 years, with most people diagnosed between the ages of 40 and 70[1]. Among the majority of patients, symptoms quickly progress into muscle paralysis and wasting, usually leading to progressive disability and death (commonly due to respiratory failure) within 1-3 years from diagnosis. Only around 10% of the patients clearly have a familial form of the disease and the vast majority appear as sporadic cases[2].

In a healthy individual, the upper motor neurons in the motor region of the cerebral cortex and the brain stem, send movement commands to the lower motor neurons, which project from the brainstem or spinal cord to the muscles. The muscles then contract, allowing voluntary movements of the body. In a patient with ALS, however, motor neuron degeneration interrupts the communication between the brain and the muscles, either at the upper motor neuron level or at the lower motor neuron level. Hence, the voluntary muscles no longer receive movement commands and atrophy as a consequence. The first symptoms of ALS are either a weakening of the extremities, so called spinal onset, or reduced capacities to speak and swallow, so called bulbar onset. Even though motor symptoms characterize ALS, cognitive and behavioral impairment affects up to 50% of ALS patients during the course of the disease.

2.2 ALS CLINICAL PRESENTATION AND DIAGNOSIS

In 1869, Jean-Martin Charcot, a French neurologist working in Paris, was able to identify ALS as a specific neurological disease with distinct pathology, and separated ALS from other disorders with similar symptoms[3,4]. Although the understanding of the disease has greatly evolved since then, due to epidemiological, molecular, and genetic discoveries, no definite test for the diagnosis of ALS is yet available, except for autopsy.

In case of a limb onset (accounting for 60% of the cases), patients start noticing the first symptoms through asymmetric spasticity and weakness (signs of upper motor neuron dysfunction), or through fasciculation, cramps, and muscle wasting (signs of lower motor neuron dysfunction)[5]. In case of a bulbar onset (about 35% of the patients), patients experience the first symptoms as progressive difficulty in talking (dysarthria), difficulty in swallowing (dysphagia), or emotional liability[5]. Only a minority of patients (5%) experience respiratory problems as initial ALS symptoms[5].

Because of the lack of valid diagnostic biomarkers[6], the diagnosis of ALS is made clinically based on the Revised El Escorial criteria, which require the evidence of lower or upper motor neuron degeneration as well as a progressive spread of signs or symptoms[7]. Revised El Escorial criteria categorize diagnostic certainty based on the burden of disease, according to the number of body regions showing signs of upper and lower motor neuron involvement. Clinicians then

classify the ALS diagnosis as definite, probable, or possible. The original version of the El Escorial criteria included a "suspected ALS" category, which would include patients showing signs of lower motor neurons involvement in two or more regions. In 1998, this category was deleted from the Revised El Escorial criteria classification system to increase the specificity of the diagnosis.

In addition to the Revised El Escorial criteria, clinicians must also rule out alternative diagnoses. The ALS diagnosis is more plausible if patients do not show electrophysiological or pathological evidence of other diseases characterized by lower or upper motor neuron degeneration, or neuroimaging evidence of diseases that could lead to specific clinical and electrophysiological signs[7]. As a result, patients have to go through a stressful diagnostic procedure, which takes on average one year to complete from the first symptoms onset[8].

2.3 ALS ETIOPATHOLOGY

The etiology of ALS, especially sporadic ALS, likely involves a complex interplay among genetics, environmental exposures, and lifestyle factors. Although the knowledge about ALS pathophysiology is still growing, the neuropathological distinctive characteristic of the disease are ubiquitinated proteinaceous inclusions in motor neurons[5]. The main constituent of these inclusions varies depending on specific ALS subtypes, but almost all ALS patients show features of a TAR deoxyribonucleic acid-binding protein 43 (*TDP43*)-associated proteinopathy[9]. Other protein aggregates that might be found are neurofilamentous hyaline congrolomerate, misfolded superoxide dismutase 1 (*SOD1*), and *TDP43*-negative inclusions[5]. Additional mechanisms involved in the disease are impaired protein homeostasis, oxidative stress, impaired deoxyribonucleic acid (DNA) repair, mitochondrial dysfunction, dysregulated vehicle transport, and aberrant ribonucleic acid (RNA) metabolism[5]. All these mechanisms seem to be interlinked and probably depend on the initial cause of the disease[5].

2.4 ALS TREATMENT

To date, there is no curative treatment for ALS. Instead, treatments are mainly tailored to slow disease progression and manage symptoms. Since 1995, the only drug approved by the United States Food and Drug Administration (FDA) to alter ALS progression has been riluzole, which prolongs the survival of ALS patients by an average of 2-3 months. Riluzole reduces the release of glutamate, the excess of which might destruct the nerve cells[10]. However, only older patients with bulbar onset seem to experience a slightly increased survival, whereas younger patients with limb onset are less likely to benefit from riluzole[11]. Additionally, riluzole must be administered in the early stages of the disease to be beneficial, as the glutamatergic action of riluzole depends on viable motor neuron activity[12].

More recently, the antioxidant drug edaravone was approved in Japan (2015) and in the United States of America (2017) to treat ALS. However, this drug seems to work only for patients with early age onset and rapidly progressing disease. Originally developed for the treatment of stroke, edaravone is a free radical scavenger administered intravenously. Although the mechanism of edaravone in ALS is unknown, it is thought to mitigate oxidative injury in the

motor neurons of the CNS at risk for degeneration by eliminating lipid peroxides and hydroxyl radicals[13]. Given the unclear effect on muscle strength, respiratory function, and quality of life, as well as the costly and labor-intensive nature of the treatment, the European Medicines Agency has not approved this drug and several countries exclude it from their health care systems[5,14].

Given the lack of a curative treatment, clinical guidelines encourage early access to symptomatic treatment, that is, palliative treatment[15]. Clinical guidelines also encourage early non-invasive ventilation, to improve the prognosis of ALS patients by compensating for weakness of the respiratory muscles[16]. Patients presenting with dysphagia (difficulty in swallowing) have the option to receive nutritional support by gastrostomy feeding[16].

Overall, the optimal management of ALS patients requires access to a multidisciplinary team that is flexible and responsive to the evolving nature of the condition[17]. The benefit of having access to a range of healthcare professionals was shown by research highlighting the longer survival of ALS patients attending multidisciplinary clinics[18].

2.5 ALS EPIDEMIOLOGY

2.5.1 Incidence and prevalence

The incidence of ALS, estimated from population-based studies in Europe, is about 2-4 cases per 100,000 individuals per year[19-22]. The incidence of ALS seems to differ based on ancestral original, with lower incidence recently reported in Beijing, China[23] (0.8 cases per 100,000 individuals per year) and South Korea[24] (1.2 cases per 100,000 individuals per year). Overall, the incidence seems to be higher among men than women, with a male/female ratio between 1 and 3[25], possibly due to a higher incidence of spinal onset ALS among males[1]. The incidence of ALS has been reported to increase during the recent decades from multiple countries across the world[21,22,26-29]. This might reflect a real increase of ALS incidence or result from improved diagnostics, reporting, or survival rate of competitive diseases[30-32]. Although the incidence appears to be increasing, given the fast disease progression, prevalence of ALS remains quite low, and was estimated at 4.1-8.4 cases per 100,000 individuals in recent population-based studies[19-22,24,29,33-36].

2.5.2 Risk factors

Historically, the established risk factors for ALS have been older age, male sex, and family history. Genetic studies have identified several important genes responsible for an increasing proportion of familial ALS cases, including chromosome 9 open reading frame 72 (*C9orf72*), *SOD1*, *TDP43*, and fused in sarcoma (*FUS*)[37]. However, given that more than 90% of ALS patients have no clear family history, the overall contribution of single genes to ALS etiology still appears weak[38]. Instead, researchers speculate that an interaction between genetic and non-genetic factors contributes to the etiology of ALS[39].

Various hypotheses, including defects in energy metabolism, chronic neuroinflammation, heavy metal exposure, strenuous physical exercise, etc., have been proposed as casual factors leading to ALS. However, it is hard to draw definitive conclusions about these factors because our knowledge of ALS pathophysiology is still developing and past studies might suffer from methodological problems. More recently, Mendelian randomization studies have suggested a causal relationship between ALS and physical activity, smoking, blood lipid levels, and education[40,41].

2.5.3 Prognostic factors

In ALS patients, death due to respiratory failure usually occurs within 1-3 years from diagnosis. Only about 10% of ALS patients present with a slower diseases progression leading to a survival of 10 years or more after diagnosis. Healthcare professionals usually measure the progression of ALS with the revised version of the ALS functional rating scale (ALSFRS-R)[42]. This scale monitors the progression of the patient disability by investigating four domains consisting of gross and fine motor tasks, bulbar functions, and respiratory function (added in the revised version of the scale)[42]. Reasons for a slower disease progression are unknown, although prognostic factors such as spinal onset, male sex, lack of cognitive dysfunction, longer diagnostic delay, and younger age at diagnosis have been associated with better survival rate[43].

2.6 NEUROINFLAMMATION IN ALS

"Neuroinflammation" is a term that refers to the inflammatory responses within the CNS that commonly accompany neurodegeneration. Signs of neuroinflammation are present in human post-mortem samples from ALS patients[44]. Additionally, key cellular modulators of neuroinflammation, such as astrocytes, microglia and immune cells, are correlated with ALS pathogenesis[45]. Although there is an association between signs of neuroinflammation and ALS, it is under debate whether neuroinflammation is a cause or consequence of motor neuron dysfunction in ALS[45]. One hypothesis is that systemic immune activation might play a role in neuroinflammation. However, studies that focus on inflammatory conditions around the motor unit might hold promise, because axons, dendrites, and synapses show the first pathological changes associated with ALS[46]. The hypothesis of a causal relationship between chronic inflammatory status in close proximity to the motor neuron unit and motor neuron degeneration is biologically plausible. For example, a normal axonal transport is indispensable for motor neurons[46]. Because axons may be up to 20,000 times larger in size than the neuronal cell body and extend up to one meter, axons are highly fragile morphologically and in terms of functionality[46], and could be damaged by increased level of neuroinflammation.

Several factors could contribute to the increased level of neuroinflammation among patients with ALS. For example, an association between intense physical activity and ALS has been proposed since long[47-54]. In addition to the recently reported shared genetic predisposition to physical activity and ALS[40], this association could possibly also be explained by the increased inflammatory status in proximity to the muscles and tendons among people who have a history of engaging in intense physical activity[55].

2.7 ALS COMORBIDITIES

ALS seems to overlap with other neurological diseases and psychiatric disorders. Neuroinflammation might be one of the possible explanations for this overlap. I speculate below on how neuroinflammation might be one of the reasons why ALS could be comorbid with a host of other neurological and psychiatric conditions.

2.7.1 Neuromuscular diseases

During the advanced stages of ALS, the diagnosis can be ascertained with a high degree of confidence, whereas in the early stages of the disease, symptoms of ALS are not specific but rather common to other neuromuscular diseases. As a result, ALS patients might be initially misdiagnosed with other neuromuscular diseases characterized by neuroinflammation in different sites, such as MS (inflammation in the CNS), myasthenia gravis (MG, inflammation in the neuromuscular junction), inflammatory polyneuropathies (IP, inflammation in the peripheral nervous system), and dermatopolymyositis (DMPM, inflammation in the muscles and skin). Although early symptoms of ALS may mimic MS, MG, IP, and DMPM[56], making diagnostic uncertainty an attractive explanation for any concurrence between these diseases, biological overlap among these diseases probably also exists[57]. Consequently, a higher-than-expected concurrence of ALS and neuromuscular diseases might be attributed to diagnostic uncertainty, real biological overlap, or perhaps both. Although a common genetic etiology might contribute to the biological overlap, it is also possible that chronic inflammation is in the causal pathway to neurodegeneration, as discussed above.

ALS has been studied in conjunction with other neuromuscular diseases, primarily MS, which is an autoimmune diseases characterized by inflammation in the CNS. This research interest started with case reports of concurrence[58-62]. Given the suspicion of an overlap between ALS and MS, researchers studied five patients from the United Kingdom with concurrent ALS/MS and identified the *GGGGCC* hexanucleotide repeat expansion of *C9orf72*, a polymorphism found in a significant proportion of both sporadic and familial ALS patients[57]. In addition to case reports, larger epidemiological studies also investigated the ALS/MS concurrence and found an association between MS and later ALS onset[63], as well as an increased risk for MS among children of ALS patients[64]. In Sweden, a high correlation between mortality rates of ALS and MS was observed between 1952 and 1992[65], but a more recent study did not confirm the correlation[66].

Similar to MS, researchers have also found overlap between ALS and MG[67-70]. MG is a disease of neuromuscular transmission in which autoantibodies bind to the neuromuscular junction. The aforementioned cohort study that investigated the risk of ALS in patients with MS, also found that MG was associated with later ALS risk; similar result was also reported for polymyositis but not dermatomyositis[63]. Polymyositis is a disease characterized by chronic inflammation of the muscles[71] and has recognized common pathological features with ALS. Despite these shared pathological features, the association between polymyositis and ALS has only been investigated in one other sample[71].

In contrast to the aforementioned neuromuscular diseases, the overlap between IP and ALS has not yet been systemically investigated. Given the similarity between the initial symptoms of ALS and IP, research has mainly focused on techniques aiming to aid a differential diagnosis between these diseases, particularly with Guillain-Barré syndrome (GBS)[72]. GBS is a form of IP, presenting with muscle weakness as a result of damage in the peripheral nervous system.

2.7.2 Neurodegenerative diseases

Clinical and pathologic overlaps between ALS and other neurodegenerative diseases have been demonstrated[2,73] and neuroinflammation is an acknowledged pathological component of numerous neurodegenerative diseases[74]. Specifically, 30-50% of ALS patients demonstrate cognitive impairment[75] and 5-10% of ALS patients fulfill criteria for frontotemporal dementia (FTD)[76]. Few studies, however, have estimated the risk of other neurodegenerative diseases among patients with ALS, probably due to the rareness of ALS and, subsequently, of its concurrence with other neurodegenerative diseases[77]. A German study found a higher prevalence of dementia and Parkinson's disease (PD) among ALS patients, compared to the general population[77]. A Swedish register-based study found an association of ALS with a higher subsequent PD risk[78]. Increased risks of PD and dementia have also been found among relatives of ALS patients[79], which suggests that there might be a partly shared etiopathogenesis between different neurodegenerative diseases[79-81].

In the familial forms of different neurodegenerative disorders, the persistent presence of a precipitating factor might help to sustain the observed inflammatory reaction[82]. The precipitating factor might be the disease-causing mutant protein in the familial dominant forms (for example, amyloid β1–42 for Alzheimer's disease, AD, mutant α-synuclein for PD, and *SOD1* for ALS). With the initial aim of combating the precipitating factor, the inflammatory reaction ends up turning into a harmful process that contributes to further neuronal damage[82].

2.7.3 Psychiatric disorders

Patients with neurodegenerative diseases might also have higher-than-expected comorbidity with psychiatric disorders[83]. A growing body of evidence suggests that many psychiatric disorders, including stress-related disorders, depression, bipolar disorder, and schizophrenia, are associated with distinct inflammatory responses in the periphery and CNS[84,85]. Thus, it is possible that the inflammatory response from microglial activation can contribute to brain pathology. In contrast to this hypothesis, an earlier study suggested that the psychological distress associated with receiving and adjusting to an ALS diagnosis might represent another underlying reason for psychiatric comorbidity in ALS[86].

Patients with ALS were shown to have increased risk of psychiatric disorders in some but not all studies[87-89] and we know little about the risk of psychiatric disorders among families of ALS patients[79]. Specifically, previous research has reported an overlap between ALS and psychosis among patients with ALS-FTD[90], and ALS patients and their relatives were reported to have an increased risk of schizophrenia and psychotic illness[79,88]. A recent population-based study further identified a clustering of neuropsychiatric disorders among relatives of ALS patients,

suggesting the presence of a common genetic basis between ALS and psychiatric disorders in the Irish population[91]. Our group reported that ALS patients had increased risk of depression and antidepressants use compared to controls, especially shortly before and after diagnosis[89]. Furthermore, individuals with abusive alcohol consumption were reported to have a reduced risk of ALS[92], whereas individuals with abusive use of opioids were reported to have a higher risk of ALS[93].

3 MS BACKGROUND

3.1 MS DISEASES CHARACTERISTICS

MS is the most prevalent chronic inflammatory disease affecting the CNS[94]. It is characterized by attacks of the immune system on myelin, which is a protective layer of fatty white substance that surrounds the axon of some nerve cells. This damage can cause a lack of communication within the brain and between the brain and the body by leading to a slower impulse transmission. The extent and severity of symptoms greatly vary among patients, depending on which and how many nerve cells are affected. Most patients receive diagnosis of MS between the ages of 20 and 40 years[95].

3.2 MS CLINICAL PRESENTATION AND DIAGNOSIS

MS was first described in 1868 by Jean-Martin Charcot, the same French neurologist that firstly distinguished ALS from other neurological diseases. Based on observations of MS pathological and clinical features, he defined the first three diagnostic criteria of MS: involuntary eye movement (nystagmus), lack of muscle control and coordination (ataxia), and difficulty in talking (dysarthria)[96].

Typically, MS initially manifests as blurred vision, sensory disturbance (such as tingling or loss of sensation), and motor impairment[97]. Subsequently, long periods of remission can follow, alternated by successive attacks with new symptoms or relapses. In the initial phases of MS, the symptoms can partially or completely regress. In the later phases, symptoms progress leads to a loss of ability to walk independently.

Giving an MS diagnosis is a challenging process, given the radiologic and clinical heterogeneity of the disease[94,98]. Currently, no externally validated blood immune marker has adequate sensitivity and specificity to be used for the diagnosis of MS, which probably reflects the genetic and environmental heterogeneity of the disease[94]. The diagnosis is therefore made clinically, according to the 2017 revised McDonald criteria[99] with the aid of imaging scans such as magnetic resonance imaging (MRI) and analysis of cerebrospinal fluid.

3.2.1 Phenotypic classification

In 1996, the phenotypic classification of MS was defined by members of the international MS community in four different groups: RRMS, primary-progressive MS (PPMS), secondary-progressive MS (SPMS), and progressive-relapsing MS (PRMS)[100]. Following a greater understanding of the clinical course of MS as well as its pathology, the clinical course of MS was re-defined in 2013[101]. The new classification system retained the RRMS, PPMS, and SPMS with some modifications, whereas the PRMS was removed and is now categorized as "active PPMS" [101].

RRMS is the most common phenotypic presentation of MS including around 85% of MS patients. RRMS is characterized by relapses that may or may not leave permanent deficits, with

no new symptoms occurring in between periods of remission. Over time, most RRMS patients convert to SPMS, a stage of progressive deterioration without periods of remissions or relapses. A minority of patients, PPMS, do not have any initial periods of remission or relapses and show from start progressive deteriorations.

3.3 MS ETIOPATHOLOGY

Despite the etiology of MS is still unclear, a mix of environmental and genetic factors seem to underlie its pathogenesis. Immunological, genetic, and histopathology studies indicate that autoimmunity appears to play a major role in MS pathogenesis[102]. However, MS is also considered a neurodegenerative condition[103].

Although most MS lesions can appear throughout the CNS, they are most easily identified in the white matter as focal areas of demyelination, inflammation, and glial reaction[94]. In some cases, lesions can reverse; however it is not well understood if the myelin sheet is reformed or if the inflammation is resolved[94]. Demyelination can also occur in gray matter[104-106]. It is also possible to observe loss of retinal ganglion cells adjoining the optic nerve[107] and retinal damage[108].

3.4 MS TREATMENT

Currently, there is no curative treatment for MS[94]. However, in contrast to ALS, several treatments are approved by the FDA to lower inflammatory disease activity and reduce the frequency of attacks in RRMS and development of new lesions in the white-matter.

These disease modulatory therapies (DMTs) include different interferon beta and glatiramer acetate preparations, monoclonal antibodies (natalizumab, alemtuzumab, daclizumab, and ocrelizumab), orally administered small-molecule agents (fingolimod, dimethyl fumarate, and teriflunomide), a chemotherapeutic agent (mitoxantrone), and hematopoietic stem cell transplantation. In Sweden, rituximab, a DMT approved for rheumatoid arthritis but not MS, has gained popularity and became the most widely used DMT for RRMS. Its popularity derives from a series of real-world studies showing that rituximab outperforms regular DMTs[109]. Other treatments commonly given for managing signs and symptoms of MS include muscle relaxants to treat muscle spasms, medications to treat fatigue, and dalfampridine to increase walking speed.

3.5 MS EPIDEMIOLOGY

3.5.1 Incidence and prevalence

Globally, the incidence of MS is about 2.5 cases per 100,000 individuals per year. However, the incidence rate is higher in Europe with 3.8 cases per 100,000 person-years, compared to only 1.5 cases per 100,000 person-years in America[110]. In Africa, the incidence is as low as 0.1 per 100,000 person-years. Although there is a hypothesis that risk for MS co-occurs with colder weather, the low incidence rate in Africa might be at least partly attributable to a lack of diagnostic tools (e.g., MRI scanners)[111]. Studies indicate that the incidence rates have more

than doubled in the past decade, perhaps due to improved diagnostics[112]. Because of the long survival time, the prevalence of MS is higher than the incidence, and is estimated as 30 cases per 100,000 individuals[110]. The prevalence is higher in North America, Europe, and Australia (over 60 cases per 100,000), and lower in South America and South East Asia (less than 20 cases per 100,000)[110].

3.5.2 Risk factors

It remains unknown if MS is caused by only one or multiple factors. However, because it is difficult to identify a common etiologic trigger behind all MS cases, it seems more plausible that a wide range of genetic and environmental factors contribute to the disease[94]. Several risk factors related to the development of inflammatory and demyelinating lesions with heterogeneous axonal loss have been identified, including Epstein-Barr virus and mononucleosis, risk genes, temperate latitude (reflecting sunlight exposure and consequently vitamin D levels but also possibly a genetic contribution), fibrinogen, toxins, trauma, low vitamin D, smoking, and obesity[94]. Furthermore, for unknown reasons, women are affected by MS twice as often as men, as commonly seen in other autoimmune diseases[94].

3.5.3 Prognostic factors

The median survival of MS patients is 40.6 years from disease onset, leading to a shortened life expectancy of seven years compared to the general population[113]. Factors associated with slower disease progression include lower grade of inflammation, fewer spinal cord lesions, endogenous repairs, preserved axons and synapses, earlier treatment, and younger age[94].

3.6 MS AND DEPRESSION

Already at diagnosis, the quality of life of RRMS patients is impacted by the presence of depression[114,115]. Severe depressive symptoms occur in 19-40% of MS patients[116-123], double that of the general population[124]. In Sweden, MS patients of working age had a higher risk of depression and selective serotonin reuptake inhibitors (SSRIs) prescription compared to individuals without MS[125]. Moreover, MS patients have an elevated risk of suicide[126,127], particularly during the year immediately after MS diagnosis[128]. This seems to suggest that receiving such diagnosis might be highly stressful for MS patients. However, there is evidence that the depression-MS association is bidirectional because depression has been suggested to predict later MS[129], and such result pattern could not be attributed to shared genetics alone[129]. One speculation is that inflammation in the CNS is the common denominator behind both depression and MS[130].

DMTs of MS appear to also have an influence on depressive symptoms. Given that depression co-occurs frequently with MS, and that DMTs might modify the probability of depression, it might be fruitful to investigate the occurrence of depression in context of different DMTs in RRMS. To date, however, there have been few such investigations. A limited number of studies indicate that fingolimod and natalizumab improve depressive symptoms in MS patients[131-135]. In contrast, other DMTs appear to worsen depressive symptoms, in particular interferon beta-

1b[136,137], which has been shown to be associated with an elevated risk of suicide in early trials[138,139]. However, the association between interferon beta-1b and suicide as reported in the early trials was later shown to be explained by a prior history of psychiatric disorders[140], and failed to replicate in later larger studies[120,141,142]. Nevertheless, all DMTs, and not just interferon beta-1b, have depression listed as a possible side effect[133].

4 THESIS HYPOTHESES AND STUDY AIMS

ALS shows signs of neuroinflammation[44,45]. However, due to a lack of large prospective epidemiological studies, the directionality of the association between neuroinflammation and ALS remains unclear (i.e. if neuroinflammation precedes or follows the onset of ALS). We hypothesized that neuroinflammation around the motor unit precedes the onset of ALS.

Several factors could contribute to the increased level of neuroinflammation among patients with ALS. We hypothesized that high levels of physical activity might be one of them, due the observed association between intense physical activity and inflammatory status in proximity to the muscles and tendons, joints, and peripheral nerves[55].

It has also been observed that ALS seems to overlap with other neurodegenerative[2,73,76-78], neuromuscular[57,58,63,67-71], and psychiatric diseases[87-89,91-93,143], all of which are associated with neuroinflammation in different body regions. We therefore hypothesized that neuroinflammation might be one of the possible explanations for such overlap.

MS is a neuromuscular diseases characterized by neuroinflammation in the CNS[74] and has been shown to overlap with ALS[57,58]. As in ALS[88,89], depression is a common comorbidity of MS patients[129]. The observation that this comorbidity in MS is not explained by genetic liability[129] implies that the etiology of depression in MS is multifactorial and might include a mix of psychological and biological factors, including neuroinflammation[124,144]. We hypothesized that treatment of MS might bring additional benefits, apart from symptomatic relief, by modulating the MS-associated inflammatory responses and thereby the risk of depression[142,145].

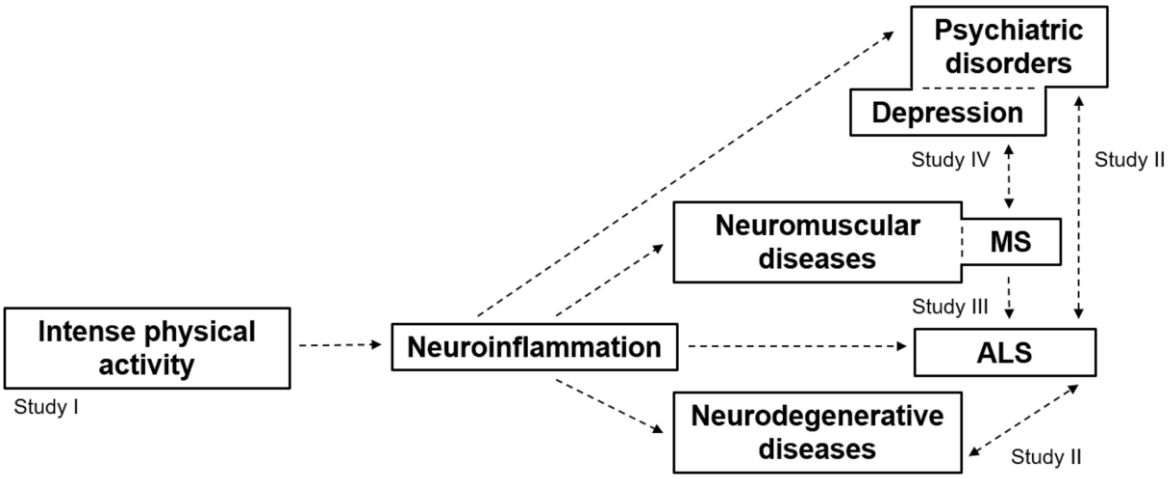


Figure 1. Schematic representation of the thesis hypotheses.

To test these hypotheses, we conducted two studies to investigate the associations of inflammation in different tissues in close vicinity to motor neurons, namely, muscles and tendons (associated with intense physical activity), muscles and skin (DMPM), neuromuscular junction (MG), peripheral nervous system (inflammatory polyneuropathies, IP), and CNS

(MS), with the risk of ALS (Studies I and III). We conducted two additional studies to assess the relationship between neuroinflammation, psychiatric disorders, and neurodegeneration in both ALS and MS (Studies II and IV).

We specifically aimed to:

- Explore whether physical and cognitive fitness at age 17-20 predicts the risk of ALS in adulthood (Study I)

- Estimate the risk of neurodegenerative and psychiatric diseases among patients with ALS and their relatives (Study II)

- Validate the concurrence of ALS and neuromuscular diseases MS, MG, IP, and DMPM; investigate the temporal relationship between the diagnoses among patients with a confirmed overlap; and investigate if the clinical characteristics of the concurrent patients are different compared to patients with only ALS (Study III)

- Estimate the risk of depression and antidepressant prescription in relation to different MS DMTs, and investigate if depression or antidepressants prescription affects DMT discontinuation or MS relapse (Study IV)

5 DATA SOURCES

We used population-based data from different sources: several Swedish National Registers[146-151], medical records review, and the Swedish MS Registry[152]. We cross-linked all the registers by using a unique identifier derived from the personal identity number, which is assigned at birth or at immigration to any person resident in Sweden for at least one year[153].

5.1 SWEDISH NATIONAL REGISTERS

Epidemiological research in Sweden owes its success to the long-standing tradition of maintaining population-based records of clinical and demographic information in national registers[154]. An overview of the Swedish National Registers is presented in Table 1.

The **Total Population Register** collects information on sex, and date and place of birth on all individuals that were resident in Sweden since 1968, when the register was established[147].

The **Migration Register**[147] and the **Causes of Death Register**[148] collect information on migration (in and out of Sweden) since 1968 and date and causes of death since 1961, respectively.

The **Population and Housing Censuses** have been conducted every five years between 1960-1990 and include socioeconomic information such as type of employment, household composition, and type of accommodation.

The **Swedish Multi-Generation Register** connects each individual born in Sweden since 1932, and alive from 1961 onward, to his/her biological parents[148]. In case of adoption, each individual is also linked to the adoptive parents.

The **Swedish Patient Register** collects hospital records data in Sweden since 1964 and has nationwide coverage since 1987[146]. Since 2001, it covers hospital-based outpatient specialist care. Diagnoses given at each hospital visit are classified according to the Swedish Revisions of the International Classification of Disease (ICD) codes (ICD-7 before 1969, ICD-8 during 1969-1986, ICD-9 during 1987-1996, and ICD-10 from 1997).

The **Swedish Prescribed Drug Register** was established in July 2005 and includes information on all prescribed medications in Sweden[151] classified according to the Anatomical Therapeutic Chemical (ATC) system. Compared to the other national Swedish registers, the Prescribed Drug Register was established relatively late because of confidentiality concerns. It includes information such as substance of the dispended item, dosage, date of prescription, and date of dispense. A limitation of this register is that it does not include over-the-counter medications, vaccines, and drugs dispensed at hospitals or nursing homes[151].

The **Swedish Conscript Register** includes all males who underwent conscription examination in Sweden during 1968-2010. Attending conscription examination was mandatory for all males when they turned 18 years old, only those with severe physical or mental disabilities were

exempt. During examination, trained healthcare professionals collected several parameters of physical and cognitive fitness to establish which individuals were fit for military service. Females attending conscription examination were also included in this register since 1975, when females were allowed in Sweden to serve in the military. However, given that female attendance to conscription examination was voluntary, the register has national coverage during 1968-2010 only on males. In 2018, mandatory conscription examination was once again implemented, this time in a gender-neutral fashion[155].

Table 1. Overview of the Swedish national registers used in the constituent studies of this thesis.

Register	Date of establishment	Date of national coverage	Information extracted	Used in
Causes of Death Register	1961	1961	Date of death	Studies I, II, and IV
Conscript Register	1968	1968-2010 for males	Physical and cognitive fitness assessed at military conscription examination	Study I
Migration Register	1968	1968	Date of migration to Sweden or emigration from Sweden	Studies I, II, and IV
Multi-Generation Register	1968	1968	Familial link for all individuals born in Sweden since 1932	Studies I and II
Patient Register	1964	1987 for inpatient care 2001 for outpatient care	Inpatient and outpatient admissions and discharges records	Studies I-IV
Population and Housing Censuses	1960	1960-1990	Socioeconomic status	Study I
Prescribed Drug Register	July 2005	July 2005	Dispensed prescribed drugs	Study IV
Total Population Register	1968	1968	Sex, date, and country of birth of Swedish residents	Studies I, II, and IV

5.2 MEDICAL RECORDS REVIEW

Population-based registers offer the opportunity to conduct unbiased investigations on several medical conditions affecting the entire Swedish population. However, national registers lack disease-specific information about the clinical course, as well as laboratory results and detailed phenotypic information. We therefore conducted a medical records review of validated ALS patients with concurrent neuromuscular diseases in order to obtain detailed information on age at disease onset, site of onset, disease progression profile, and cognitive impairment among this group of patients.

5.3 THE SWEDISH MS REGISTRY

In Study IV, we used information from the Swedish MS Registry. The Swedish MS Registry is a web-based quality register that includes high quality healthcare data for all MS patients in Sweden since 2001[152]. In addition to demographic data, several parameters of clinical relevance are included in the Swedish MS Registry, for example, onset and diagnosis date, fulfillment of McDonald's criteria[99], MS clinical course, adverse events, DMT (type, dosage, date of start, date and reason of discontinuation), laboratory results, neuroimaging exams, and functional and severity scores (Expanded Disability Status Scale, EDSS[156], and MS Severity Score, MSSS[157]). Despite participation in the registry being voluntary, both for the patients and their attending neurologists, the registry includes about 80% of MS patients in Sweden with approximately 18,000 patients in total. The success of the registry is due to its use as clinical support tool for the attending neurologists and healthcare personnel. Patients also feel involved in their own care by registering patients reported outcome (MS impact scale, MSIS-29[158]) and being able to see a simplified overview of the clinical course of the disease. Data collected in this registry appears to be both accurate and complete[159].

5.4 MAIN MEASURES

5.4.1 Clinical diagnoses

The Swedish Patient Register[146] was the main sources of information to identify patients with ALS, other neurodegenerative diseases, psychiatric disorders, and neuromuscular diseases in the thesis. We defined individuals as being diagnosed with a certain disease if they had at least one recorded discharge diagnosis in the Swedish Patient Register, according to the corresponding ICD codes listed in Table 2. We defined as date of diagnosis the date of the first hospital contact regarding that diagnosis. Specialists assign all diagnoses in the Swedish Patient Register, which have been shown to have high accuracy[146]. Additional studies specifically validated ALS and MS diagnoses in the Swedish Patient Register and concluded that it is a valuable instrument to correctly identify such patients[19,160].

Table 2. ICD codes used to identify ALS, neurodegenerative diseases, psychiatric disorders, and neuromuscular diseases in the Swedish Patient Register.

	ICD-8	ICD-9	ICD-10
Amyotrophic lateral sclerosis	348.00	335.C	G12.2
Frontotemporal dementia	290.11	331.B	F02.0, G31.0
Alzheimer's disease	290.10	290.A[a], 290.B[a], 331.A	F00, G30
Other or unspecific dementia	290[b], 293.0, 293.1	290.A, 290.B, 290.E, 290.W, 290.X, 294.B, 331.C, 331.X	F01, F02[c], F03, F05.1, G31.1, G31.8A[d]
Parkinsonian disorders	342.00, 342.08, 342.09	332.A, 333.A	F02.3[d], G20, G21.4, G21.8, G21.9, G23.1, G23.2, G23.9, G25.9, G31.8A[d]
Schizophrenia	295	295	F20
Bipolar disorder	296.1, 296.3, 296.8	296.A, 296.C-296.E, 296.W	F30, F31
Depression	300.4	300.E, 311	F32[e], F33[f], F34, F38, F39
Neurotic disorders	300.1-300.3, 300.5-300.9	300.A-300.D, 300.F-300.H, 300.W, 300.X	F40-F42, F44, F45, F48
Stress-related disorders	307	308, 309	F43
Alcohol abuse/dependence	303	303, 305.A	F10[g]
Drug abuse/dependence	304	304, 305.X	F11-F19
Multiple sclerosis	n/a	340	G35
Myasthenia gravis	n/a	358.A, 358.B, 358.W	G70.0
Inflammatory polyneuropathies	n/a	357.A, 357.B, 357.W, 357.X	G61.0, G61.8, G61.9
Dermatopolymyositis	n/a	710.D, 710.E	M33.0-M33.2, M33.9, G72.4, G73.7

[a] If found as primary diagnosis

[b] Except 290.10 (Alzheimer's disease) and 290.11 (Pick's disease)

[c] Except F02.0 (Dementia in Pick's disease)

[d] ICD-10 codes considered both dementia and parkinsonian disorder diagnoses

[e] Except F32.2 (depressive psychosis)

[f] Except F33.3 (depressive psychosis)

[g] Except F10.5 (psychotic state)

n/a = ICD-8 not applicable during the study period

5.4.2 Physical and cognitive fitness

In Study I, we extracted information on different physical and cognitive fitness variables from the Swedish Conscript Register.

Physical fitness was measured at conscription with a test called "the maximal work test"[161]. During this test, the physical fitness of a person is measured by recording in Watts the maximum working capacity (WMAX) that this person can sustain for six minutes on an electric bicycle with gradually increasing resistance[162,163]. As previously suggested[52], we analyzed WMAX adjusted for weight (WMAX/kg). We defined high levels of weight-adjusted physical fitness as above the highest tertile (≥4.25 W/kg).

We additionally extracted information on body mass index (BMI) and resting heart rate (RHR). We derived **BMI** from weight (kg) and height (m) measured at conscription by dividing weight for height in meters squared. We coded individuals as underweight (BMI <18.5 kg/m^2), normal weight (BMI 18.5-24.99 kg/m^2), and overweight or obese (BMI ≥25 kg/m^2). **RHR** was measured in beats per minute after 5-10 minutes of rest in the supine position with a cuff positioned at the heart level[164].

We included intelligence quotient (IQ) and stress resilience in our definition of cognitive fitness. **IQ** was assessed by several cognitively challenging subtests of progressive difficulty measuring general knowledge and verbal, visuospatial, and mechanical ability[165]. By summing the scores of all different tests a general score of IQ was obtained. This score was standardized to fit a Gaussian distribution of values ranging from 1 to 9 (so called "stanine scale") with higher values reflecting higher intellectual abilities. We defined high IQ as scores 7-9 on the stanine scale.

Stress resilience was measured during a 20-25 minutes semi-structured interview conducted by a trained clinical psychologist[166]. The aim of this interview was to assess the ability to cope with stress during armed combat[167] by evaluating different psychological functions domains (mental energy, emotional control, social maturity, and active/passive interests). For example, psychologists gave a high stress resilience score if during the interview they assessed emotional stability, persistence, ability to contribute to group cohesion, or being able to cope with loss of personal freedom. Akin to IQ, this score was standardized into a stanine scale with higher values reflecting better abilities to cope with stress. We defined high stress resilience as scores 7-9 on the stanine scale. The Swedish National Defense Research Institute found a high interrater-reliability of stress resilience by comparing the scores with army service records at the end of military service[168].

5.4.3 Antidepressants prescription

In Study IV, we identified patients undergoing pharmacological treatment for depression from the Swedish Prescribed Register[151]. Our definition of antidepressants was restricted to SSRIs (ATC: N06AB). We restricted our definition to SSRIs because they are first-line treatment for

depression in MS[169-171], and because other antidepressants such as tricyclic antidepressants (TCAs) and serotonin–norepinephrine reuptake inhibitors (SNRIs) are commonly prescribed among MS patients for neuropathic pain. To increase validity, we only coded this variable as positive for individuals who had been prescribed SSRIs twice. Identifying patients undergoing pharmacological treatment for depression also allowed us to capture patients who might not have received a diagnosis from a specialist in the Swedish Patient Register (i.e., depressed individuals who only met with the general practitioner).

6 METHODS

6.1 STUDY DESIGNS AND CORRESPONDING STATISTICAL METHODS

Because the etiology of ALS and MS remains unknown, large-scale population-based observational studies are important to help uncover clues to their origins. Population-based observational studies include patients of all ages and with all forms of the disease, in contrast to clinical studies that are often biased toward including younger ALS patients with slower disease progression[172] or MS patients with variable disease course[173].

6.1.1 Cohort studies, Cox model, and flexible parametric model

A population-based prospective cohort design, with complete and long-term follow-up, allows to identify potential precursors several years or decades before the disease is diagnosed, and to collect information about exposure variables long before the outcomes of interest have occurred. Such data might offer important clues, as they are collected independently from the later outcome development and are not hampered by recall bias.

Working with longitudinal data allows us to use time-dependent statistical models such as Cox regression, which compares the hazard function of different exposure levels while allowing for controlling of multiple potential confounders and the underlying time-scale. The Cox regression model, however, assumes that the difference between the hazard rates of different exposure groups is proportional over time, which is not always the case. This assumption can be circumvented by using flexible parametric models, which involve fitting an interaction between the covariates and time. Other advantages of flexible parametric models over Cox models include the possibility to observe how the hazard functions might change over time[174]. This is of great medical interest, as the association of certain risk factors with a specific disease might be directly related to the time-course of a specific disease.

6.1.2 Nested case-control studies and logistic regression

Although the aforementioned prospective cohort design has many advantages, when presented with a large cohort and a rare outcome such as ALS, the nested case-control design is a computationally efficient alternative. The nested case-control design includes all cases and a pre-defined number of randomly selected outcome free-controls, sampled from a parent cohort. In this study design, logistic regression is suitable to compare the risk of ALS in individuals exposed to certain factor, compared to individuals who are unexposed to the factor. In our case, it is possible to use logistic regression because the outcome investigated can be classified as a binary variable, having or not having a diagnosis of ALS[175].

6.1.3 Medical records review and descriptive statistics

Large-scale Swedish register-based studies rarely collect detailed phenotypic, clinical, or biological information, given the (usually) nationwide and administrative nature of data collection. However, it is possible to retrospectively collect detailed information among a smaller set of individuals included in the register-based studies, through medical records

review. One can compare frequency differences between groups of individuals using the Chi-square test and estimate mean differences using Student's t-test. This kind of analysis can provide detailed information on specific subset of patients, which is relevant for diseases of uncertain etiology such as ALS.

6.2 METHODS STUDY I

We identified all males aged 17-20 who attended conscription examination from 1968 to 2010, during which time military conscription was mandatory for all Swedish males. This ALS-free cohort consisted of 1,901,807 males. We excluded conscripts who died (N=6,531) or emigrated (N=10,634) before the beginning of follow-up. We also excluded conscripts without information on physical fitness, i.e. WMAX/kg, BMI, RHR, IQ, and stress resilience (N= 46,266), leading to a final sample of 1,838,376 conscripts (97.5% of the initial cohort).

We set the beginning of follow-up to January 1st 1987. If the conscripts attended conscription examination after January 1st 1987, we followed them from the date of conscription examination. We set the end of follow-up at December 31st 2013. If the conscripts were diagnosed with ALS, died, or emigrated out of Sweden before the end of follow-up, we stopped following them on the first date of ALS diagnosis, death, or emigration (whichever came first).

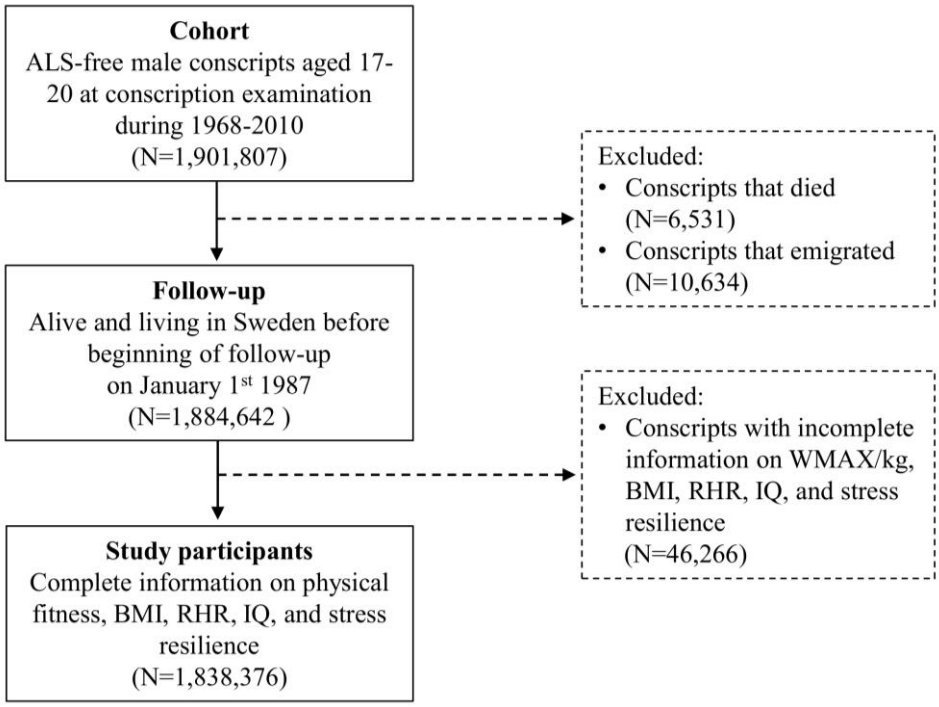


Figure 2. Flow-chart of identification of the study participants of Study I.

Statistical analysis

We computed the correlation among the exposure variables (WMAX/kg, BMI, IQ and stress resilience) using Spearman correlation coefficient (ρ). We used Spearman correlation coefficient because it does not require continuous-level assumptions.

We conducted two separate flexible parametric models to examine if high levels of physical (WMAX/kg and BMI) and cognitive fitness (IQ and stress resilience) would affect the age-specific risk of ALS, compared to lower levels of fitness. The full model for physical fitness included as covariates calendar period of conscription examination, parental socioeconomic status (SES), RHR, and was mutually adjusted for WMAX/kg and BMI. The full model for cognitive fitness included calendar period of conscription examination, parental SES, and was mutually adjusted for IQ and stress resilience. We used attained age as the time scale in all models and derived hazard ratios (HRs) and corresponding 95% confidence intervals (CIs). We picked five degrees of freedom for the baseline hazard and three degree of freedom for the time-dependent effect[174].

The adjustment for calendar period of examination (1968-1980, 1981-1993, and 1994-2010) was necessary because the procedures of assessment of the different parameters in the Conscript Register varied during the years, for example tests to assess IQ changed in 1994[176]. We added RHR into the physical fitness model because of its suggested association with ALS[177] and correlation with physical fitness[178]. Because all conscripts were aged 17-20, we adjusted for their parental SES instead of their own SES, after identifying mothers and fathers of the conscripts from the Swedish Multi-Generation Register and identifying the parents' SES information through the Swedish Population and Housing Censuses.

We considered statistically significant associations with two-sided P-values <0.05.

6.3 METHODS STUDY II

In Study II, we followed all individuals identified from the Swedish Multi-Generation Register, who were born in Sweden during 1932-2013 (N=8,575,515), from January 1st 1990 until December 31st 2013. If an individual was born after January 1st 1990, we followed him/her from date of birth. Individuals who were diagnosed with ALS (N=662), died (N=120,612), or emigrated out of Sweden (N=186,670) before the beginning of follow-up were not included in the study base (N=8,269,319). If an individual was diagnosed with ALS, died, or emigrated out of Sweden before end of follow-up, we stopped following him/her on date of first ALS diagnosis, death, or migration (whichever came first).

Within the study base, we conducted a **nested case-control study** and defined as cases all the patients diagnosed with ALS during follow-up (N=3,648). By incidence density sampling, ten controls were randomly selected and individually matched to each case by sex, year and month of birth, and county of birth (N=36,480). To serve as an eligible control, an individual had to be without ALS, alive, and living in Sweden on the date of ALS diagnosis of the corresponding ALS case. For cases and controls, we defined as index date the date of ALS diagnosis of the

cases. In this nested case-control study, we assessed the association between neurodegenerative and psychiatric diseases and the future risk of ALS.

We also assessed the association between neurodegenerative and psychiatric diseases and being a relative of an ALS patient. Hence, we conducted a **nested case-control study of the relatives** of the cases (N=19,760) and controls (N=198,794) of the above nested case-control study. We identified the parents, siblings, half-siblings, and children of the cases and controls through the Swedish Multi-Generation Register.

We also examined the association between being exposed to an ALS diagnosis and the risk of subsequently being diagnosed with neurodegenerative and psychiatric diseases. After excluding all individuals from the above nested case-control studies who were diagnosed with any neurodegenerative or psychiatric diseases before ALS diagnosis, we conducted a **follow-up study** of the ALS patients (N=3,169) and ALS-free individuals (N=33,110), and a **follow-up study of the relatives** of the ALS patients (N=13,313) and the relatives of the ALS-free individuals (N=130,321). In both follow-up studies, the participants were followed from index date to date of diagnosis of neurodegenerative or psychiatric diseases, emigration, death, or end of follow-up on December 31st, 2013 (whichever came first).

Statistical analysis

In the nested case-controls studies, to measure the association between neurodegenerative and psychiatric diseases with future risk of ALS, we fitted conditional logistic regression models to estimate odds ratios (ORs), and corresponding 95% CIs of ALS and of becoming a relative of an ALS patient. In the nested case-control study, the models were automatically adjusted for sex, year and month of birth, and county of birth, as these were the variables the controls were matched to the cases on. In the nested case-control study of the relatives, we adjusted for both sex, year and month of birth, and county of birth of the index person, as well as for sex, year and month of birth, and county of birth of the relatives.

In the follow-up studies, to measure the risk of being diagnosed with neurodegenerative or psychiatric diseases after being exposed to ALS, we fitted Cox proportional hazard regression models to derive HRs and corresponding 95% CIs by comparing ALS patients to ALS-free individuals, and relatives of ALS patients to relatives of ALS-free individuals. We set attained age as the underlying time scale in all models. In the follow-up study, the models were further adjusted for sex and county of birth, and in the follow-up study of the relatives the models were further adjusted for sex and county of birth of the index person, as well as for sex, year and month of birth, and county of birth of the relatives.

We tested the assumption of proportional hazards using Schoenfeld residuals and considered statistically significant associations with two-sided P-values ≤ 0.05.

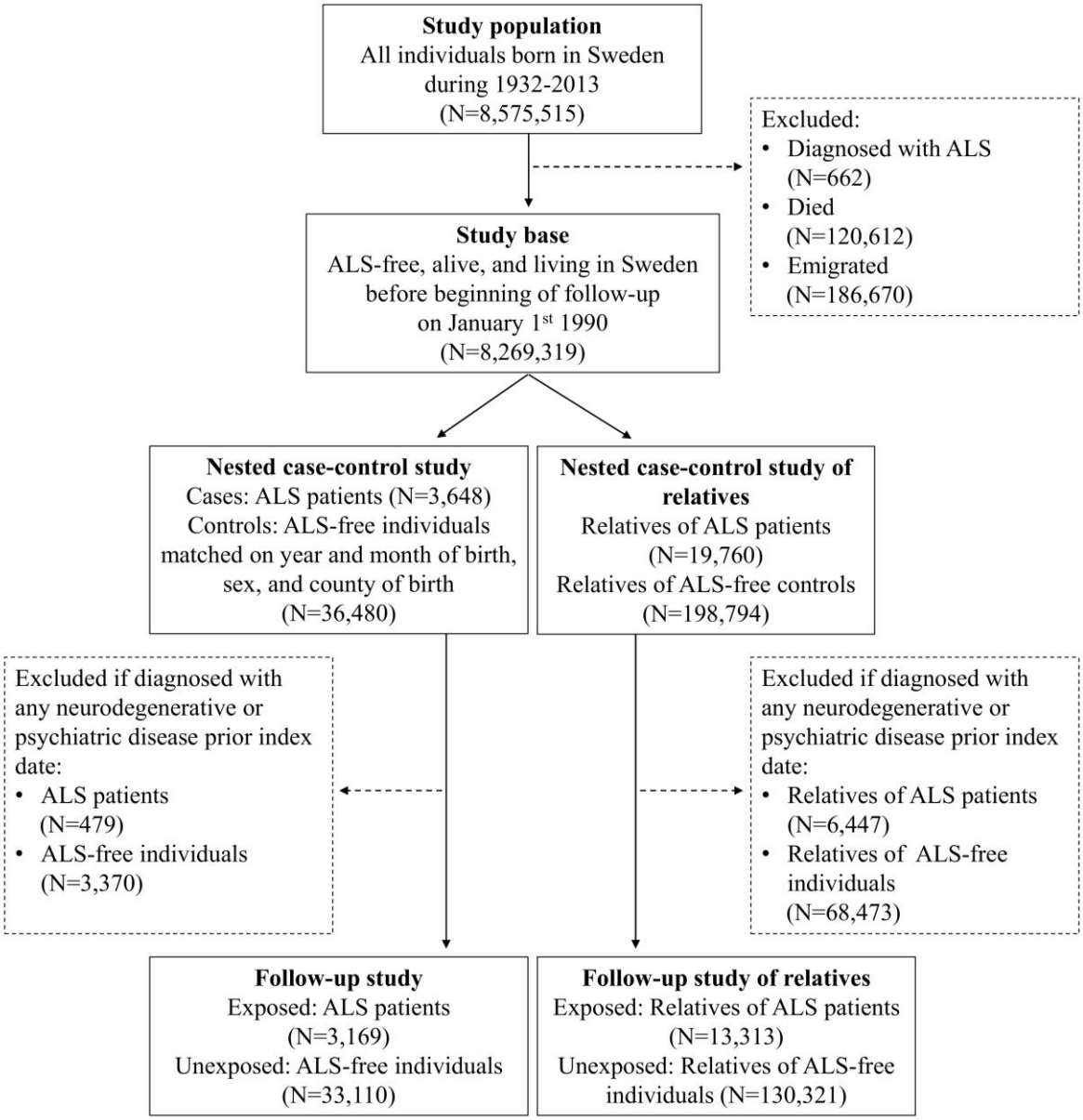


Figure 3. Flow-chart of identification of the study participants of the nested case-control studies and follow-up studies of Study II.

6.4 METHODS STUDY III

We requested from the Swedish National Board of Health and Welfare a list of personal identity numbers of all the patients in the Swedish Patient Register who had recorded diagnoses of both ALS and either MS/MG/IP/DMPM during 1991-2014 (N=263). From the Swedish National Board of Health and Welfare, we also received a list of all the hospitals and departments that these patients were visited at. We then mailed the chiefs of departments of all the hospitals and requested the medical records of these patients, identified by their personal identity numbers. We sent reminders to the chiefs of departments who did not get back to us within three months. Once we received all the medical records available for each patient (N=245), three experienced neurologists, specialized in ALS and neuroinflammatory diseases, independently reviewed the medical records. Decisions of diagnostic accuracy were made by consensus. Because PLS is

gaining recognition as a sub-phenotype of ALS, the neurologists validated a patient meeting criteria of PLS as an ALS patient[179].

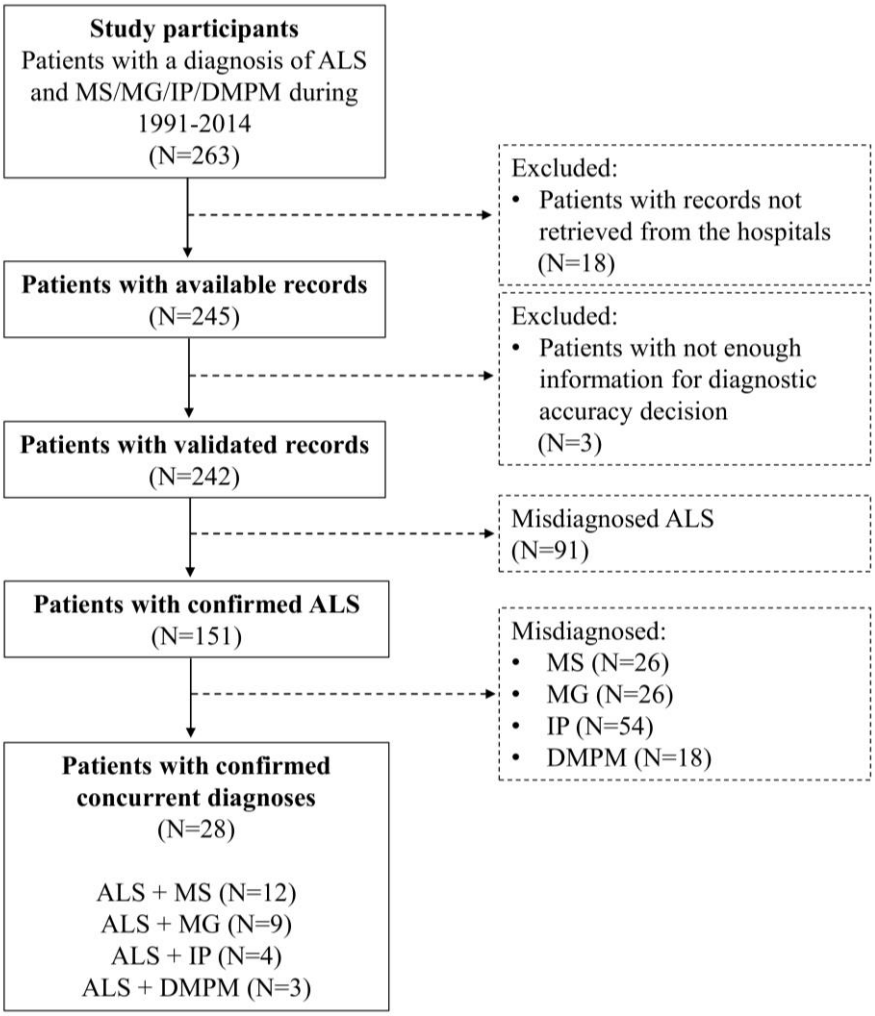


Figure 4. Flow-chart of data collection and diagnostic accuracy decisions of the study participants of Study III.

Statistical analysis

We extracted detailed clinical data from the medical records of patients with validated concurrent ALS and MS/MG/IP/DMPM. We summarized their clinical ALS characteristics, the order in which the concurrent diseases occurred (i.e., which diagnosis was given first), and how much time passed in between diagnoses.

Additionally, we examined if patients with a concurrent diagnosis of ALS and MS/MG/IP/DMPM represented a special group of patients that differed in terms of clinical characteristics of ALS. To assess this, we compared patients with concurrent ALS and MS/MG/IP/DMPM against an independent sample of patients who were diagnosed with only ALS. This group consisted of 271 ALS patients visited in Stockholm during 2013-2014. Our group previously performed a validation study of ALS diagnosis of these patients based on a detailed extraction of data from medical records[19]. For the categorical variables, we used Chi-

square test or Fisher's exact test (if the expected frequencies were less than or equal to five) to test the differences between two groups, and for non-normally distributed continuous variables we used the Wilcoxon test. We considered statistically significant associations with two-sided P-values <0.05.

6.5 METHODS STUDY IV

In Study IV, our study population consisted of all patients diagnosed with RRMS in the Swedish MS Registry, who were born in Sweden and living in Sweden during January 2005-September 2018 (N=6,000). We followed this cohort from start of their first DMT until end of follow-up on December 31st 2018. We did not include in the study participants individuals with either a depression diagnosis in the Swedish Patient Register or an antidepressants prescription in the Prescribed Drug Register before start of their first DMT (N=789). We also excluded individuals who withdrew from the MS Registry before start of their first DMT (N=2), or the ones that did not have any recorded DMT (N=342), leaving 4,867 RRMS patients in the final analyses.

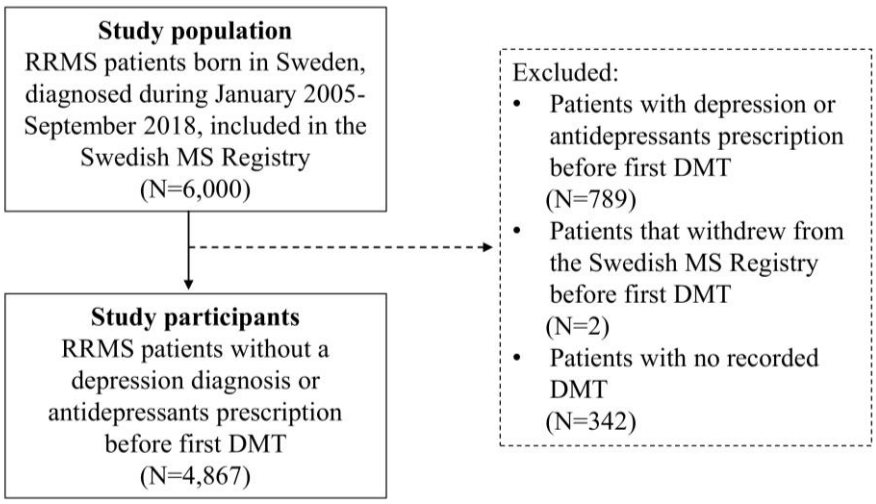


Figure 5. Flow-chart of identification of the study participants of Study IV.

Statistical analysis

Our main aim was to assess if the risk of depression or prescription of antidepressants varied according to different DMTs. Hence, we fitted Cox proportional hazard regression models and calculated HRs of depression or antidepressant prescription and corresponding 95% CIs in relation to DMTs. We considered DMTs as time-varying exposure because RRMS patients frequently switch DMTs during the course of the disease for various lengths of time. A patient was considered exposed to a certain DMT from the date of DMT start to the date of DMT discontinuation. We compared all the different time periods when patients were on dimethyl fumarate, fingolimod, natalizumab, rituximab, or other DMTs, against the time periods when

patients were on interferons, which were our reference DMT. Other DMTs analyzed as a group were alemtuzumab, daclizumab, glatiramer acetate, hematopoietic stem cell transplantation, novantrone, and teriflunomide. In this analysis, patients re-entered the study on the start date of each DMT. Each patient was followed until date of depression diagnosis or antidepressants prescription, DMT discontinuation, loss to follow-up (withdrawal from the Swedish MS registry, death, or migration out of Sweden), or end of follow-up.

We also examined if being exposed to depression or antidepressant prescription had an effect on DMT discontinuation or MS relapse after DMT initiation. Again, we fitted Cox proportional hazard regression models and separately calculated HRs of DMT discontinuation or MS relapse and corresponding 95% CIs. We considered depression or prescription of antidepressants as time-varying exposures. A patient was considered exposed from date of depression diagnosis or from date of first prescription of antidepressants. Similar to the above analysis, each patient re-entered the study on the start date of each DMT. Each patient was followed until date of DMT discontinuation, date of MS relapse, loss to follow-up (withdrawal from the Swedish MS registry, death, or migration out of Sweden), or end of follow-up. Because we aimed to examine if exposure to depression or antidepressants prescription had an effect on DMT adherence, we censored follow-up for observations where the reason of DMT discontinuation included stable condition, pregnancy, or planned pregnancy, instead of using these as discontinuation outcomes. Two patients were excluded from the models where the outcome of interest was MS relapse (N=4,865) because they experienced an MS relapse on the day of DMT start.

We set time since start of DMT as the underlying time scale in all analyses so that all models would be automatically adjusted for it. Additionally, we progressively built multivariable Cox models including other potential confounders: sex, age at start of DMT, geographic region of treatment (as an indicator of SES), bipolar disorder diagnosis (given its association with depression[180] and potential association with MS[122]), MS severity (assessed via the scales EDSS, MSIS-29, and MSSS), MS relapse at symptoms onset, and line of DMT. Given that EDSS, MSIS-29, and MSSS are repeatedly recorded in the Swedish MS Registry, we selected in all analyses the closest scores that were assessed prior to DMT start of each observation. In the models where we assessed the risk of DMT discontinuation or MS relapse, if a patient was exposed to depression or prescription of antidepressants, we selected the closest EDSS, MSIS-29, and MSSS scores recorded both prior to DMT start and date of depression diagnosis or date of prescription of antidepressants.

In all analyses, given that each patient contributed to multiple time periods of observation every time a patient started a new DMT, we used a sandwich estimator to estimate unbiased standard errors.

We considered statistically significant associations with two-sided P-values ≤ 0.05.

7 ETHICAL CONSIDERATIONS

In Studies I and II we used linkage of different existing population-based Swedish registers that contain data on intelligence, neurodegenerative and psychiatric diagnoses, familial links, as well as a large amount of other sensitive information. The Swedish national registers have been established without individual informed consent with the aim of benefiting the society. In the Nordic countries, large-scale epidemiological studies have been conducted since the establishment of national registers with the general approval from the population, mainly due to a high degree of public trust in research[154]. A Swedish qualitative study, investigating consent issues in register-based data linkage, reported that study participants wished to be contacted prior to the beginning of the study[181]. However, the participants themselves did not see active consent as of primary importance and expressed concern over the possibility of missing data due to withdrawal[181].

In Study IV we also used a data linkage of population-based Swedish registers, but clinical and treatment data on the study population was extracted from the Swedish MS Registry. Unlike the Swedish national registers, participation in the Swedish MS Registry is voluntary, both for the patients and their attending neurologists. Specifically, the clinical documentation is collected locally and merged into a compiled data set that constitutes the Swedish MS Registry. Patients are informed about this procedure and have the possibility to withdraw from the registry at any time, if desired.

The record linkages used for Studies I, II, and IV, have obtained approval by the Regional Ethical Review Board in Stockholm, which recognized that although active consent fulfills the moral and legal premises of study participants' autonomy, technical and economical limitations arise from having to inform millions of individuals. Overall, the decision to not seek informed consent for the record linkages has been approved after taking into account the balance between gain of knowledge, positive long-term effects on the community, and violation of integrity of the participants.

In Study III, we used sensitive data from a medical records review containing detailed medical information. Although by accessing medical records we were subjected to the Secrecy Act, the project was granted ethical permit by the Regional Ethical Review Board in Stockholm, which waived us from seeking informed consent. This exemption, regulated in the Personal Data Act (in place at the time of ethical application for this project) states that data is personal when it is possible to connect it to a person who is alive. Because ALS is a disease with fast and fatal progression, with patients dying on average within three years after diagnosis, we expected the majority of our study participants to no longer be alive at the time of the study.

When working with register-based data the risk of violation of integrity is reduced since data are de-identified with an internally created identifier. In Study III, we did receive the actual personal identity numbers of all the study participants, in order to access their medical records. However, once we obtained the medical records, we de-identified the patients and assigned

them an internally created identifier to secure the privacy of the participants during data analysis.

Further security measures are in place in order to ensure a correct processing of personal data in according to the European General Data Protection Regulation. Anyone who handles personal data is subject to confidentiality, and only the assigned database administrator has the access to all data, whereas researchers only have limited access to the information strictly necessary for the project. We implemented additional appropriate technical and organizational actions, such as IT protection and good data management by keeping data stored on secure servers and following good data management guidelines in place at the department.

8 MAIN RESULTS

8.1 STUDY I – PHYSICAL AND COGNITIVE FITNESS ARE ASSOCIATED WITH ALS

At conscription examination, 65.40% of the 1,838,376 conscripts included in the study were aged 18, 37.32% went through conscription examination during 1981-1993, 33.27% had a WMAX/kg between 3.63 and 4.24 W/kg, 74.26% had a normal BMI, 54.20% had a medium level IQ, and 57.77% had a medium level stress resilience.

At conscription examination, the correlation between WMAX/kg and BMI was negative (ρ=-0.23; $p<0.001$), whereas the correlation between IQ and stress resilience was positive (ρ=0.37; $p<0.001$). Correlation coefficients among all physical and cognitive fitness variables used in the analyses are reported in Table 3, all of which are statistically significant ($p<0.001$).

Table 3. Spearman correlation coefficients between physical and cognitive fitness variables.

	WMAX/kg	**BMI**	**IQ**	**Stress resilience**
WMAX/kg	n/a	-0.23	0.15	0.30
BMI	-0.23	n/a	-0.05	0.11
IQ	0.15	-0.05	n/a	0.37
Stress resilience	0.30	0.11	0.37	n/a

We identified 439 newly diagnosed ALS patients during follow-up. On average the age at ALS diagnosis was 48 years (range 21-62 years, standard deviation=8.74, SD).

Conscripts with high WMAX/kg (≥4.25 W/kg) had a 66-75% increased risk of being diagnosed with ALS before age 44 (range of HRs= 1.66, 95% CI 1.01-2.82 and 1.75, 95% CI 1.01-3.05), compared to conscripts with lower WMAX/kg, after adjusting for BMI and RHR (Figure 6). Overweight or obese conscripts had a 50-58% reduced risk of being diagnosed with ALS after age 41 (range of HRs= 0.42, 95% CI 0.19-0.96 and 0.50, 95% CI 0.26-0.94), as compared to normal weight and underweight conscripts, after adjusting for WMAX/kg (Figure 6). The association between WMAX/kg and ALS was statistically significant at age 41-43 years, whereas the association with BMI was statistically significant at age 42-48 years.

Conscripts with high IQ (i.e., those scoring above the highest tertile) had a 33-81% increased risk of being diagnosed with ALS after age 55 (range of HRs= 1.33, 95% CI 1.00-1.78 and 1.81, 95% CI 1.01-3.30), compared to conscripts with lower IQ, holding stress resilience constant (Figure 7). Conscripts with high stress resilience (above the highest tertile) had a 29-53% reduced risk of being diagnosed with ALS before age 56 (range of HRs= 0.47, 95% CI 0.28-0.79 and 0.71, 95% CI 0.53-0.97), as compared to conscripts with lower stress resilience,

holding IQ constant (Figure 7). Specifically, the association for IQ was statistically significant at age 56-61 years, whereas the association for stress resilience was statistically significant at age 46-55 years.

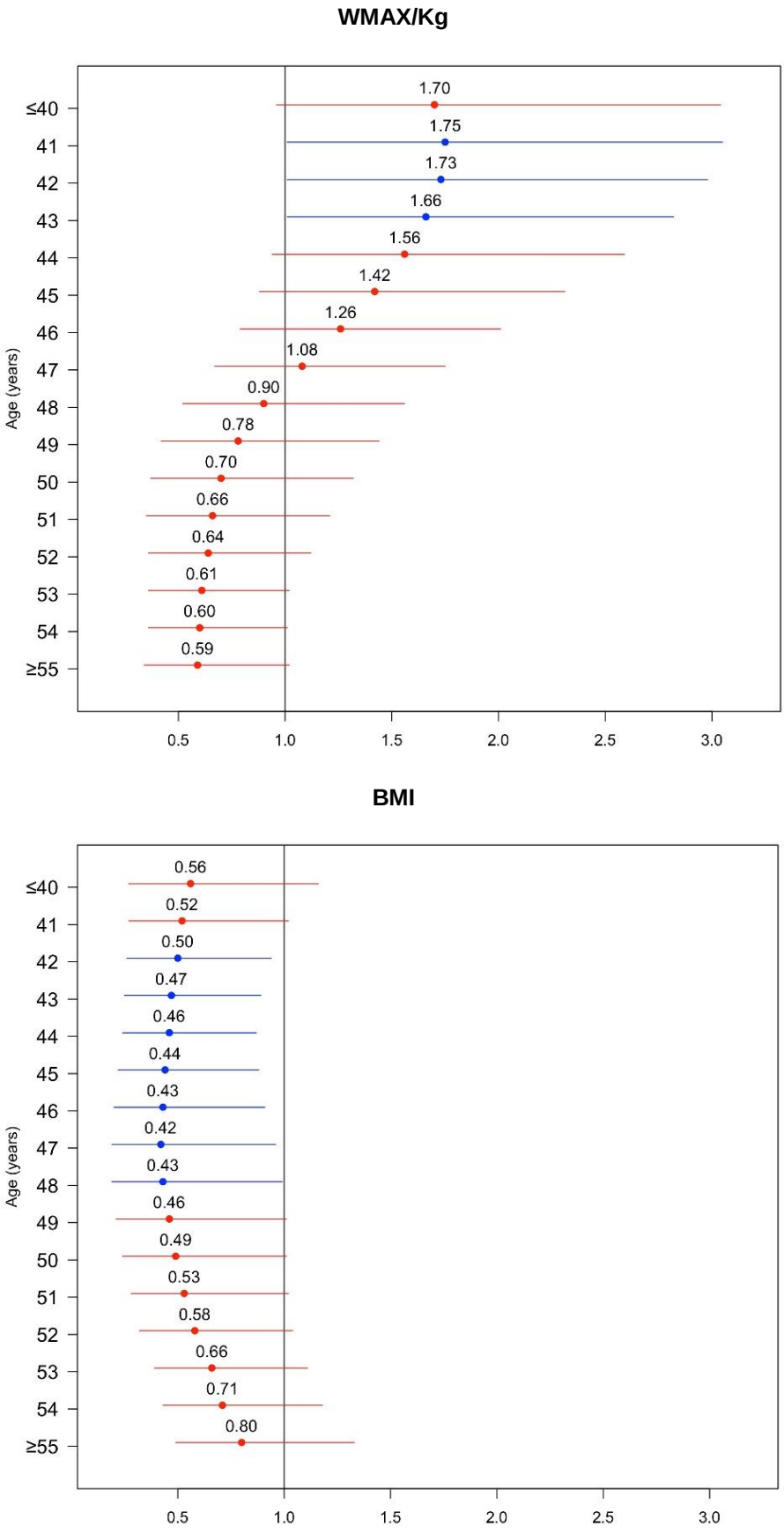


Figure 6. HRs and corresponding 95% CIs of ALS, for WMAX/kg (≥4.25 W/kg versus <4.25 W/kg; model adjusted for calendar period of conscription examination, parental SES, BMI, and RHR), and for BMI (overweight or obese vs. lower BMI; model adjusted for calendar period of conscription examination, parental SES, and WMAX/kg).

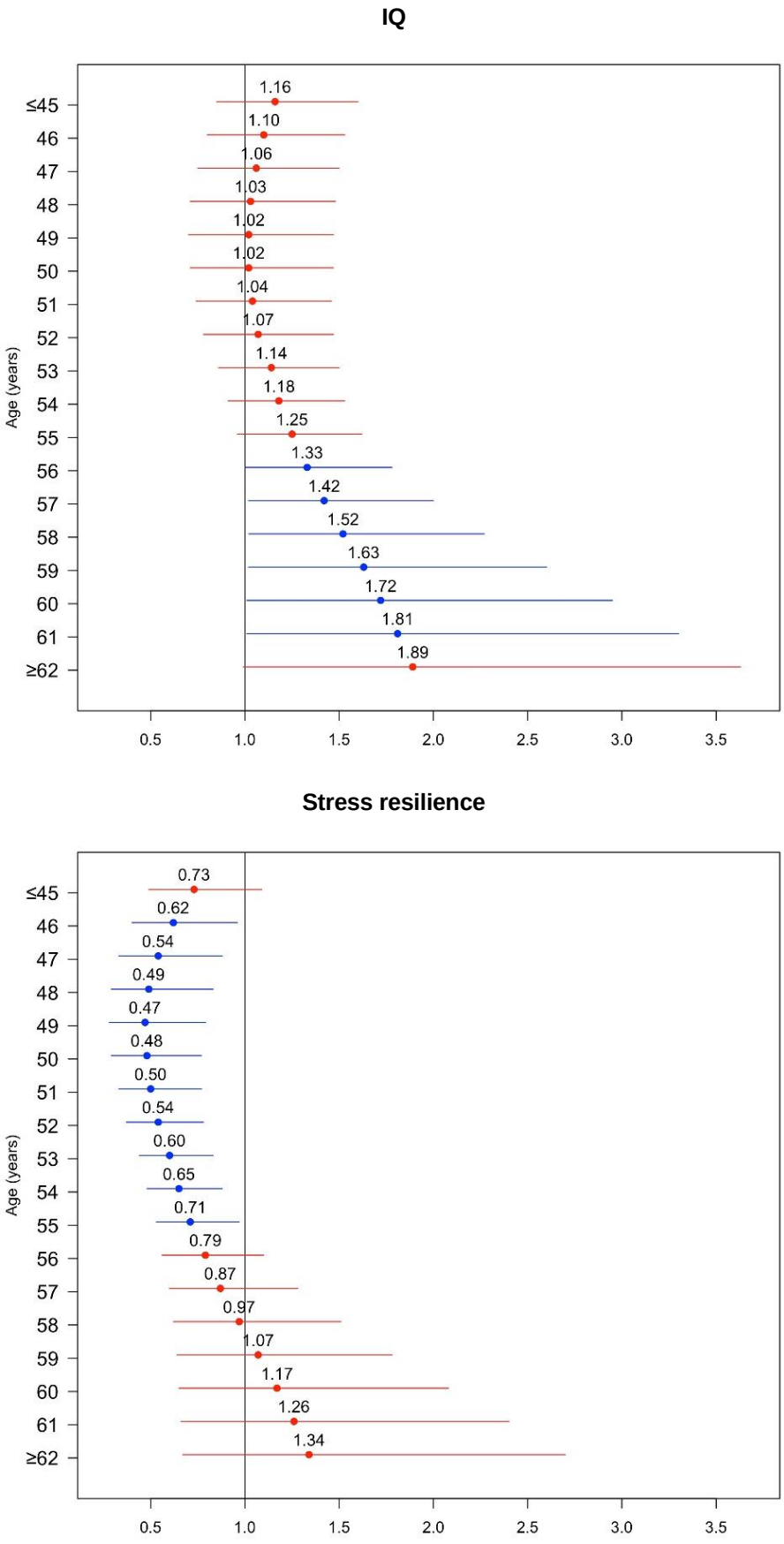


Figure 7. HRs and corresponding 95% CIs of ALS, for IQ and stress resilience above the highest tertile versus lower IQ and stress resilience (model adjusted for calendar period of conscription examination, parental SES, and mutually adjusted for IQ and stress resilience).

8.2 STUDY II – OVERLAP OF NEURODEGENERATIVE AND PSYCHIATRIC DISEASES AMONG ALS PATIENTS AND THEIR FAMILIES

We identified 3,648 ALS patients during the study period, who on average were diagnosed with ALS at age 60 years (SD=11.30). The majority of the patients were males (59.90%). Because the 36,480 controls were matched to the cases on sex, year and month of birth, and county of birth, they had the same sex, age at index date, and county of birth distribution. The age at index date and sex of parents, siblings, half-siblings, and children of ALS patients were comparable to the ones of parents, siblings, half-siblings, and children of ALS-free controls. On average, we identified 1.8 parents, 1.5 siblings, 0.2 half-siblings, and 1.9 children for each ALS patient and for each ALS-free control.

ALS patients, compared to ALS-free controls, had increased risk of all neurodegenerative diseases investigated (OR=3.58, 95% CI=2.89-4.44 before index date; HR=3.95, 95% CI=2.92-5.34 after index date), as well as depression (OR=1.51, 95% CI=1.28-1.77 before index date; HR=2.78, 95% CI=2.05-3.79 after index date), neurotic disorders (OR=1.53, 95% CI=1.27-1.84 before index date; HR=3.07, 95% CI=2.23-4.24 after index date), and drug/abuse dependence (OR=1.80, 95% CI=1.36-2.38 before index date; HR=2.0, 95% CI=1.2-3.4 after index date; Figure 8 and Figure 9). The diseases with the strongest associations with ALS in descending order of magnitude were FTD, PD, other or unspecific dementia, and AD.

The year before ALS diagnosis was the time window with the strongest associations of FTD (OR=40.0, 95% CI=11.3-141.8), PD (OR=9.5, 95% CI=5.2-17.2), other or unspecific dementia (OR=12.1, 95% CI=7.0-20.7), AD (OR=4.7, 95% CI=2.5-9.0), depression (OR=4.8, 95% CI=3.3-7.0), neurotic disorders (OR=4.8, 95% CI=3.0-7.5), drug abuse/dependence (OR=2.8, 95% CI=1.5-5.4) with ALS risk. These associations were also statistically significant up to five years before ALS diagnosis, although of lower magnitude. Similarly, the year after ALS diagnosis was the time window with the strongest associations between ALS and any neurodegenerative or psychiatric diseases (HR=5.02, 95% CI=3.91-6.45), extending up to five years after ALS diagnosis (HR=2.31, 95% CI=1.75-3.06). During the year before ALS diagnosis we also found a positive association of schizophrenia (OR=5.0, 95% CI=1.2-20.1) and stress-related disorders (OR=2.6, 95% CI=1.3-5.5) with ALS risk. The association between ALS diagnosis and stress-related disorders was statistically significant up to five years after ALS diagnosis (HR=2.9, 95% CI=1.2-6.9), with again the strongest association observed during the year following ALS diagnosis (HR=5.5, 95% CI=2.3-13.4).

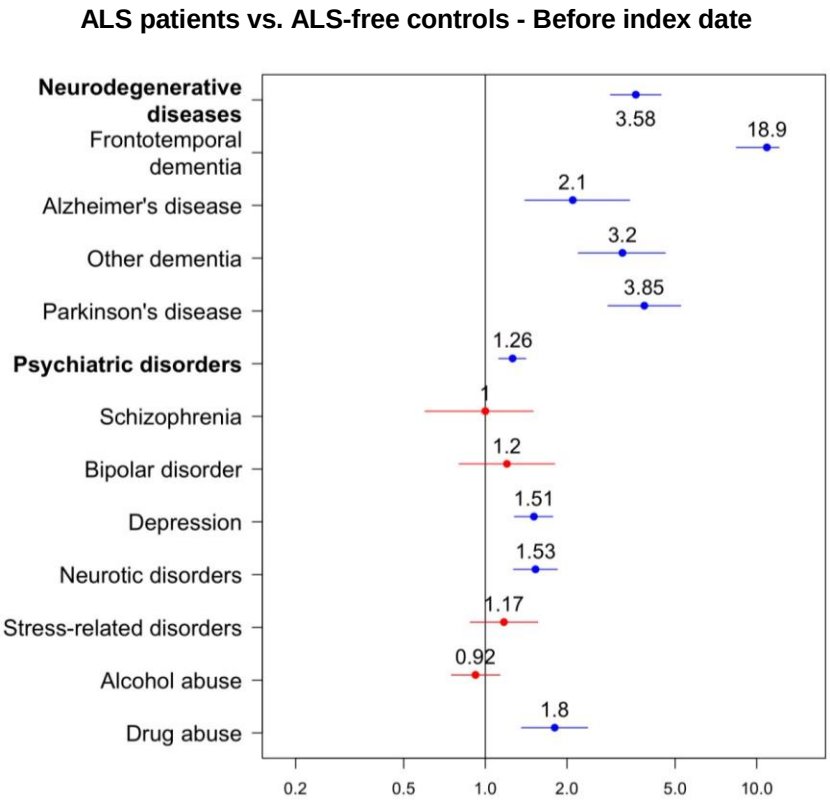


Figure 8. ORs and corresponding 95% CIs of neurodegenerative and psychiatric diseases among ALS patients and matched controls before index date (model adjusted for age, sex, and county of birth).

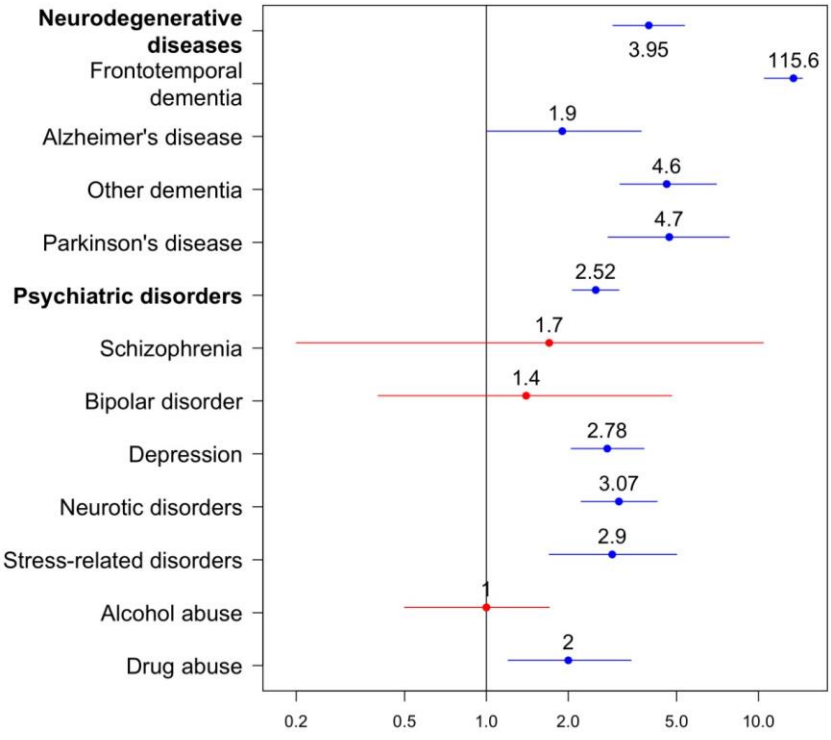


Figure 9. HRs and corresponding 95% CIs of neurodegenerative and psychiatric diseases comparing ALS patients against ALS-free individuals after index date (model adjusted for age, sex, and county of birth).

Relatives of ALS patients, compared to relative of ALS-free individuals, had a higher risk of neurodegenerative diseases before (Figure 10) and after (Figure 11) the index date. However, this association was only statistically significant for siblings (OR=1.41, 95% CI=1.02-1.96 before index date; HR=1.76, 95% CI=1.31-2.36 after index date), but not for parents and children. In contrast, children of ALS patients, compared to children of ALS-free individuals, had a higher risk of psychiatric diseases before (OR=1.11, 95% CI=1.01-1.23; Figure 10) and after (HR=1.11, 95% CI=1.00-1.25; Figure 11) the index date. Half-siblings of ALS patients did not differ in terms of risks of any neurodegenerative or psychiatric diseases, compared to half-siblings of ALS-free individuals, neither before (OR=0.66, 95% CI=0.42-1.07) nor after (HR=1.2, 95% CI=0.6-2.5) the index date.

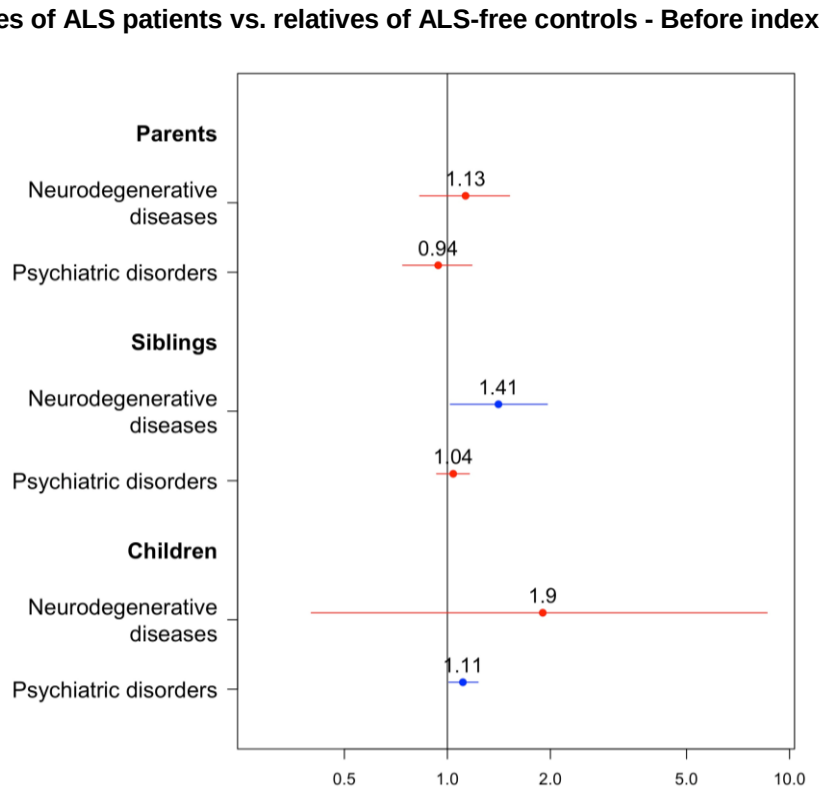


Figure 10. ORs and corresponding 95% CIs of neurodegenerative and psychiatric diseases among relatives of ALS patients and relatives of matched controls before index date (model adjusted for age, sex, and county of birth).

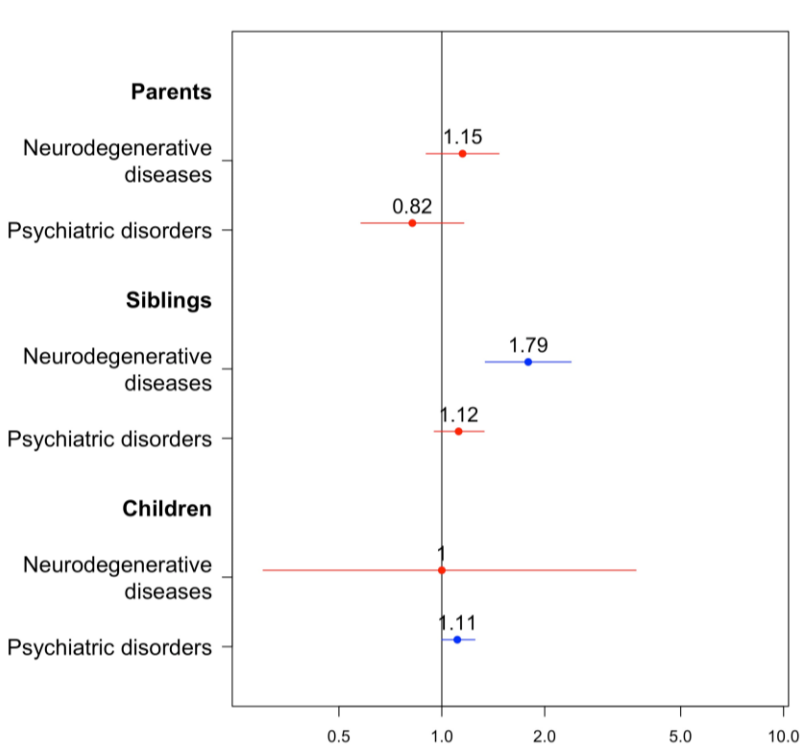


Figure 11. HRs and corresponding 95% CIs of neurodegenerative and psychiatric diseases comparing relatives of ALS patients against relatives of ALS-free individuals after index date (model adjusted for age, sex, and county of birth of the relatives, as well as age, sex, and county of birth of the proband individuals).

8.3 STUDY III – OVERLAP OF NEUROMUSCULAR DISEASES AND ALS

After receiving medical records from 245 out of the 263 patients who were recorded in the Swedish Patient Register as having a diagnosis of both ALS and MS/MG/IP/DMPM, we had enough medical information to validate the diagnoses for 242 of them. We first validated the ALS diagnoses of these patients. Among the 151 patients who were confirmed as having an ALS diagnosis (62.4%), we validated the concurrent diagnoses of MS/MG/IP/DMPM. We found that 26 patients were misdiagnosed with MS, 26 patients were misdiagnosed with MG, 54 patients were misdiagnosed with IP, and 18 patients were misdiagnosed with DMPM. The diagnoses of concurrent ALS and MS/ MG/IP/DMPM were confirmed for 12 patients with ALS/MS, nine patients with ALS/MG, four patients with ALS/IP, and three patients with ALS/DMPM.

Of the 28 patients with a confirmed concurrent diagnosis of ALS and MS/MG/IP/DMPM, 17 were women. The majority of patients (79%) were first diagnosed with MS/MG/IP/DMPM, and then, after a median of 6 years (range 0-53), with ALS. Among the minority of patients diagnosed with ALS first (21%), the median time interval to MS (N=2) or MG (N=4) diagnosis was shorter than one year (range 0-8).

Five ALS patients had the sub-phenotype “PLS”, three had concurrent PLS/MS, and two had concurrent PLS/MG. Among the 12 patients with concurrent ALS/MS, there was enough

information to assess MS subtypes for nine of them, divided among PPMS (N=3, 33.3%), SPMS (N=3, 33.3%), and RRMS (N=3, 33.3%). Seven of the nine patients with concurrent ALS/MG had generalized MG (77.8%), whereas two presented with bulbar MG (22.2%). Among the four patients with concurrent ALS/IP there was enough information to assess IP subtypes for three of them: two presented with Acute Motor Axonal Neuropathy (66.7%), a variant of GBS, whereas one had Chronic Inflammatory Demyelinating Polyneuropathy (CIDP, 33.3%). Polymyositis accounted for two of the three ALS/DMPM patients (66.7%), whereas unspecified myositis for one of them (33.3%).

The patients with confirmed concurrent ALS and MS/MG/IP/DMPM differed in terms of clinical characteristics of ALS from patients diagnosed with only ALS. They were 8.5 years older at ALS onset (median age 70.5 years; P=0.014), presented with a 17.6% higher prevalence of bulbar ALS onset (46.4%; P=0.037), and had a 23.5% lower prevalence of riluzole treatment (64.3%; P=0.001). When treated with riluzole, they had an 18 months shorter median time between ALS diagnosis and first riluzole prescription (1 month; P<0.0001), compared to patients with only ALS. They had also a 30.1% lower prevalence of treatment with non-invasive ventilation (17.9%; P=0.002), compared to patients with only ALS. However, sex (P=0.174), time between ALS onset and diagnosis (P=0.571), cognitive impairment symptoms before ALS diagnosis (P=0.450), use of invasive ventilation (P=1.000), and survival (P=0.579) did not differ between patients with concurrent ALS and MS/MG/IP/DMPM and patients with only ALS.

8.4 STUDY IV – DEPRESSION IN RRMS

Of the 4,867 RRMS patients included in Study IV, there were more women (69.24%) than men (30.76%). On average, the age at RRMS diagnosis was 35.98 years (SD=10.89), and the age at start of first DMT was 36.30 years (SD=10.84). More than half of the patients (55.62%) had interferons as their first DMT, followed by rituximab (12.68%), dimethyl fumarate (10.19%), natalizumab (9.97%), other DMTs (9.08%), and fingolimod (2.47%).

When treated with rituximab, patients had a 47% lower risk of being diagnosed with depression or receiving a prescription of antidepressants, as compared to treatment periods with interferons (HR=0.53, 95% CI 0.36-0.78; Figure 12), holding constant sex, age at start of DMT, geographic region of treatment, bipolar disorder diagnosis, MS severity, relapse at MS debut, and line of DMT. When treated with “other DMTs”, these patients also had a reduced risk of depression and antidepressants prescription (HR=0.63, 95% CI 0.41-0.97; Figure 12).

DMTs and risk of depression or antidepressants prescription among RRMS patients

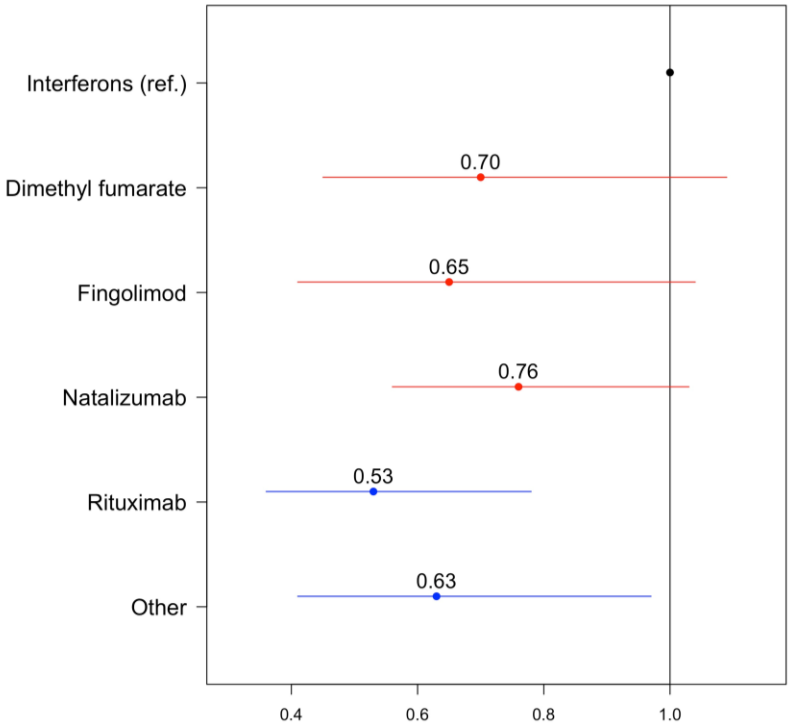


Figure 12. HRs and corresponding 95% CIs of depression or antidepressants prescription during treatment periods with dimethyl fumarate, fingolimod, natalizumab, rituximab, or other DMTs, compared to treatment periods with interferons (model adjusted for time since DMT start, sex, age at start of DMT, geographic region of treatment, bipolar disorder, MS severity, relapse at MS debut, and line of DMT).

In the full model adjusted for sex, age at start of DMT, geographic region of treatment, bipolar disorder diagnosis, MS severity, MS relapse, and line of DMT, we did not find a risk alteration of DMT discontinuation or MS relapse after DMT initiation when comparing patients with a diagnosis of depression or a prescription of antidepressants to patients without a diagnosis of depression or a prescription of antidepressants (Table 4).

Table 4. Associations of depression or antidepressants prescription with the risk of DMT discontinuation and MS relapse among RRMS patients.

	DMT discontinuation	**MS relapse**
	HR (95% CI)[a]	HR (95% CI)[a]
No depression diagnosis	Ref.	Ref.
Depression diagnosis	1.12 (0.86-1.44)	0.82 (0.40-1.66)
No antidepressants prescription	Ref.	Ref.
Antidepressants prescription	1.05 (0.88-1.26)	0.72 (0.44-1.18)

[a] Model adjusted for time since DMT start, sex, age at start of DMT, geographic region of treatment, bipolar disorder, MS severity, relapse at MS debut, and line of DMT.

9 DISCUSSION

9.1 PHYSICAL AND COGNITIVE FITNESS PREDICT EARLY ONSET ALS

Using a nationwide representative cohort of Swedish male conscripts, we found that a specific profile of physical and cognitive fitness already in young adulthood is associated with subsequent diagnosis of ALS.

Specifically, we found that high levels of physical fitness and IQ, but lower BMI and stress resilience, were associated with early onset ALS. We concluded that these associations were significant for early onset ALS because in our cohort we identified a group of ALS patients who were on average 48 years old at ALS diagnosis, whereas the mean age at diagnosis of ALS reported in European population-based studies is about 65 years[182]. Patients with early-onset ALS differ from other ALS patients in terms of pathological features and survival[183].

Our findings confirm the results of Mattsson et al.[52], who found an association between physical fitness and ALS. We further show that the association of exposure to greater physical fitness in early life might be a specific risk factor for ALS diagnosis before age 45 years, but not thereafter. This finding dovetails with the observation that soccer players are at an increased risk of early onset ALS[47-51]. Intensive exposure to non-genetic risk factors in early life, such as physical activity, might be one of the underlying reasons for early onset ALS, in addition to a genetic predisposition[183].

We also confirmed that BMI is inversely associated with ALS risk[184] and showed that metabolic changes among ALS patients might occur as early as at age 18.

We are the first to empirically confirm the clinical impression that ALS patients have a specific cognitive profile by showing that high levels of IQ, before secondary education is obtained, are associated with an increased risk of ALS. IQ is therefore possibly one of the reasons leading to high educational attainment among some ALS patients[185]. We are also the first to show that a lower level of stress resilience at young age is associated with increased risk of later ALS. This provides further support to the hypothesis that psychological stress might contribute to the initiation of motor neuron degeneration[186].

It is interesting to note that our findings of a specific physical and cognitive fitness profile in early life might be specific to ALS but not to other neurodegenerative diseases. For example, similar studies conducted in the Swedish Conscript Register found that high levels of physical fitness in early life was associated with a lower risk of MS, and that there was no association of BMI, IQ, or stress resilience with the risk of MS[187].

9.2 SHARED ETIOLOGY OF NEURODEGENERATIVE DISEASES AND STRESS REACTION OF ALS DIAGNOSIS

Using two nested case-control and follow-up studies from a population-based study sample, we showed that ALS patients suffered from an increased risk of neurodegenerative and

psychiatric diseases both before and after ALS diagnosis. The increased risk of neurodegenerative diseases was also found among first-degree relatives of ALS patients, whereas the increased risk of psychiatric disorders was observed only among children of ALS patients.

We confirmed previous research showing an association between some neurodegenerative diseases after ALS diagnosis[77,78] and further extended these findings by highlighting that the increased risk of several neurodegenerative diseases is present years before until years after ALS diagnosis. We found that this association was not limited to ALS patients but also, as shown previously[79], to their first-degree relatives. Because first-degree relatives share about 50% of the segregating genes, these results provide evidence for a shared genetic etiology between different neurodegenerative diseases[79-81]. The accumulation of protein aggregates commonly found in the brain of patients with neurodegenerative diseases could lead to neuroinflammation[188] and might therefore be one of the possible underling mechanisms. The observation that the association between neurodegenerative diseases and ALS was present even five years before ALS diagnosis is a unique finding, which argues against misdiagnosis of neurodegenerative diseases and surveillance bias as the pure explanations for these findings.

We also confirmed the results of previous studies regarding an association between psychiatric disorders and ALS[88,89]. Our unique contribution includes showing that this association was present already five years before diagnosis and peaked during the year before and the year after ALS diagnosis. This might imply that in addition to non-motor symptoms of ALS mimicking psychiatric disorders[83], a stress reaction might occur along with the onset of ALS symptoms and in relation to receiving a fatal diagnosis. The significantly increased risk of schizophrenia during the year preceding ALS diagnosis might be explained by shared genetics, as suggested by a GWAS study[189].

We did not confirm the previously reported inverse association between alcohol abuse and ALS risk[92,190-192]. Our results might have suffered from lack of adjustment for smoking and diet. However, we did confirm the previously suggested association between use of opioids and ALS[93], and further extended it to drug abuse/dependence including medicines, cocaine, caffeine, opioids, and cannabis. Although depression and stress-related disorders might increase the risk of drug abuse/dependence[193-195], the association remained after excluding individuals with concurrent diagnoses of stress-related disorders and drug abuse/dependence.

We also found an increased risk of psychiatric disorders among children of ALS patients, but not among the other first-degree relatives. This implies that the association might not be driven by shared genetics. Instead, the association might be attributable to the psychological distress due to high involvement of children of ALS patients in the care of their parents[196].

9.3 NEUROINFLAMMATION AROUND THE MOTOR UNIT MIGHT TRIGGER ALS

By following the entire Swedish population for 23 years, we were able to confirm a concurrent diagnosis of ALS and MS/MG/IP/DMPM in 28 patients. Because we showed that the majority

of these patients were diagnosed with neuroinflammatory disorders years before ALS diagnosis, this provides evidence for the role of neuroinflammation around the motor unit in triggering the onset of ALS. We further showed that these patients had different clinical characteristics of ALS compared to patients with ALS alone, and they might hence represent a specific albeit very small subgroup of ALS.

Our study, which is the largest to date, replicates past case-reports and epidemiological studies showing an overlap between ALS and MS, ALS and MG, and ALS and DMPM[57-63,67-70,197-201]. We are the first to report on the overlap between ALS and GBS as well as ALS and CIDP, suggesting that both chronic and acute neuroinflammation around the motor unit might play a role in ALS onset.

Because we were able to describe onset and date of diagnosis of the concurrent neuroinflammatory diseases with respect to ALS, this implies that neuroinflammation appears to precede rather than follow ALS onset. Furthermore, in a minority of patients where ALS was diagnosed before MS and MG, the median time interval between diagnoses was less than one year. This might suggest that MS and MG in such patients were formally diagnosed after ALS, but were in fact already present before ALS onset.

When we compared patients with concurrent ALS and MS/MG/IP/DMPM to patients with only ALS, we found that concurrent patients were more likely to have bulbar ALS onset, they were older at ALS onset, and they were treated less frequently with riluzole and non-invasive ventilation. Patients with bulbar onset of ALS present with neurofibrillary tangles, basophilic inclusions, and unusual pathology of the extra motor cortical regions[202]. The higher prevalence of bulbar onset patients in the concurrent ALS and MS/MG/IP/DMPM group might explain why we found that concurrent patients were less frequently treated with non-invasive ventilation, given that they are less likely to tolerate non-invasive ventilation than patients with limb onset[203]. However, it is also possible that this difference might be due to fewer respiratory symptoms or a slower disease progression. The hypothesis of neuroinflammation around the motor unit playing a role in triggering ALS onset is further supported by the older age at ALS onset as a possible consequence of longer induction time in this subgroup of patients.

By validating the medical records of the patients with a register-based diagnosis of ALS and MS/MG/IP/DMPM in Sweden, we showed that in most cases one of the two diseases had been misdiagnosed. This is a result that might not be specific to the Swedish diagnostic system. Hence, we recommend that researchers exercise caution when using register-based diagnosis to study the concurrence between these diseases. However, we do not aim to discredit the accuracy of the Swedish Patient Register, or register-based research for ALS in general, as the high percentage of misdiagnosis in our study is not an accurate representation of overall quality of ALS diagnoses in the Swedish Patient Register. We believe that this finding is specific to this particular and very small group of patients with unspecific symptoms at disease onset. Indeed, our research group previously validated the register-based diagnosis of ALS in the Stockholm county and found a positive predictive value of 91%, which extended to 97% when PLS was included in the definition of ALS[19].

9.4 RITUXIMAB LOWERS THE RISK OF DEPRESSION IN RRMS

By prospectively following a nationwide population-based cohort of RRMS patients, we showed that when patients were prescribed rituximab, the risk of depression or antidepressants prescription was lower compared to when patients were prescribed interferons. We did not find evidence for an effect of depression or antidepressants on DMT discontinuation, nor on MS relapse after DMT start.

We are the first to identify an inverse association between rituximab and depression or antidepressants prescription in RRMS. We speculate that rituximab might act on inflammatory pathways related to depression, in addition to the inflammatory pathways of MS. It is also possible that patient satisfaction of rituximab, an effective drug with good tolerability profile and lower discontinuation than other DTMs[204-207], might be one of the explanations for the observed lower risk of depression or antidepressants prescription.

In contrast to previous research reporting an inverse association between depression and MS relapses[208-210], we did not find an association between receiving a depression diagnosis or being prescribed antidepressants and DMT discontinuation or MS relapse. The fact that we excluded the TCAs and SNRIs from our definition of antidepressants might have explained to some extent such contrasting findings.

9.5 STRENGTHS

The findings discussed in this thesis are based on population-based nationwide studies with prospective design. We were able to conduct extensive follow-up on all study participants, with virtually no attrition (except in the rare case when individuals emigrated out of Sweden, which is believably non-differential). Therefore, with the exception of Study I (where we only investigated males), the findings should be generalizable to the entire Swedish population.

All exposure variables were assessed independently of the later outcomes and are therefore not influenced by biases in relation to outcome status (such as recall bias). Moreover, all variables have satisfactory validity because they were assessed and validated by health professionals or systematically recorded by governmental agencies.

Furthermore, we had the unique advantage of being able to objectively identify family members, as well as their disease diagnoses, making it possible to assess potential genetic and environmental contributions to the associations of interest. Overall, the large sample size of all studies allowed for sufficient statistical power in detecting effect sizes of smaller magnitudes, despite the low prevalence of ALS. We were also able to validate and study an extremely small subgroup of ALS patients that were concurrently diagnosed with other neuromuscular diseases, resulting in the largest study to date on the concurrence of ALS and MS/MG/IP/DMPM.

9.6 LIMITATIONS

Despite our efforts to account for all available confounding factors, we lacked information about life-style factors such as smoking and dietary habits. Hence, residual confounding

remains a possibility. Life-style factors might also have acted as mediators, and studying such pathways might have led to a greater understanding of the underlying mechanisms for some of the observations made in the thesis.

We also lacked information about the genetic characteristics of the ALS patients identified. It was therefore not possible to examine if our findings are specific to sporadic ALS or ALS with known mutations. In Studies I and II, we were also unable to examine if our findings were applicable to ALS of different clinical subtypes.

We might have underestimated the prevalence of some psychiatric disorders not requiring specialist care. For example, some patients with psychiatric disorders manifesting with less severe symptoms might not get referred by their general practitioner, or might choose not to engage with a specialist. Such individuals would not end up in the Swedish Patient Register. Hence, the results pertaining to psychiatric disorders might only be generalizable to the more severe forms of the disorders.

When estimating the age-specific associations between physical and cognitive fitness and ALS, we did not have a sufficiently large number of ALS patients to gain adequate statistical power for each age band investigated. Therefore, caution is needed when interpreting the results in terms of specific ages at risk.

We were not able to follow the children of ALS patients and ALS-free individuals long enough to reach the peak age for the diagnosis of neurodegenerative diseases. Hence, the analysis of risk of neurodegenerative diseases among children of ALS patients might have suffered from a so-called "right truncation effect" which would lead to an underestimation of the true estimate.

It is possible that the reported differences in terms of ALS treatment between patients with only ALS, and patients with concurrent ALS and MS/MG/IP/DMPM, are due to a cohort effect. Nevertheless, most of the patients with concurrent diagnoses received the ALS diagnosis in 1997 and thereafter, when the practice of non-invasive ventilation treatment was already common[211], as well as treatment with riluzole[212]. We compared patients with concurrent ALS and MS/MG/IP/DMPM visited at hospitals from the entire Sweden to patients with only ALS attending hospitals of the Stockholm area. It is therefore possible that factors like SES or genetics might contribute to some of the observed differences.

Surveillance bias might have played a role in the protective effect of rituximab treatment on the risk of depression or antidepressants prescription among RRMS patients. Because rituximab is administered twice a year as an infusion, whereas natalizumab is administered every month, a less frequent contact with healthcare staff might not have prompted the detection of depressive symptoms. On the other hand, in comparison to other DMTs such as fingolimod and dimethyl fumarate (which are taken orally) and interferons (which are self-administered injections), rituximab requires more contacts with the clinics.

10 CONCLUSIONS

In summary, based on collective evidence from the present thesis, we found evidence that neuroinflammation might precede the onset of ALS (Figure 13).

In a small subgroup of ALS patients, motor neuron degeneration might be triggered by chronic or acute neuroinflammation around the motor unit (Study III).

High level of physical fitness in young adulthood, possibly reflecting intense levels of physical activity and the resultant inflammatory status in proximity to the muscles, tendons, joints, and peripheral nerves, is associated with a higher risk of early onset ALS (Study I).

Different neurodegenerative diseases and ALS might share common disease mechanisms including neuroinflammation, which might in part explain the increased risk of neurodegenerative diseases among ALS patient from years before ALS diagnosis (Study II).

Neuroinflammation might also be a contributing factor to the increased risk of psychiatric disorders among ALS patients, in addition to a strong stress response to early symptoms of ALS and receiving the fatal diagnosis. The severe stress response to ALS diagnosis might also have contributed to the increased risk of psychiatric disorders observed among children of ALS patients (Study II).

Treatment with rituximab might decrease the risk of depression and antidepressants prescription among RRMS patients. One potential explanation might be through modulating both the MS- and the depression-related inflammatory responses in RRMS (Study IV).

Thesis hypotheses

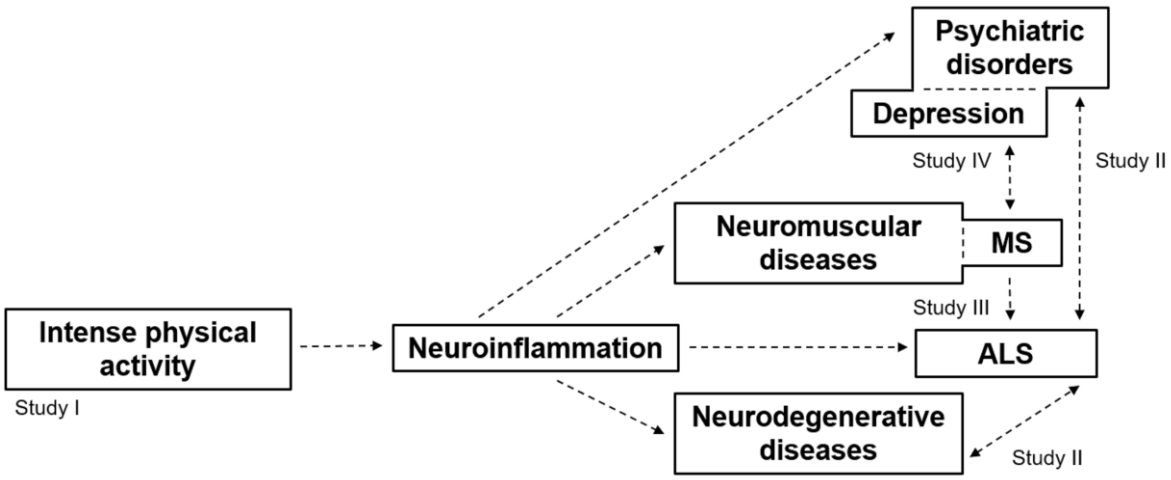
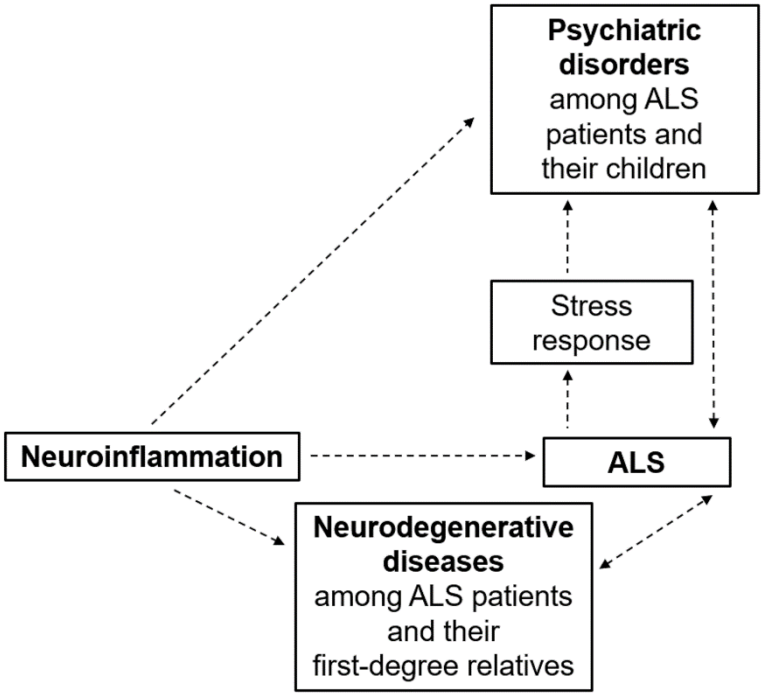


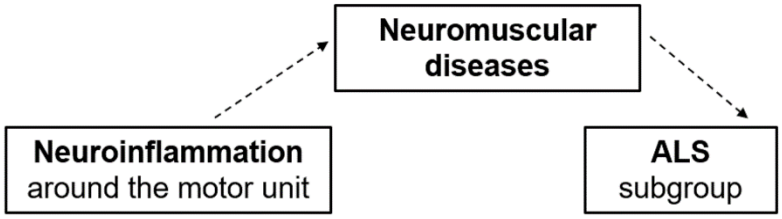
Figure 1. Schematic representation of the thesis hypotheses (reprinted for convenience).

Conclusions Study I

Conclusions Study II



Conclusions Study III



Conclusions Study IV

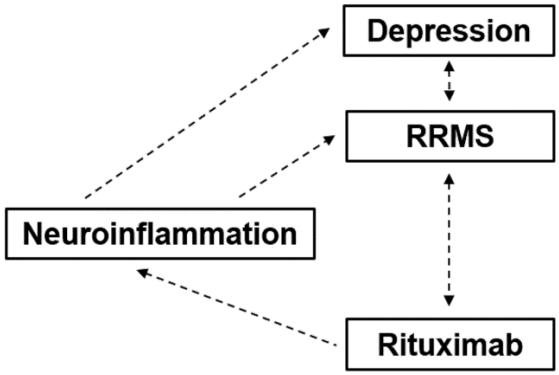


Figure 13. Schematic representation of the conclusions drawn from the results of the constituent papers of this thesis. Decomposed and adapted from the schematic representation of the thesis hypothesis depicted in Figure 1.

10.1 FUTURE RESEARCH AND CLINICAL IMPLICATIONS

Because of intrinsic limitations of the studies of this thesis, further research is needed to continue exploring the role of neuroinflammation in ALS and MS and ultimately help to draw clinical recommendations. We recommend the following:

1. Replicate all findings outside Sweden to test if these results are generalizable to other populations.
2. Investigate if high level of physical activity in young adulthood predicts risk of ALS among females. It would also be interesting to investigate if physical fitness later in life also predicts ALS diagnosis. Should genetic prediction improve, it might be advisable to refrain from engaging in intense physical activity among individuals with high susceptibility of ALS.
3. Pool together patients with concurrent ALS and MS/MG/IP/DMPM from different countries in order to obtain a sufficiently large sample size to examine if patients with such rare concurrences differ in terms of other important clinical characteristics. Studying potential differences in outcomes such as survival might eventually lead to further identification of therapeutic options.
4. Conduct further studies to confirm the protective effect of rituximab on depression as well as on overall quality of life. If confirmed, it might be worthwhile to promote the psychological benefits of rituximab as effective DMT for RRMS patients.

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