Cognition, disease activity and MRI changes in early multiple sclerosis

PhD thesis

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A series of studies have found that a feeling of gratitude is associated with subjective well-being (Emmons and McCullough 2003; Wood et al. 2008, 2010). Whether these phenomena really are separate entities, whether there is a causal relationship between them and whether this proposed association is valid also for PhD students is outside the scope of this thesis. However; I am grateful for having had the opportunity to work on this project and to all the people who have helped me and kept me company while I worked on it.

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Abbreviations

BDI	Beck Depression Inventory
BVMT	Brief Visuospatial Memory Test-Revised
CNS	Central nervous system
CVLT	California Verbal Learning Test
FSS	Fatigue Severity Scale
FLAIR	Fluid-attenuated inversion recovery
HLA	Human Leukocyte Antigen
LC	Locus coeruleus
LCS	Low Cognitive Score
GM	Gray matter
OUS	Oslo University Hospital
MPRAGE	magnetization-prepared rapid gradient echo
MS	Multiple sclerosis
MRI	Magnetic resonance imaging
NA	Noradrenaline
NAWM	Normal-appearing white matter
NP	Neuropsychological
RRMS	Relapsing-remitting MS
SDMT	Symbol Digit Modalities Test

UiO	University of Oslo
WASI	Wechsler's Abbreviated Scale of Intelligence
WM	White matter

1. List of publications included

Paper I

Gro O. Nygaard, Kristine B. Walhovd, Piotr Sowa, Joy-Loi Chepkoech, Atle Bjornerud, Paulina Due-Tonnessen, Nils I. Landro, Soheil Damangir, Gabriela Spulber, Andreas B. Storsve, Mona K. Beyer, Anders M. Fjell, Elisabeth G. Celius and Hanne F. Harbo.

Cortical thickness and surface area relate to specific symptoms in early relapsing-remitting multiple sclerosis. Multiple Sclerosis Journal, 2015 Apr;21(4):402–14.

Paper II

Gro O. Nygaard, Elisabeth G. Celius, Sigrid A. de Rodez Benavent, Piotr Sowa, Marte W Gustavsen, Anders M. Fjell, Nils I. Landrø, Kristine B. Walhovd and Hanne F. Harbo.

A longitudinal study of disability, cognition and gray matter atrophy in early multiple sclerosis patients according to evidence of disease activity. PLoS One, 2015 Jan; 10(8):e0135974.

Paper III

Gro O. Nygaard*, Sigrid A. de Rodez Benavent*, Hanne F. Harbo, Bruno Laeng, Piotr Sowa, Soheil Damangir, Kristian Bernhard Nilsen, Lars Etholm, Siren Tønnesen, Emilia Kerty, Liv Drolsum, Nils I. Landrø, Elisabeth G. Celius

Eye and hand motor interactions with the Symbol Digit Modalities Test in early multiple sclerosis. Multiple Sclerosis and Related Disorders 2015; 4(6):MSARDD1500117

Paper IV

Sigrid A. de Rodez Benavent*, Gro O. Nygaard*, Hanne F. Harbo, Siren Tønnesen, Piotr Sowa, Nils I. Landrø, Marte W. Gustavsen, Lars Etholm, Kristian B. Nilsen, Liv Drolsum, Emilia Kerty, Elisabeth G. Celius, Bruno Laeng

Pupillary responses to problem-solving in early multiple sclerosis patients. Submitted.

*The authors contributed equally

2. Introduction

2.1 Impact of the disease

Multiple sclerosis (MS) is a neurological disease of unknown cause most often affecting young adults with a peak incidence around age 30 (Koch-Henriksen and Sørensen 2010). The disease may lead to a range of different neurological signs and symptoms, including motor, sensory, visual, cognitive, energy-related and depressive symptoms (Compston and Coles 2008). It frequently leads to working inability, especially in the patients with a severe disease course (Kobelt et al. 2006; Pfleger et al. 2010; Glad et al. 2011), to a reduced quality of life both in patients (Miller and Allen 2010) and caregivers (Patti et al. 2007), and to excess mortality (Smestad et al. 2009; Kingwell et al. 2012). Around 380 000 individuals were affected by MS in Europe in 2005, resulting in an estimated total annual cost of €12.5 billion (Sobocki et al. 2007). Estimates from 2013 indicated that 2.3 million people were afflicted with the diagnosis worldwide (Http://www.msif.org/about-us/advocacy/atlas/). Thus the disease has major consequences, both to patients, health care systems and society (Naci et al. 2010).

2.2 Epidemiology

There are large differences in the reported prevalence of MS, both within and between countries. Prevalence estimates from Norway are among the highest in the world, with 203 per 100 000 inhabitants in a recent study (Berg-Hansen et al. 2014a). In contrast prevalence rates in regions of Asia are below five per 100 000 inhabitants (Koch-Henriksen and Sørensen 2010). A latitude gradient of prevalence has been identified, with a high prevalence in Northern Europe, North America, Australia and New Zealand, and a lower prevalence in South-America, Africa and Asia (Figure 1) (Http://www.msif.org/about-us/advocacy/atlas/; Koch-Henriksen and Sørensen 2010; Simpson et al. 2011). There's a tendency towards higher prevalence and incidence in all investigated regions with time (Koch-Henriksen and Sørensen 2010).

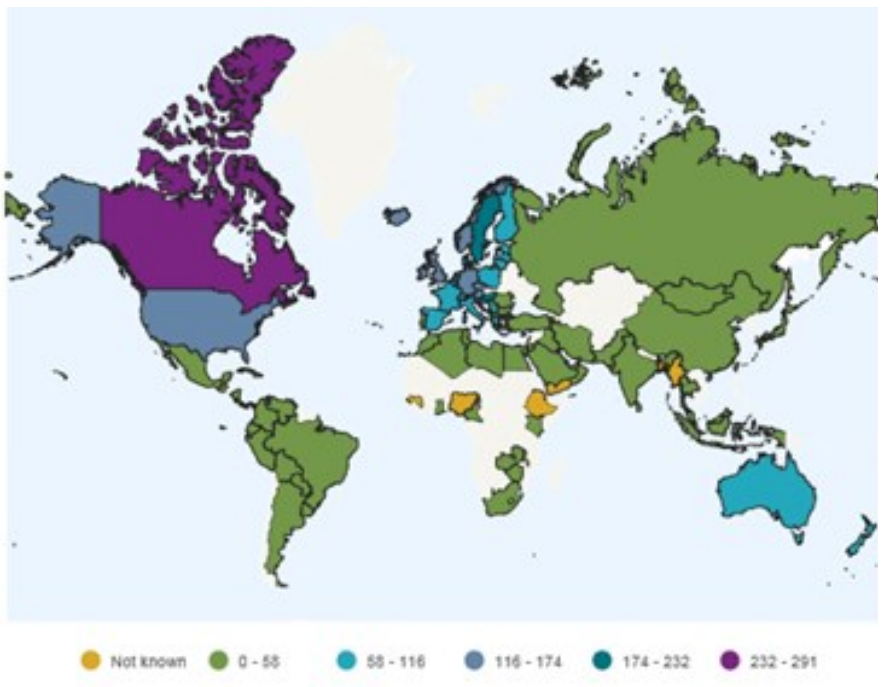

Figure 1. World prevalence of MS in 2013. The figure is based on published studies in 2008-2013. The color codes indicate prevalence per 100 000 inhabitants. Printed with permission from Multiple Sclerosis International Federation.

During the last century an increased female-to-male ratio has been observed, and in Norway the most recent study reports a female-to-male ratio of 2.2:1 (Koch-Henriksen and Sørensen 2010; Berg-Hansen et al. 2014a).

Early epidemiological studies identified a difference in MS prevalence between the coastal and rural regions of Norway (Swank et al. 1952). However, the most complete prevalence study to date neither found any difference in prevalence between different regions of Norway nor a latitude gradient (Berg-Hansen et al. 2014a).

Difference in MS risk between different ethnic groups have been reported worldwide (Milo and Kahana 2010), and a lower risk of MS has been identified in the Sami population compared to Caucasians in Norway (Grønlie et al. 2000; Harbo et al. 2007). Migration studies indicate that migration from low-risk to high-risk regions may alter the individual MS risk, but that migration from high-risk to low-risk countries may not give the corresponding risk-reduction (Milo and Kahana 2010). Recent studies from Norway indicate that in ethnic groups with low MS risk in their home country, second generation immigrants develop a higher MS risk (Smestad et al. 2008; Berg-Hansen et al. 2014b). Hence epidemiological studies indicate that both environmental and genetic factors are involved in the risk of acquiring the disease.

Further, ethnicity may influence the manifestation of symptoms at onset or the disease course. Most studied are the differences between African American and White Americans in the USA. These studies show that African Americans often have a higher magnetic resonance imaging (MRI) lesion burden, multiple symptoms at onset and a more severe disease compared to Caucasian Americans (Cree et al. 2004; Kister et al. 2010; Weinstock-Guttman et al. 2010). A recent Norwegian study found that disease severity was higher in the immigrants studied than in the native Norwegian population (Berg-Hansen et al. 2013). Thus ethnicity may not just affect the risk of the disease, but also the disease course.

2.3 Risk factors for MS

The large differences in the reported prevalence of MS indicate that there are regional risk factors for disease susceptibility. Extensive research has shown that both genetic and environmental factors contribute to the risk of acquiring the disease (McKay et al. 2015).

The most important environmental

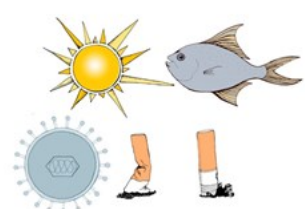

Figure 2. The most important environmental factors associated with MS disease susceptibility are low levels of vitamin D, infections and smoking. Illustration: MS Research Group OUS/Gro Owren Nygaard

factors associated with increased disease susceptibility are low levels of Vitamin D, infections and smoking (Figure 2) (Ascherio 2013). Longitudinal studies have observed associations between serum levels, dietary or supplemental intake of Vitamin D and MS risk later in life (Munger et al. 2004, 2006). A recent review systematically collected material from systematic reviews and meta analyses in the field, and found a summary odds ratio (OR) of 4.5 for anti-Epstein-Barr nuclear antigen sero-positivity, an OR of 2.17 for infectious mononucleosis and an OR of 1.52 for smoking (Belbasis et al. 2015). Body size, gut-microbiota and nutritional salt intake may also be contribute to disease susceptibility or modulate the disease course (Munger et al. 2009; Berer et al. 2011; Kleinewietfeld et al. 2013; Wesnes et al. 2014; Farez et al. 2015). These environmental factors probably all contribute to increased MS risk though modulations of the immune system (Ascherio 2013; Kutzelnigg and Lassmann 2014).

Both migration studies and studies of disease risk in families support a role for susceptibility genes in the development of multiple sclerosis (Milo and Kahana 2010; Westerlind et al. 2014). More than forty years ago genetic studies found associations between certain HLA alleles and MS risk (Jersild et al. 1972). It is now established that the HLA allele DRB1:1501 is the major genetic risk factor in MS, increasing the risk of developing MS threefold among Northern Europeans (Compston and Coles 2008). Recent years large international collaborations have led to the identification of 110 single nucleotide polymorphisms (SNPs) associated with increased MS risk (Figure 3) (Sawcer et al. 2011; International Multiple Sclerosis Genetics Consortium 2013). With these findings around a quarter of the reported heritability in MS can be accounted for (Sawcer et al. 2014). The

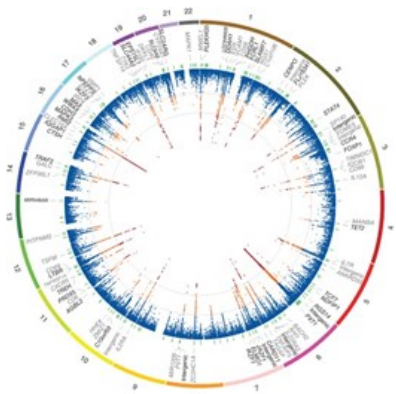

Figure 3. Circus plot of 110 SNPs associated with increased MS risk. International Multiple Sclerosis Genetics Consortium 2013, printed with permission.

identified SNPs are mainly located in the previously reported HLA regions or in non-coding regions of the genome and are probably involved in the regulation of the adaptive immune system (Sawcer et al. 2014). Most of these SNPs add minimally to the disease susceptibility, with odds ratios below 1.5. However, an accumulation of risk genes is related to a younger age at onset and may increase risk of disease and presence of cerebrospinal fluid OCBs (De Jager et al. 2009; Harbo et al. 2013). Further, the interaction between genetic and environmental risk factors may lead to accumulated MS risk in some patients, as has been shown for smoking and childhood obesity combined with unfavorable HLA genotypes (Hedström et al. 2011, 2014).

2.4 Pathology of MS

We do not know the cause of multiple sclerosis, but the leading hypotheses concerning the initiation of the disease may be explained as illustrated in Figure 4. The risk of disease increases with certain genetic variants, as well as environmental factors (Sawcer et al. 2011). Disease is triggered by a stimulus either external (the *outside-in hypothesis*) or internal to the CNS (the *inside-out hypothesis*) (Hemmer et al. 2015).

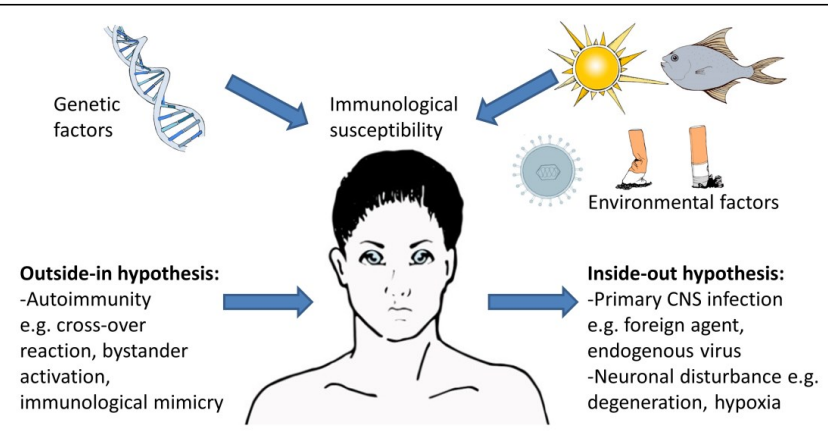

Figure 4. Genetic and environmental risk factors contribute to disease susceptibility. Illustration: MS Research Group OUS/Gro Owren Nygaard

This event initiates a cascade of pathological events, involving both the innate and adaptive immune system.

2.4.1 Inflammation, demyelination and reparative mechanisms

Pathology studies of MS patients date back to the 19th century, with macroscopic studies and drawings of lesions of the brainstem and spinal cord accompanied by atrophy (figure 5) (Carswell 1838; Cruvhelhier 1842) and microscopic description of CNS veins with surrounding lesions (Rindfleisch 1863). Disruption of the blood-brain barrier, focal demyelinating lesions with axonal damage and reactive glial scar formation in gray and white matter all contribute to axonal, synaptic and neuronal loss (Figure 6) (Kutzelnigg and Lassmann 2005, 2014; Dutta and Trapp 2007; Bø 2009). This damage leads to the irreversible neurological disability associated with the disease (Tallantyre et al. 2010; Popescu et al. 2013).

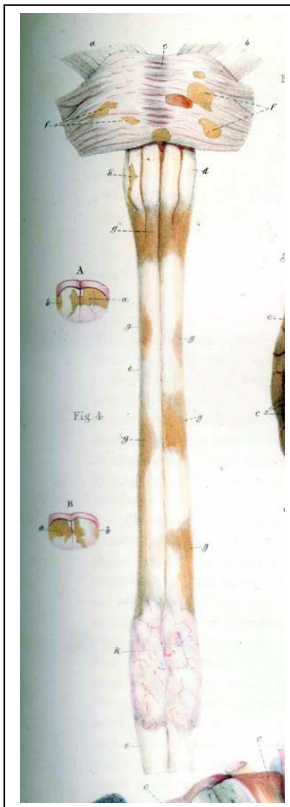

Figure 5. Macroscopic drawing of MS lesions in brainstem and medulla. Carswell, 1838. Printed with permission from Glasgow University.

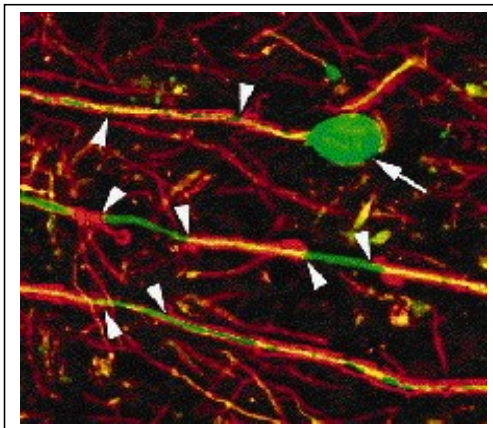

Figure 6. Demyelination and axonal damage. Confocal microscopy of axons with active demyelination, transection and axonal swelling. Printed with permission from Trapp BD et al. 1998, Copyright Massachusetts Medical Society.

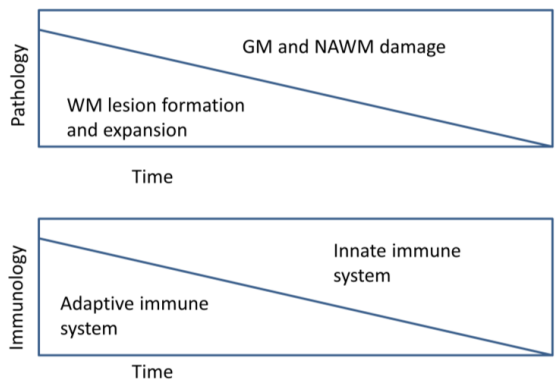

Figure 7. Pathological and immunological mechanisms change with MS disease duration. Adapted from Kutzelnigg 2014 and Hemmer 2015. Illustration: Gro Owren Nygaard

Focal inflammatory demyelinating lesions in the WM dominate the pathology in acute and relapsing MS, while cortical lesions, atrophy and damage to NAWM dominate in the progressing disease (Figure 7) (Kutzelnigg and Lassmann 2014; Mahad et al. 2015).

Variable degrees of remyelination have been identified in MS plaques. While almost all plaques are remyelinated in some patients with very long disease duration, remyelination can hardly be identified in others (Barkhof et al. 2003; Patrikios et al. 2006; Goldschmidt et al. 2009). Remyelination seems to be a continuous reparative process, contributing to the formation of new, thin myelin sheaths surrounding axons both in the relapsing and the progressive phase of the disease (Franklin and Ffrench-Constant 2008; Mahad et al. 2015). This remyelination may protect the axons against chronic damage by reducing energy demand for signal conduction and the reestablishment of a protective extracellular environment (Trapp and Stys 2009).

2.4.2 White matter damage in MS

White matter lesions are typically round or oval, and are mainly located periventricularly, juxtacortically, infratentorially and in the spinal cord (Figure 8) (Brownell and Hughes 1962; Polman et al. 2011). Inflammatory cells from both the adaptive and innate immune system contribute to demyelination, damage to the myelin-forming oligodendrocytes, axonal damage and neuronal death in active lesions (Trapp et al. 1998). Combined MRI and pathology studies have shown a high correspondence between T2 and FLAIR detectable lesions and these sites of

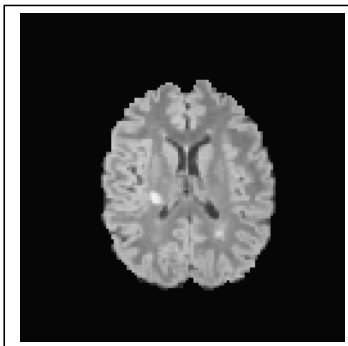

Figure 8. WM lesions on axial FLAIR image of MS patient. Illustration: Piotr Sowa/Gro Owren Nygaard

inflammation, demyelination and remyelination (Filippi et al. 2012). A higher T2 lesion load, i.e. a larger volume of white matter lesions visible on T2 weighed MRI images, increases the risk of conversion from CIS to MS (Kuhle et al. 2015). Radiological progression is also associated with a higher future relapse rate (Sormani et al. 2013). Furthermore, new or enlarging WM lesions, together with hypointense T1 lesions and brain atrophy, predict future disability progression (Bermel et al. 2013; Giorgio et al. 2013; Popescu et al. 2013).

While new active WM lesions mainly appear in patients with acute and relapsing multiple sclerosis, diffuse injury of the normal-appearing white matter (NAWM) is more prominent in primary and secondary progressive multiple sclerosis (Kutzelnigg et al. 2005). A pathology study of MS patients, mainly in the progressive stage of the disease, found that 72 % of NAWM investigated was histologically abnormal (Allen and McKeown 1979). The abnormalities included gliosis, demyelination, immune cell infiltration and perivascular deposits (Allen and McKeown 1979).

2.4.3 Gray matter damage in MS

Damage to gray matter (GM) in MS patients has been described for more than a century (Bø 2009). However, the impact of GM lesions and atrophy on disability has become evident through extensive research the last decades (Geurts et al. 2012).

Cortical GM lesions are found in all disease stages, but are most prominent in patients with a long disease course (Figure 9) (Giorgio et al. 2011; Filippi et al. 2013b), Most cortical lesions are intracortical or located at the border between GM and WM (Bø et al. 2003). However, in a subset of patients subpial cortical lesions dominate, indicating meningeal involvement in the disease process (Bø et al. 2003; Calabrese et al.

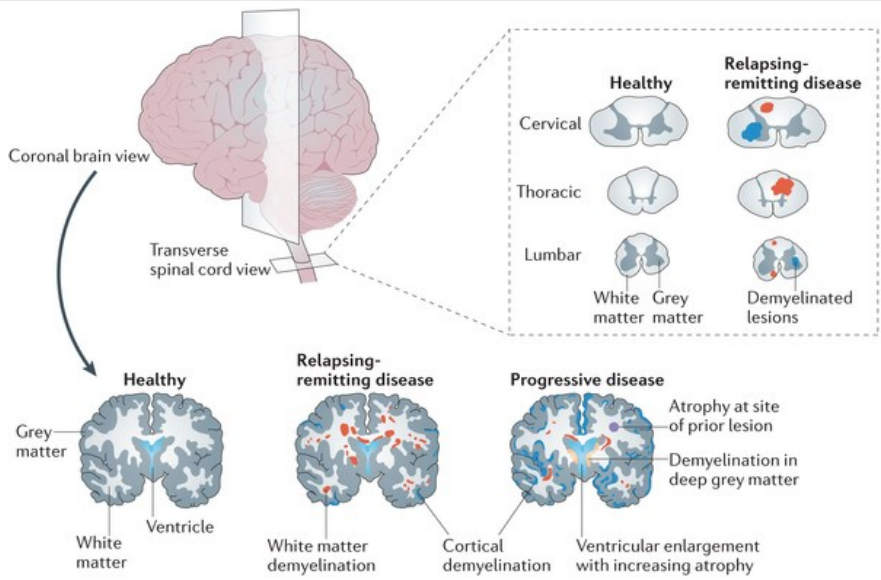

Figure 9. Damage to white and gray matter in MS. Printed with permission from Dendrou, Fugger, and Friese 2015 and Nature Reviews Immunology.

2015). A more rapidly progressing disease course is associated with more meningeal inflammation and GM damage (Magliozzi et al. 2010; Howell et al. 2011; Calabrese et al. 2013).

In addition to focal GM lesions, widespread cortical and subcortical GM atrophy is identified in MS patients (De Stefano et al. 2003; Sailer et al. 2003; Fisher et al. 2008). Subcortical atrophy is found even in patients with clinically isolated syndrome (CIS) indicative of MS (Henry et al. 2008). The first studies of RRMS patients with a short disease duration (less than three and five years respectively) showed conflicting results concerning cortical thinning in patients compared to controls, while studies of total gray matter fraction found smaller values in the patient group (Chard et al. 2002; De Stefano et al. 2003; Sailer et al. 2003). In patients with SPMS, GM atrophy is generalized and includes both cortical, subcortical, cerebellar and brainstem atrophy (Ceccarelli et al. 2008). A longitudinal study has found that the GM atrophy rate

increases with time, from 3.4 times the normal rate in CIS patients to 14 times the normal rate in SPMS (Fisher et al. 2008). Thus both cerebral location and GM atrophy rate change with disease duration.

GM atrophy is associated with cognitive impairment (Calabrese et al. 2010b; Riccitelli et al. 2011) and high atrophy rates early in the disease course may be the best predictor available for a worse long-term disability (Filippi et al. 2013a; Popescu et al. 2013).

Studies of the association between GM atrophy and WM lesions have shown conflicting results (Bö et al. 2007; Bendfeldt et al. 2010). Recent studies indicate that diffuse damage to white matter tracts may be associated with damage to the corresponding cortical and subcortical GM (Gorgoraptis et al. 2010; Kolasinski et al. 2012; Cappellani et al. 2014; Bergsland et al. 2015). Furthermore, MR spectroscopy studies have found that damage to NAWM predicts future brain atrophy and neurological disability evolution (Llufriu et al. 2014). However, in the progressive phase of the disease, GM atrophy escalates, while increase in WM lesion load is similar to rates in RRMS (Fisher et al. 2008). Thus GM atrophy may evolve in parallel or be secondary to WM damage in the early disease stages, while in the progressive stage of the disease other mechanisms dominate.

The main pathological substrate of neocortical GM atrophy is neuronal, axonal and glial degeneration and synaptic loss (Wegner et al. 2008; Popescu et al. 2015). Axonal damage has been identified as the factor most closely associated with irreversible disability in MS (Tallantyre et al. 2010). Thus GM atrophy is an indicator of irreversible brain damage and early GM atrophy may serve as a marker of long-term disability outcomes in MS patients.

2.5 MS diagnosis

There are anecdotes and patient histories of disease courses that fit with MS dating back to the 12th century (Holmøy 2006). Jean-Martin Charcot (1825-1893)

defined the disease clinically and pathologically, after following the disease progression of his housemaid until her death. He described “Sqlerose en Plaque” with a triad of clinical symptoms (nystagmus, scanning speech and intentional tremor), and he also noted cognitive impairments in some of the patients (Charcot 1868; Clanet 2008).

The disease was later systematically characterized by Schumacher in 1965 and the diagnostic criteria were revised in a Workshop on the Diagnosis of Multiple Sclerosis in 1983 (Poser and Paty 1983). Expanding knowledge in the field and a need for clarifying criteria in research and clinical care has led to several revisions of the disease criteria. There is international consensus for the use of the current diagnostic MS criteria, the 2010 revisions to the McDonald criteria (McDonald et al. 2001; Polman et al. 2005, 2011). With these criteria RRMS can be diagnosed after a single relapse if there is clinical or imaging evidence of dissemination of the disease in space and time (Table 1). Oligoclonal bands in the cerebrospinal fluid, not identified in serum, is found in 80-95 % of MS cases, but are not part of the criteria for RRMS (Polman et al. 2011; Goris et al. 2015). The diagnosis of progressive MS is more complex and requires disease progression over time (Table 1) (Polman et al. 2011).

MRI was mentioned as a possible paraclinical tool guiding the diagnosis of MS in the Poser criteria shortly after the introduction of MRI in clinical practice (Poser and Paty 1983). In the 2001 McDonald Criteria MRI assessment was established as a diagnostic tool, and MRI assessments have increasing weight in the subsequent revisions of the criteria in 2005 and 2010 (McDonald et al. 2001; Polman et al. 2005, 2011). However, radiological evidence alone is not sufficient for the diagnosis of MS. The identification of radiological lesions indicative of MS without clinical symptoms or signs, Radiological Isolated Syndome (RIS), will only lead to an MS diagnosis within the next two years in around a third of the cases, even after extensive clinical and paraclinical examinations (Lebrun et al. 2014). In contrast, a Danish post-mortem study published in 1988, before the everyday use of MRI, verified the clinical MS diagnosis made by neurologists in 94 % of the patients investigated (Engell 1988). Thus the

Clinical Presentation	Additional Data Needed for MS Diagnosis
≥2 attacks[a]; objective clinical evidence of ≥2 lesions or objective clinical evidence of 1 lesion with reasonable historical evidence of a prior attack[b]	None[c]
≥2 attacks[a]; objective clinical evidence of 1 lesion	Dissemination in space, demonstrated by: ≥1 T2 lesion in at least 2 of 4 MS-typical regions of the CNS (periventricular, juxtacortical, infratentorial, or spinal cord)[d]; or Await a further clinical attack[a] implicating a different CNS site
1 attack[a]; objective clinical evidence of ≥2 lesions	Dissemination in time, demonstrated by: Simultaneous presence of asymptomatic gadolinium-enhancing and nonenhancing lesions at any time; or A new T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI, irrespective of its timing with reference to a baseline scan; or Await a second clinical attack[a]
1 attack[a]; objective clinical evidence of 1 lesion (clinically isolated syndrome)	Dissemination in space and time, demonstrated by: For DIS: ≥1 T2 lesion in at least 2 of 4 MS-typical regions of the CNS (periventricular, juxtacortical, infratentorial, or spinal cord)[d]; or Await a second clinical attack[a] implicating a different CNS site; and For DIT: Simultaneous presence of asymptomatic gadolinium-enhancing and nonenhancing lesions at any time; or A new T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI, irrespective of its timing with reference to a baseline scan; or Await a second clinical attack[a]
Insidious neurological progression suggestive of MS (PPMS)	1 year of disease progression (retrospectively or prospectively determined) plus 2 of 3 of the following criteria[d]: 1. Evidence for DIS in the brain based on ≥1 T2 lesions in the MS-characteristic (periventricular, juxtacortical, or infratentorial) regions 2. Evidence for DIS in the spinal cord based on ≥2 T2 lesions in the cord 3. Positive CSF (isoelectric focusing evidence of oligoclonal bands and/or elevated IgG index)

If the Criteria are fulfilled and there is no better explanation for the clinical presentation, the diagnosis is "MS"; if suspicious, but the Criteria are not completely met, the diagnosis is "possible MS"; if another diagnosis arises during the evaluation that better explains the clinical presentation, then the diagnosis is "not MS."

[a]An attack (relapse; exacerbation) is defined as patient-reported or objectively observed events typical of an acute inflammatory demyelinating event in the CNS, current or historical, with duration of at least 24 hours, in the absence of fever or infection. It should be documented by contemporaneous neurological examination, but some historical events with symptoms and evolution characteristic for MS, but for which no objective neurological findings are documented, can provide reasonable evidence of a prior demyelinating event. Reports of paroxysmal symptoms (historical or current) should, however, consist of multiple episodes occurring over not less than 24 hours. Before a definite diagnosis of MS can be made, at least 1 attack must be corroborated by findings on neurological examination, visual evoked potential response in patients reporting prior visual disturbance, or MRI consistent with demyelination in the area of the CNS implicated in the historical report of neurological symptoms.

[b]Clinical diagnosis based on objective clinical findings for 2 attacks is most secure. Reasonable historical evidence for 1 past attack, in the absence of documented objective neurological findings, can include historical events with symptoms and evolution characteristics for a prior inflammatory demyelinating event; at least 1 attack, however, must be supported by objective findings.

[c]No additional tests are required. However, it is desirable that any diagnosis of MS be made with access to imaging based on these Criteria. If imaging or other tests (for instance, CSF) are undertaken and are negative, extreme caution needs to be taken before making a diagnosis of MS, and alternative diagnoses must be considered. There must be no better explanation for the clinical presentation, and objective evidence must be present to support a diagnosis of MS.

[d]Gadolinium-enhancing lesions are not required; symptomatic lesions are excluded from consideration in subjects with brainstem or spinal cord syndromes.

MS = multiple sclerosis; CNS = central nervous system; MRI = magnetic resonance imaging; DIS = dissemination in space; DIT = dissemination in time; PPMS = primary progressive multiple sclerosis; CSF = cerebrospinal fluid; IgG = immunoglobulin G.

Table 1. Diagnostic criteria of MS. Printed with permission from Polman et al 2011 and Archives of Neurology.

diagnosis of MS, though guided by MRI and other paraclinical examinations, should still be based on clinical assessment.

2.6 Disease course

The Advisory Committee on Clinical Trials of MS and the MS Phenotype Group have published recommendations for definition of the clinical course of MS (Lublin et al. 1996, 2014). The patients investigated in this thesis were evaluated according to the suggestions published in 1996 (Lublin et al. 1996). According to those criteria the disease course of MS patients could be divided into four subtypes. In relapsing-remitting (RRMS) the patients experienced episodes of relapses followed by total or partly recovery. In Primary Progressive Multiple Sclerosis (PPMS) the disease was progressive with no relapses. Secondary Progressive Multiple Sclerosis denoted the

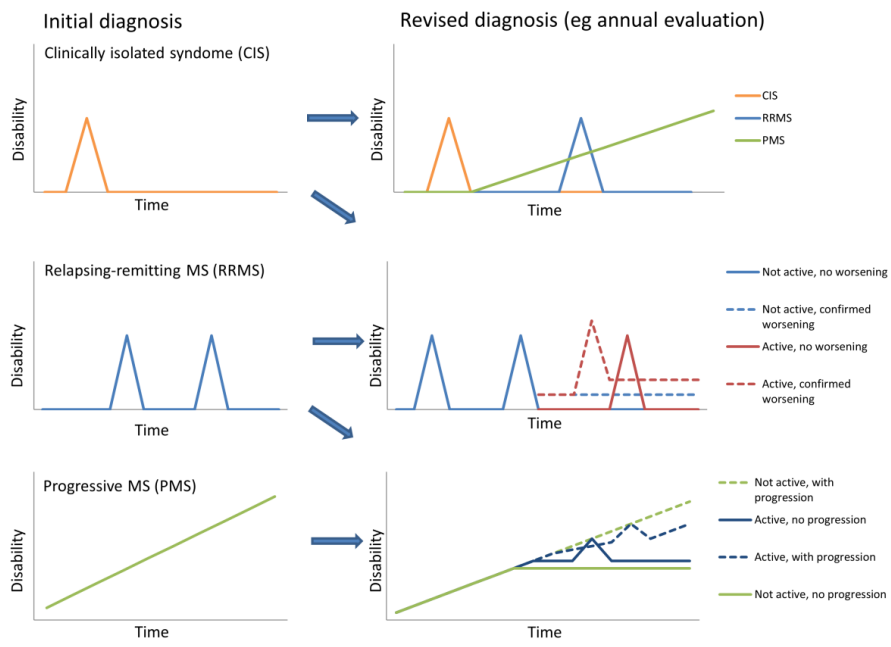

Figure 10. Definitions of disease course in MS. Recommendations published by The Advisory Committee on Clinical Trials of MS and the MS Phenotype Group (Lublin et al. 2014). Illustration: Gro Owren Nygaard

conversion from RRMS to PPMS, and Progressive-Relapsing Multiple Sclerosis (PSMS) denoted a phenotype with a dominance of progressive disease with occational relapses (Figure 10) (Lublin et al. 1996). The latest update of clinical course by the Advisory Committee on Clinical Trials of MS and the MS Phenotype Group was published after the end of the data collection in this thesis (Lublin et al. 1996, 2014). In those guidelines CIS was included as a part of the MS diagnosis spectrum, and the other diagnoses were limited to RRMS and PMS (including both PPMS and SPMS). Annual evaluations of disease activity (defined as MRI evidence of new or expanding lesions or clinical relapses) and clinical worsening or disability progression were recommended. They suggested the term "confirmed worsening" for clinical symptoms remaining after relapses and "disability progression" for the progressive disability accumulation in PMS (Figure 10) (Lublin et al. 2014).

2.7 The natural history of MS

Around 85 % of MS patients present with a clinically isolated episode of neurological disability (Confavreux and Vukusic 2006). The presence of OCB, multiple T2 WML and a younger age increase the probability of conversion to clinically definite MS within the next five years (Kuhle et al. 2015). Fifteen to twenty percent of the patients present with a primary progressive disease and experience gradual increase in disability with no definite relapses (Myhr et al. 2001; Confavreux and Vukusic 2006). Both natural history studies and pathological studies suggest that primary progressive MS succeeds a period of "silent" relapses, and represents the same disease as secondary progressive MS (Figure 11) (Confavreux and Vukusic 2006; Kutzelnigg and Lassmann 2014; Dendrou et al. 2015; Mahad et al. 2015).

Most RRMS patients convert to SPMS after 5-15 years (Scalfari et al. 2014). A gradual or stepwise increase in disability is the rule, but disability regression has been reported in a subset of patients (Tremlett et al. 2012). A higher age at onset, male sex, involvement of multiple systems, high relapse rate and a rapid increase in EDSS the first five years are prognostic markers for a worse long-term outcome, as measured by

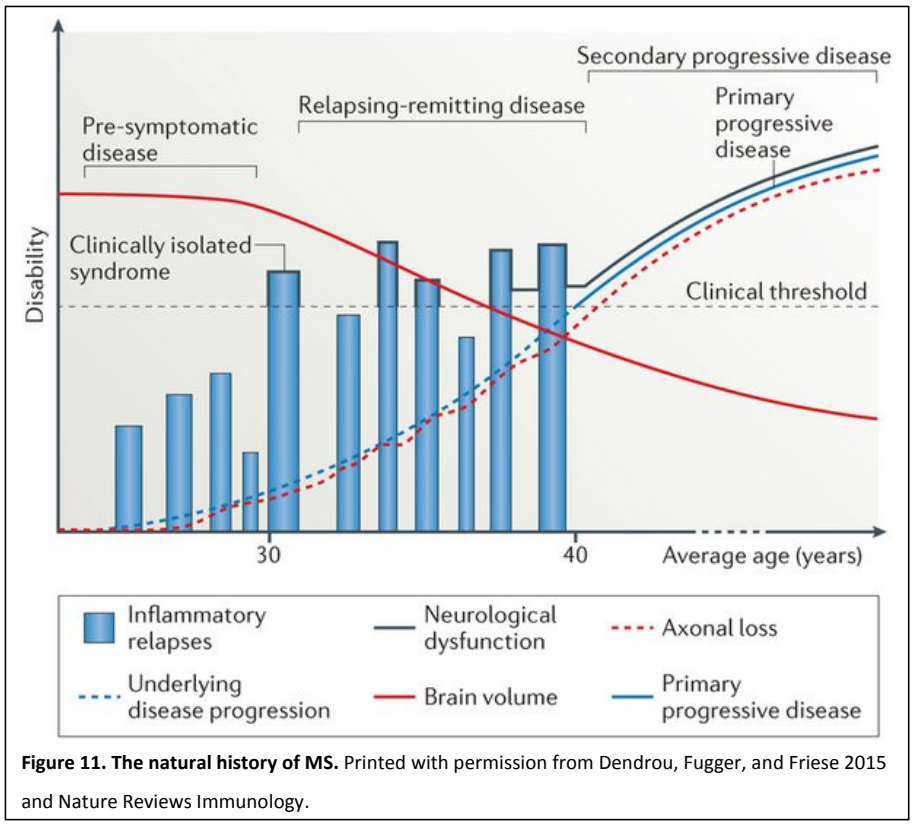

Figure 11. The natural history of MS. Printed with permission from Dendrou, Fugger, and Friese 2015 and Nature Reviews Immunology.

conversion to SP MS and higher EDSS scores (Degenhardt et al. 2009; Scalfari et al. 2010, 2014). However, patients with low EDSS after a long disease duration ("benign MS") may be bothered with symptoms less accounted for on the EDSS scale, like cognitive impairment, depression and fatigue, and may also be severely affected by the disease (Amato et al. 2006; Smestad et al. 2010; Glad et al. 2011).

2.8 Evidence of disease activity in MS

In order to predict clinical course, decide on treatment and to inform patients, it is important to have reliable and user-friendly tools describing disease activity in MS patients. The most readily available measures are information about relapses, disability and MRI changes.

Relapses can be defined as any new neurological symptoms, not associated with fever or infection, lasting for at least 24 hours and accompanied by new neurological signs (Havrdova et al. 2009). Relapses are most frequent early in the disease course (Compston and Coles 2008). A high relapse rate during the first years after diagnosis is associated with a shorter time to reach EDSS 6, faster conversion to progressive MS and higher disability levels (Weinshenker et al. 1989; Scalfari et al. 2010, 2014). Further, incomplete remission from relapses may lead to long-term disability accumulation (Lublin et al. 2003). Also, relapses on interferon treatment increase the risk of sustained disability progression (Bosca et al. 2008).

The most common measure of disability in MS is the Expanded Disability Status Scale (EDSS) (Kurtzke 1983). *Disability progression* can be defined as an increase in EDSS≥1 compared to baseline in the absence of a relapse the last six weeks before examination (Giovannoni et al. 2011). Disability progression early in the disease course is correlated with long-term disability (Degenhardt et al. 2009). Indeed, early disability progression was reported to be the strongest predictor of long term disability in patients treated with interferons (Río et al. 2006).

MRI measures of MS has had major influences on MS diagnostic criteria, treatment and research (Rovira et al. 2015). In clinical practice *radiological progression* can be defined as at least one new or enlarging T2 or Fluid Attenuated Inverse Recovery (FLAIR) white matter lesion (WML) (with or without gadolinium enhancement on T1) compared to MRI at baseline (Havrdova et al. 2009). As mentioned previously, higher T2 lesion load increases the risk of conversion from CIS to MS and radiological progression is associated with a higher future relapse rate (Sormani et al. 2013; Kuhle et al. 2015). Furthermore radiological progression, together with hypointense T1 lesions and brain atrophy, predict future disability progression (Bermel et al. 2013; Giorgio et al. 2013; Popescu et al. 2013).

No Evidence of Disease Activity (NEDA), i.e. the absence of relapses, disability progression or radiological progression, was first introduced as an outcome measure in

post-hoc studies of disease modifying treatment (DMT)s in clinical trials (Havrdova et al. 2009; Giovannoni et al. 2011; Lublin et al. 2013). This outcome measure has separated patients treated with DMTs from those on placebo (Havrdova et al. 2009; Giovannoni et al. 2011; Lublin et al. 2013). Furthermore, NEDA has been used as outcome measure in patients with active MS treated with autologous hematopoietic cell transplantation (HCT) (Nash et al. 2015). A recent population-based cohort study found that almost half of the patients fulfilled the NEDA criteria after one year, and that NEDA status at two years predicted stability of disability the following five years better than any of the individual measures alone (Rotstein et al. 2014).

2.9 Symptoms in MS

2.9.1 Neurological symptoms

A range of different symptoms are associated with MS (Figure 12). The presenting symptoms often include motor, sensory, visual or cerebellar symptoms (Compston and Coles 2008). Later in the disease course the patients often have multiple neurological symptoms. Specific signs typical of MS include l'Hermitte's sign, i.e. sensory symptoms

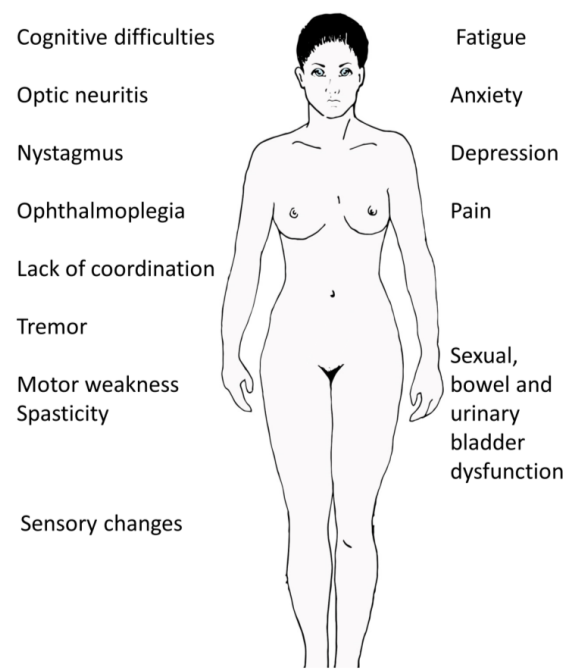

Figure 12. Common symptoms in MS. Illustration: MS Research Group OUS/Gro Owren Nygaard

with neck flexion, and Uthoffs phenomenon, i.e. transient worsening of symptoms with increased body temperature (Compston and Coles 2008). Pyramidal, sensory, and mental disabilities (including both cognitive impairment, fatigue and depression) all have a large impact on MS patients' quality of life (Nortvedt et al. 1999).

Autonomic symptoms in MS patients include disturbed control of the bladder and bowel, disturbed cardiovascular function, sleep disturbancies, sexual problems and dysregulation of sweat organs (Haensch and Jörg 2006). Studies of autonomic dysregulation in MS patients have been conflicting concerning the association with fatigue (Egg et al. 2002; Niepel et al. 2013). However, recent studies indicate that autonomic nervous system (ANS) dysfunction may contribute to MS-related fatigue (Cortez et al. 2015).

2.9.2 Cognitive symptoms

Cognitive symptoms in MS were described already in the 19th century (Clanet 2008). However, the first population-based study of cognitive impairments in MS patients was not performed before the early 1990s (Rao et al. 1991). Rao and colleagues identified cognitive impairments in 43 % of the MS patients investigated (Rao et al. 1991). Amato and colleagues examined MS patients with a short disease duration (less than two years), and they found that these patients performed worse than healthy controls, most notably in verbal memory and abstract reasoning (Amato et al. 1995). More recent studies have found cognitive impairment (CI) in patients at the very early disease stages: in RIS patients (Lebrun et al. 2010) and in CIS patients (Achiron and Barak 2003; Feuillet et al. 2007).

Cognitive dysfunction in MS patients increases with disease duration (Amato and Ponziani 2001; Van Schependom et al. 2014b). A population-based study in Stockholm, Sweden, found that 50 % of the MS patients investigated had cognitive problems (Einarsson et al. 2006). Similarly, a study in Oslo, Norway, identified CI in 50 % of the MS patient with a long disease duration (>20 years) (Smestad et al. 2010).

Several cognitive domains may be affected in MS patients (Figure 13). Processing speed and memory are the most commonly impaired functions, followed by executive functions and visuospatial perception (Benedict et al. 2006). Processing speed may be the primary domain affected in most patients (Demaree et al. 1999; DeLuca et al. 2004; Covey and Zivadinov 2011; Van Schependom et al. 2014b).

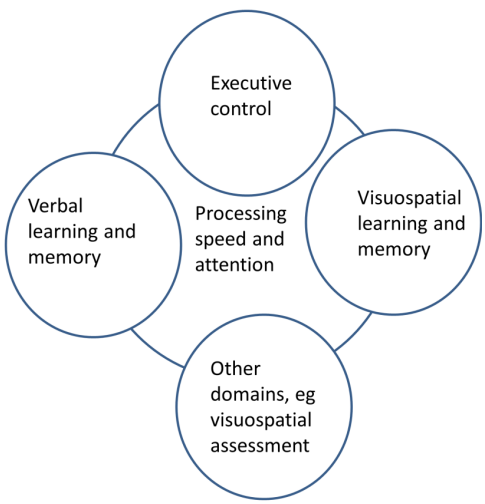

Figure 13. Cognitive symptoms in MS. Illustration: Gro Owren Nygaard

Impairments in this cognitive domain may contribute to a poorer test performance in other domains, like verbal memory (Lengenfelder et al. 2006; Forn et al. 2008; Urbanek et al. 2010) and executive functions (Denney and Lynch 2009; Roth et al. 2015).

Predictors for cognitive impairments include young age at MS onset and a severe disease course. Some studies have found that CI is associated with a higher EDSS (Amato and Ponziani 2001), while others have not confirmed such an association (Smestad et al. 2010). Several MRI studies have found that a higher WM lesion load, damage to NAWM, GM atrophy and GM lesions are all associated with more cognitive symptoms (Goodin et al. 2012; Rocca et al. 2015).Cognitive dysfunction at diagnosis predicts disability progression and conversion to SP MS ten years later (Moccia et al. 2015). Furthermore, cognitive dysfunction itself has severe consequences: it is associated with lower working abilities and with reduced quality of life (Nortvedt et al. 1999; Glad et al. 2011).

Studies of cognitive reserve in MS have shown that high premorbid intelligence levels moderate or delay the negative effects of structural brain damage on cognition (Benedict et al. 2010). Higher education and more premorbid intellectual leisure

activities are associated with a lower risk of cognitive dysfunction (Sumowski et al. 2010; Martins Da Silva et al. 2015). Also a larger maximal lifetime brain growth measured by estimates of intracranial volume protects against CI, a phenomenon termed “brain reserve” (Sumowski et al. 2013). Results are conflicting as to whether these protective effects are sustained when the disease progresses (Amato et al. 2013; Sumowski et al. 2014).

Structural brain damage, most notably damage to deep gray matter, but also cortical gray matter atrophy, cortical lesions, increased WMLL and damage to NAWM increase the risk of CI in MS patients (Rao et al. 1989; Dineen et al. 2009; Calabrese et al. 2012). Results from functional neuroimaging have been more conflicting. Functional MRI (fMRI) studies have indicated that patients with MS or clinically isolated syndromes (CIS) show a different cerebral resting-state activation as well as task-related cerebral activation patterns compared to healthy controls (Staffen et al. 2002; Audoin et al. 2003; Penner et al. 2003; Forn et al. 2006; Roosendaal et al. 2010; Rocca et al. 2014b). MS patients with both normal cognitive function (Staffen et al. 2002) and with mild cognitive impairment (CI) (Penner et al. 2003) may recruit more cortical areas during cognitive tasks than controls. Such an increased activation could be a sign of functional recruitment or plasticity early in the disease course compensating and delaying cognitive difficulties. This “functional reorganization” theory fits with results of fMRI studies of motor tasks in MS patients, where patients with motor impairments show more extensive activations in cortical areas than participants with normal cognitive scores (Rocca et al. 2005). However, cerebral activation may also be normal in patients with mild CI and altered activation may be evident only in more severely impaired patients (Rocca et al. 2014b). The altered cerebral recruitment among MS patients may thus alternatively be a sign of dysfunctional reorganization caused by the disease (either directly because of structural changes, i.e. lesions and atrophy or by an altered activation pattern caused by cerebral stress or inflammation), leading to less appropriate brain activation, and contributing to cognitive difficulties. Recently a hypothesis of cerebral network collapse as a cause for developing cognitive impairment

in MS has been raised (Schoonheim et al. 2015). They suggest that network collapse is induced after a critical threshold dependent on both structural and functional brain changes (Schoonheim et al. 2015).

2.9.3 Fatigue

Fatigue is a broad term used to describe a feeling of excessive tiredness or exhaustion (Krupp 2010). A distinction between *fatigue* as the subjective sensation of tiredness and *performance fatigability* as the objective deterioration in performance with time has been suggested to define a unified taxonomy for neurological disorders (Kluger et al. 2013).

Fatigue is a common and often disabling symptom in MS patients (Krupp 2010). It afflicts more than 80 % of MS patients and 40 % report fatigue as their most disabling symptom (Bakshi 2003; Minden et al. 2006). A longitudinal study found that patient-reported severity of fatigue symptoms were not static, rather they varied considerably throughout the observation period in most patients (Johansson et al. 2008). Fatigue is associated with depressive symptoms, a worse quality of life, not living with a partner and not working (Bakshi et al. 2000b; Johansson et al. 2008; Smedal et al. 2011).

The first MRI studies of fatigue in MS reported conflicting results concerning associations between whole brain atrophy, lesion load and fatigue (Codella et al. 2002; Tedeschi et al. 2007). However, further studies have found that localized WM lesion load, GM atrophy of frontotemporal regions, damage to NAWM in frontotemporal regions, and damage to corpus callosum, thalamus and basal ganglia, respectively, are associated with fatigue in these patients (Bakshi et al. 2000b; Calabrese et al. 2010a; Pellicano et al. 2010; Gobbi et al. 2014; Rocca et al. 2014a; Yaldizli et al. 2014). This evidence from imaging studies has led to the suggestion of a thalamo-striato-cortical explanatory model of fatigue in MS (Leocani et al. 2008). This model suggests that circuits involving thalamus, basal ganglia, and frontoparietal cortex could be disrupted either structurally by MS lesions or functionally by inflammation and lead to symptoms of fatigue (Leocani et al. 2008).

2.9.4 Depressive symptoms

The life-time risk of major depression is 25-50 % in MS patients, which is two to five times the risk in the general population (Feinstein et al. 2014). In cross-sectional population-based studies 30-40% of MS patients report depressive symptoms (Chwastiak et al. 2002; Beiske et al. 2008). Depression is also more common in MS patients than in patients with other chronic diseases (Patten et al. 2003).

A recent diagnosis of MS, major changes in functioning, or limited social support increase the risk of depression (Chwastiak et al. 2002). MRI studies have found that WM lesion burden, ventricular enlargement and frontotemporal GM atrophy are associated with depressive symptoms in MS (Pujol et al. 1997; Bakshi et al. 2000a; Feinstein et al. 2004; Gobbi et al. 2014). Together these studies indicate that both social factors and structural damage to fronto-parietal brain regions contribute to depression in these patients.

2.9.5 Other psychiatric symptoms

Other psychiatric symptoms, particularly anxiety, are also common in MS (Beiske et al. 2008). The incidence of psychiatric comorbidity in this patient group remains understudied and is probably also undertreated (Beiske et al. 2008; Marrie et al. 2015).

2.9.6 Vision and vison-related symptoms

Optic neuritis (ON) is common in patients with MS. It is the first symptom of the disease in 25 % of MS patients and occurs during the disease in about 70 %, usually in the relapsing–remitting phase (Toosy et al. 2014b). High contrast visual acuity is often affected in acute ON and recovers only partly in most patients (Balcer et al. 2014). Retinal Nerve Fiber Layer (RNFL), assessed by Optic Coherence Tomography (OCT) is thinner in MS patients than controls, even early in the disease course of MS. Furthermore, RNFL thinning is associated with MRI measures of CNS GM atrophy (Balcer et al. 2014). Visual deficits, structural loss of retinal axonal and neuronal integrity and delayed VEP can frequently be found in the patients even without a

history of ON (Balcer et al. 2014). Eye-movement disorders are less studied, but a range of disorders, including nystagmus, disruption of saccades, diplopia and internuclear ophthalmoplegia frequently afflict the patients (Frohman et al. 2005). These decrements in vision and eye-movements contribute to disability and to a worse vision-related quality of life (Schinzel et al. 2014).

2.10 Neuroimaging in MS

Magnetic resonance imaging is a relatively new imaging modality. The high sensitivity to a broad range of tissues, good contrast, relative safety and non-invasive nature all contribute to the popularity and usefulness of the technique. Advances in physics throughout the last century led to the development of the first MRI platform for clinical examinations of humans in 1980. The development in the field of MRI has led to several Nobel prizes in medicine and physics and to implementations of imaging both in clinical medicine and in research (Haacke et al. 1999).

In the field of MS, the introduction of MRI has been particularly important. Pathological studies have long identified white matter lesions and brain atrophy in MS brains (Kutzelnigg and Lassmann 2014). However, prior to the MRI technology no good imaging modalities were available to detect these changes in the patients *in vivo*. In 1981 Young and colleagues scanned ten MS patients by means of CT and MRI. They identified a total of 19 cerebral lesions on the CT scans, all in the periventricular regions, and 112 further lesions on the MRI scans (Young et al. 1981). Studies comparing MRI and pathological findings confirmed that T2 weighed MRI captured histologically confirmed demyelinated WM lesions (Stewart et al. 1984).

The first studies of clinical and MRI findings in MS patients reported an incomplete association between the two, termed the “clinico-radiological paradox” (Barkhof 2002). This gap probably had several explanations. The use of EDSS as disability outcome led to imprecise clinical assessments (Cohen et al. 2012). Further, the early MRI studies did not image damage to gray matter and normal-appearing

white matter (Filippi and Rocca 2013). Also, premorbid brain resources, both cognitive reserve and brain reserve, that could modulate the association between brain pathology and clinical outcomes were not accounted for (Sumowski et al. 2014). Recent years this gap has diminished because of advances in the field of clinical assessments, MRI and cognitive reserve (Cohen et al. 2012; Filippi et al. 2014; Sumowski et al. 2014).

Today MRI scans are parts of the routine examinations both at diagnosis and clinical follow-up of MS patients in Norway and internationally (Myhr et al. 2011; Rovira et al. 2015). Furthermore, MRI changes are used as primary or secondary outcome in clinical trials, and new MRI sequences are considered for future assessments in MS patients (Filippi et al. 2014).

2.11 Treatment

To date there is no cure for MS. The treatment is limited to pharmacological treatment of relapses, disease course and symptoms, as well as non-medical rehabilitation strategies.

The intravenous or oral treatment of relapses with methylprednisolone significantly reduces the severity and longevity of relapses in CIS, ON and MS patients. However, no evidence of long term effect of such treatment has been found (Beck et al. 1992; Miller et al. 2000; Toosy et al. 2014b). Thus this treatment is indicated if symptoms are bothersome to the patients or interfere with daily function (Myhr et al. 2011).

Disease modifying treatments (DMTs) for RRMS have been available since the 1990s (Paty et al. 1993; The IFNB Multiple Sclerosis Study Group 1993). Most disease-modifying treatments (DMTs) reduce the annual relapse rate in RRMS and the number of new MRI lesions, but do not stop the disease progression completely (Piehl 2014; Galea et al. 2015).

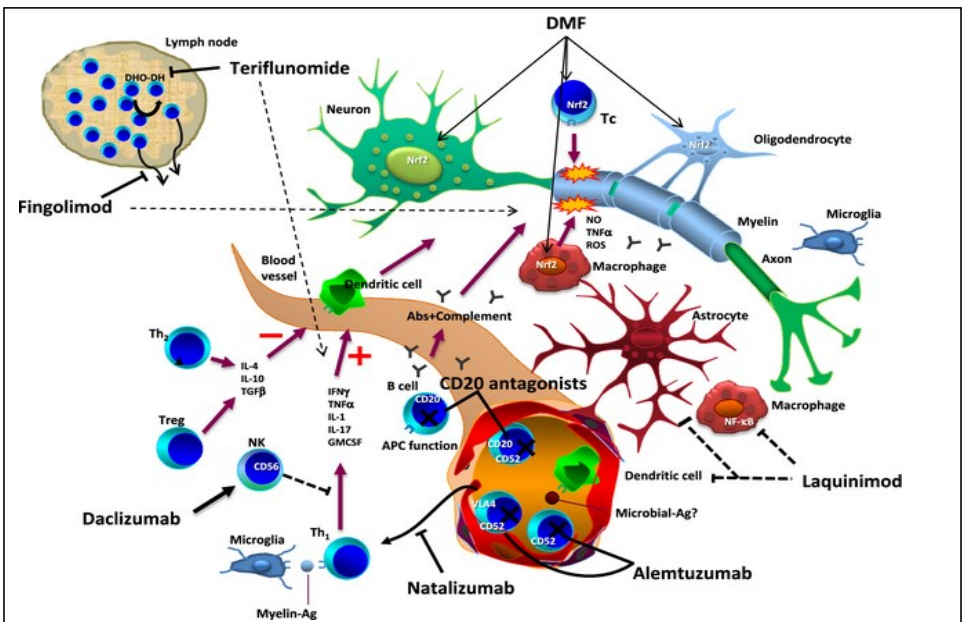

Figure 14. Mode of action of DMTs used in MS. From Piehl 2014, printed with permission.

Subcutaneous Interferonβ (IFNβ) 1b injections were the first DMTs with clinical and MRI effect on disease course, with a 30 % reduction in relapses and new T2 enhancing lesions (The IFNB Multiple Sclerosis Study Group 1993). Subcutaneous glatiramer acetate, a myelin basic protein peptide-analogue followed in 1996, with similar effects (Johnson 2012). These early DMTs may reduce time to conversion to definitive MS (Tedeholm et al. 2013), but early treatment with these drugs does not generally affect disability progression five years later (Kappos et al. 2009). A large Canadian population-based study neither found long-term effects on disability (Shirani et al. 2012). When both clinical and neuroradiological evidence of continued disease activity were taken into account, more than half of the patients who started treatment with these first-line drugs had an inadequate response within the first 2 years (Rudick and Polman 2009). Thus both short-term and long-term effects of the early first line treatments for MS are modest.

The first phase III study of Natalizumab was published in 1996 (Polman et al. 2006). This is a humanized monoclonal antibody that binds to the a4-integrin of very late antigen-4, a surface marker present on immune cells, and is given by monthly IV infusion (Piehl 2014). This drug reduced the relapse rate after one year by 68 percent and led to an 83 percent reduction in radiological progression compared to placebo (Polman et al. 2006). Fingolimod was the first oral treatment approved for RRMS and also the first drug to target the sphingosine-1-phosphate receptors, arresting immune cells in peripheral lymph nodes (Piehl 2014). Fingolimod showed a reduction of relapses of 53–60% compared to placebo and of 38–52% compared to IFNb, respectively (Cohen et al. 2010; Kappos et al. 2010). However, risk of severe side-effects, most notably Progressive Multifocal Leucoencephalopathy (PML) when using Natalizumab and cardiac arrhythmias when using Fingolimod have limited the use of these treatments (Piehl 2014). After the data collection for this thesis, several new treatments have become available, including Alemtuzumab (a humanized monoclonal antibody directed against CD52, a surface antigen expressed on certain subtypes of lymphocytes, originally approved for the treatment of haematological malignancies), Dimetylfumarate (the methyl ester of fumaric acid, an intermediate in the citric acid cycle, previously used for the treatment of psoriasis) and Teriflunomide (an inhibitor of the enzyme dihydroorotate dehydrogenase, thought to exert cytotoxic effects on proliferating lymphocytes and possibly involved in the modulation of cytokine production) (Piehl 2014). The mode of action of these and other proposed drugs are illustrated in Figure 14 and the trade name and administration of different treatments in use in Norway are illustrated in Table 2.

In addition to these established treatments, recent publications of the effects of autologous hematopoietic cell transplantation have shown very promising results, though randomized controlled studies are still lacking (Burman et al. 2014; Burt et al. 2015; Nash et al. 2015).

Treatment of symptoms, like spasticity, pain, bladder dysfunction, walking disability, depression and epileptic seizures may alleviate the patients' symptoms

Drug	Trade name	Administration	1st, 2nd or 3rd line treatment
Interferonβ1b	Betaferon®/ Extavia®	250 µg sc every other day	1
Interferonβ1a	Avonex®/ Rebif®	30 µg sc weekly/ 22/44 µg sc three times per week	1
Glatiramer acetate	Copaxone®	20 mg sc daily	1
Fingolimod	Gilenya®	0.5 mg p.o. daily	2
Natalizumab	Tysabri®	300 mg i.v. monthly	2
Teriflunomide	Aubagio®	14 mg po daily	1
Dimetylfumarate	Tecfidera®	240 mg p.o. twice daily	1
Alemtuzumab	Lemtrada®	12 mg iv x5/x3 two series of treatment a year apart	1 and 2
Autologous hematopoietic cell transplantation		Research protocol at Haukeland University Hospital	3

Table 2. Disease modulatory treatments in Norway in 2015. Illustration: Gro Owren Nygaard

(Toosy et al. 2014a; Feinstein et al. 2015). However, MS patients often have multiple symptoms and pharmacological treatment targeting one symptom may lead to the worsening of another. Thus in practice, an effective response may be hard to achieve (Feinstein et al. 2015). For these patients, multidisciplinary rehabilitation may be useful and improve daily function (Grasso et al. 2005).

Neuropsychiatric rehabilitation is still in its infancy (Rosti-Otajärvi and Hämäläinen 2014). Studies of daily physical activity (Rietberg et al. 2014) and cognitive reserve-building activities (Schwartz et al. 2015) show that MS patients are less active, both physically and cognitively, than healthy controls. There are few studies of the effect of lifestyle interventions on cognition and other symptoms in MS patients. However, a pilot study has found that physical exercise may improve cognition in progressive MS (Briken et al. 2014), and some studies indicate that reserve-building activities like cognitive training may improve cognitive test results and alter functional cortical networks (Penner et al. 2007; Hubacher et al. 2015).

3. Aims of the study

The overall aim of the study was to explore the association between neurological, cognitive, fatigue and depressive symptoms and MRI characteristics in early RRMS. This was sought through clinical, paraclinical and experimental examinations of a cohort of recently diagnosed RRMS patients. A better understanding of these associations will improve future treatment of this patient group.

Specific aims were:

1) To investigate the differences in cortical structure between healthy controls and early MS patients, and to assess whether the cortical structure of the RRMS patients were associated with neurological, cognitive, fatigue or depressive symptoms.

2) To determine whether RRMS patients with evidence of disease activity at one year follow-up had similar change in disability, cognition and brain volumes as patients without evidence of disease activity and healthy controls.

3) To investigate whether the results of a common test of processing speed in MS, the Symbol Digit Modalities Test (SDMT), was affected by disease specific confounders like saccadic initiation time or hand motor speed in early MS patients.

4) To assess whether RRMS patients and controls had similar pupillary responses to problem-solving, and to assess whether these responses differed according to cognitive scores, fatigue levels or depressive symptoms.

4. Summary of results

4.1 Paper I

Cortical thickness and surface area relate to specific symptoms in early relapsing-remitting multiple sclerosis

Cortical atrophy is common in early RRMS. Whether this atrophy was caused by changes in cortical thickness or cortical surface area was not known prior to this study, nor was the correlation between these measures and clinical symptoms. The objective of this study was to investigate the difference in cortical surface area, cortical thickness and cortical volume between early RRMS patients and healthy controls, and to study the relationship between these measures and neurological disability, cognitive decline, fatigue and depression. RRMS patients (n = 61, mean age 34 years, mean disease duration 2.4 years) underwent MRI, neurological and neuropsychological examinations. We estimated cortical surface area, thickness and volume and compared them with matched healthy controls (n = 61, mean age 34 years). We estimated the correlations between clinical symptoms and cortical measures within the patient group. We found no differences in cortical surface area, but widespread differences in cortical thickness and volume between the groups. Neurological disability was related to regionally smaller cortical thickness and volume. Better verbal memory was related to regionally larger surface area, and better visuospatial memory related to regionally larger cortical volume. Higher depression scores and fatigue were associated with regionally smaller cortical surface area and volume. We concluded that cortical thickness, but not cortical surface area, was affected in early RRMS and identified specific structural correlates to the main clinical symptoms in early RRMS

4.2 Paper II

A longitudinal study of disability, cognition and gray matter atrophy in early multiple sclerosis patients according to evidence of disease activity

New treatment options may make “no evidence of disease activity” (NEDA: no relapses or disability progression and no new or enlarging MRI lesions, as opposed to “evidence of disease activity” [EDA] with at least one of the former), an achievable goal in relapsing-remitting multiple sclerosis (RRMS). The objective of this study was to determine whether early RRMS patients with EDA at one-year follow-up had different disability, cognition, treatment and gray matter (GM) atrophy rates from NEDA patients and healthy controls (HC). RRMS patients (mean age 34 years, mean disease duration 2.2 years) were examined at baseline and one-year follow-up with neurological (n=72), neuropsychological (n=56) and structural MRI (n=57) examinations. Matched HC (n=61) were retested after three years. EDA was found in 46 % of RRMS patients after one year independent on treatment strategy. EDA patients used more first line and less second line disease modifying treatment than NEDA (p=0.004). The patient groups differed in disability at follow-up (p=0.010); EDA patients progressed (EDSS: 1.8-2.2, p=0.010), while NEDA patients improved (EDSS: 2.0-1.7, (p<0.001). Cognitive function was stable in both patient groups. Subcortical GM atrophy rates were higher in EDA patients than HC (p<0.001). These results support the relevance of NEDA as outcome in RRMS and indicate that pathological neurodegeneration in RRMS mainly occur in patients with evidence of disease activity.

4.3 Paper III

Eye and hand motor interactions with the Symbol Digit Modalities Test in early multiple sclerosis

Eye and hand motor dysfunction may be present early in the disease course of RRMS, and can affect the results on visual and written cognitive tests. We aimed to test for differences in saccadic initiation time (SI time) between RRMS patients and healthy controls, and whether SI time and hand motor speed interacted with the written version of the Symbol Digit Modalities Test (wSDMT). Patients with RRMS (n=44, mean age 35 years), time since diagnosis < 3 years and matched controls (n=41, mean age 33 years) were examined with ophthalmological, neurological and neuropsychological

tests, as well as structural MRI (white matter lesion load (WMLL) and brainstem lesions), visual evoked potentials (VEP) and eye-tracker examinations of saccades. We found that SI time was longer in RRMS than controls ($p<0.05$). SI time was neither related to WMLL, nor to the presence of brainstem lesions. Both SI time and 9 hole peg test (9HP) correlated negatively with the results of wSDMT ($r=-0.32$, $p<0.05$, $r=-0.47$, $p<0.01$), but none correlated with the results of the Paced Auditory Serial Addition Test (PASAT). We concluded that RRMS patients had an increased SI time compared to controls. Cognitive tests results, exemplified by the wSDMT, could be confounded by eye and hand motor function.

4.4 Paper IV

Pupillary responses to problem-solving in early MS patients

Cognitive impairment and fatigue in early multiple sclerosis (MS) patients are frequent and troublesome symptoms, probably related to both structural and functional brain changes. An altered regulation of central neuropeptides could lead to changes in regulation of autonomic function, cognitive difficulties and fatigue. However, whether there is a connection between these symptoms in early MS patients is currently unknown. We tested whether measurements of pupil size during problem-solving in early MS patients could detect early functional brain changes associated with cognitive load. Early MS patients (n= 49, mean disease duration 2.6 years) and healthy controls (n=43) with no prior eye pathology were included. Neurological impairment, MRI, visual evoked potentials (VEP), depression and fatigue were assessed in all the patients. In both groups we assessed processing speed and retinal imaging. Pupil size was recorded with an eye-tracker while patients and controls were orally presented with seven multiplication tasks of increasing difficulty. We found that both groups performed well on the cognitive test. The groups showed similar pupillary responses to cognitive tasks at a group level with 15.8 % dilation in patients and 15.7 % dilation in controls for the easy tasks ($t=0.02$, $p=0.982$). However, while controls with low cognitive scores (LCS) (n=9) had an increased pupillary response to cognitive tasks, LCS

MS patients (n=6) did not show such an increased response (Analysis of variance (ANOVA) between-groups effect: $F(2, 25)=8.10$, $p=0.009$). There was a trend towards a smaller pupillary response in patients with fatigue and depression (ANOVA between-groups effect: $F(2, 30)= 2.60$, $p=0.118$) and $F(2, 30)= 1.14$, $p=0.294$). This is the first study to investigate pupillary responses to cognitive tasks in MS patients. Our results suggest that MS-related changes in cognition and fatigue are associated with changes in the autonomic regulation of task-related pupillary responses. This supports the theory of a link between cognition and fatigue in MS.

5. Methodological considerations

This thesis is based on clinical quantitative research, i.e. we have raised scientific hypotheses and used statistics to test whether the corresponding null-hypotheses could be discarded (Laake et al. 2008). There are several points to consider when evaluating such research. First, the population tested should be suitable to for testing our hypotheses. Secondly, the measures should be relevant and precise. And at last, the statistical methods used should provide estimates of effect sizes and their uncertainty, and guide us to decide whether our null-hypotheses should be discarded (Laake and Hjartåker 2007).

5.1 Study design

Internal validity relates to the extent to which the results reflect the populations tested, for example whether we were able to capture the true depressive symptoms in our patient cohort. Selection bias, information bias and wrong statistical methods may threaten internal validity (Laake and Hjartåker 2007). External validity relates to whether the results can be generalized to another population, e.g. whether our cohort resembles a cohort of patients in another city or another country. A good study design would lead to good external validity (Laake and Hjartåker 2007).

The studies in this thesis were observational, i.e. the participants had not been exposed to any systematic medical intervention, except for treatment according to national guidelines, that could alter their risk of disease or disease progression (Laake and Hjartåker 2007). However, in both Paper III and Paper IV, we have used experimental tasks, i.e. examinations not in common use. These tasks have been designed specifically for the purpose of the study to investigate the potential differences in outcome between the participants.

The material in this thesis is provided from baseline and follow-up examinations of a cohort of early MS patients collected in a hospital clinic and with a time since diagnosis of less than four years, and matched controls. Paper I, III and IV had a cross-

sectional design; patients and controls were examined at one time-point, matched on group level, and compared. The patients were identified retrospectively, i.e. after the diagnosis of RRMS. In cross-sectional studies a careful selection of patients and controls is necessary to avoid a selection bias, as discussed in the next section.

Also, there is a potential to encounter an information bias. An information bias may occur if a variable is measured on a categorical scale, and the error leads the variable to be placed in an incorrect category. Correspondingly, if a variable is measured on continuous scale the error may lead the variable to be over- or underestimated. A non-differential misclassification or information mistake is a misclassification unrelated to other study variables. In contrast, in a differential misclassification the wrongly classified variable is related to other study variables (Rothman 2012). Cross-sectional studies with a retrospective design are susceptible to the differential misclassification called recall bias; i.e. people with a certain disease may have remembered more previous symptoms than healthy people (Laake and Hjartåker 2007). Disease duration, i.e. the time from the first probable symptom of the disease, may thus have been over-estimated in this study because of recall bias. Most of the outcome variables of the studies were, however, unrelated to the patients' subjective recall of events.

Paper II had a prospective longitudinal design, where the main outcome was change in clinical and MRI measures from baseline to follow-up. Here the patients were followed prospectively from baseline to follow-up. The main advantage of prospective cohort studies is the possibility to study causality: e.g. whether differences in exposure at baseline cause different outcomes at follow-up. The disadvantages are that longitudinal studies are time consuming and costly. They are prone to selection bias, i.e. a non-random skewness in the inclusion of participants, as not all the invited participants may wish to participate, either at baseline or follow-up. This could lead to a reduction in the representativeness, i.e. the ability of the study population to reflect the source population, of the cohort. Such a skewness could reduce the external validity of the study (Laake and Hjartåker 2007).

5.2 Patients and controls

5.2 1 Patient recruitment

The patients of these studies were included in the period from January 2012 to December 2012. We aimed to capture all patients who received the diagnosis of RRMS from January 2009 to October 2012 and who lived in regions of Oslo receiving public neurological services from Department of Neurology, Oslo University Hospital (OUH), Ullevål, at the time (Figure 15).

The abundancy of sources for patient identification secured that most of the RRMS patients diagnosed in 2009-2012 in the relevant region were identified (Figure 15). The low number of patients who declined to participate was a methodological strength of the study. However, we did not examine all identified cases because of limited MRI capacity. These unexamined cases may have contributed to selection bias.

The inclusion criteria were a diagnosis of RRMS in the period 2009-2012, age 18-50, and clinical follow-up at the Department of Neurology, OUH, Ullevål. The exclusion criteria were a history of other psychiatric or neurological diseases, drug abuse, previous adverse Gadolinium reaction, pregnancy or breast feeding at inclusion, or non-fluency in Norwegian. The main advantage of these stringent criteria was a well-defined homogenous patient sample, reducing the impact of confounding, i.e. the confusion of effects (Rothman 2012). The disadvantage of these criteria was a possible lower representativeness; the patients investigated may not have represented the average MS patient at the department. Furthermore, the inclusion of patients from only one neurological department, may have led to some selection bias. This may reduce the external validity of the study. However, we did not aim at including all RRMS patients in the region; our main focus was to ensure that we could carefully compare patients and controls, and identify MS as the key factor for the clinical and paraclinical differences observed between the groups.

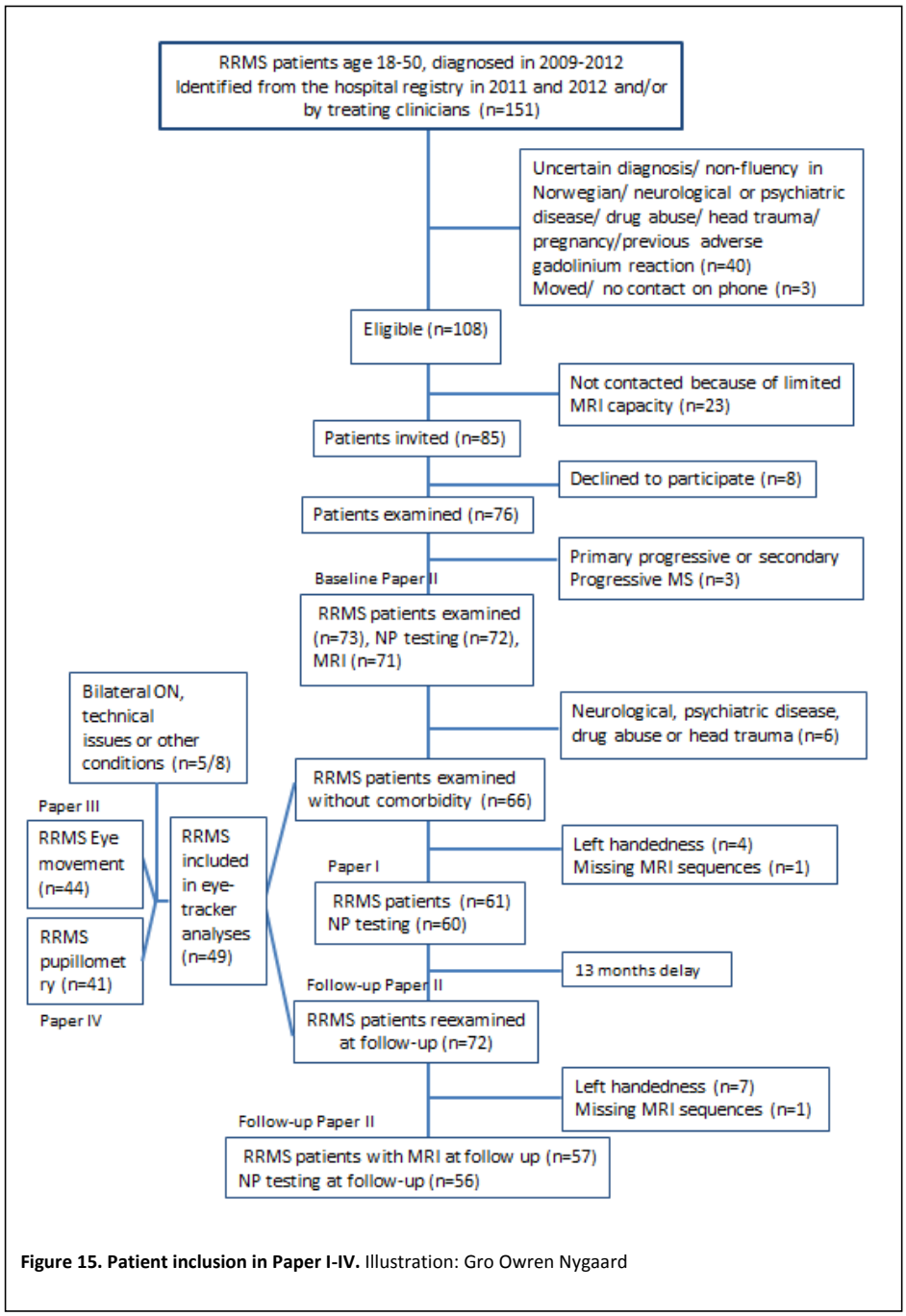

Figure 15. Patient inclusion in Paper I-IV. Illustration: Gro Owren Nygaard

5.2.2 Healthy Control recruitment

Two different control groups were used in these studies. In Paper 1 and II, healthy controls were selected from the ongoing project "Cognition and plasticity through the lifespan" at the Department of Psychology, University of Oslo, from a pool of approximately 150 eligible participants (Walhovd et al. 2013). Inclusion criteria were right handedness, fluency in Norwegian, normal or corrected to normal vision and hearing and MRI at two timepoints. Exclusion criteria were neurological or psychiatric disease, drug abuse, head trauma, depressive symptoms (BDI>16), Mini Mental State Examination (MMSE) score <26 or subjective worries concerning cognitive function. These controls were identified through newspaper advertisements, among students and employees at the University of Oslo. They were matched with the RRMS patients on group level at baseline, based on age, gender and availability of MRI at baseline and follow-up. The method of control recruitment may have led to an excessively healthy or otherwise resourceful sample, and thus to a selection bias. People with high awareness of health and cognitive abilities may have been recruited, i.e. a "Healthy Worker Effect" (Laake and Hjartåker 2007). As can be seen from the background and demographic tables of Paper I, the controls had high general ability levels and high levels of education. They did, however, match our patient sample quite well on these points (see Paper I), so that comparability was ensured.

In Paper III and Paper IV another control group was used. These controls were mainly recruited from the hospital and university environment after direct inquiry by email. The inclusion criteria were age 18-50, fluency in Norwegian and no medical conditions known to affect the visual pathways. These controls were matched on age and gender on a group level to the patient samples of Paper III and IV. Because of the recruitment procedure, we would expect a similar selection bias as with the first control group, i.e. we could have recruited controls with a better health and higher cognitive functions than the patients. As illustrated in these papers, the controls had a slightly higher education and better verbal memory than the patients. However, a

similar proportion of patients and controls were classified with low cognitive scores, because they failed at least one of three cognitive tests (Smith Aron 1982; Delis et al. 2000; Benedict et al. 2012a). We therefore believe that for our purposes, the groups were comparable.

5.3 MS diagnosis

The patients included in the study were diagnosed by clinicians at the Department of Neurology, OUH, Ullevål, in 2009-20012 according to the diagnostic criteria at the time (Polman et al. 2005, 2011). The diagnosis was confirmed for study purposes first by patient journal examination, and then by detailed patient interviews, MRI evaluations and clinical investigations. At the start of the inclusion to the study, a relapsing-remitting disease course was not a part of the inclusion criteria. Two patients with primary progressive MS and one with secondary progressive MS were therefore examined. These patients have been followed-up with the same examinations as the included patients, but have been excluded from the studies.

The diagnostic criteria changed during the time of diagnosis of the patients. Most of the patients included received the diagnosis in 2011 according to the 2010 McDonald Criteria. However, some were diagnosed in 2009 and 2010, and were thus subject to slightly different diagnostic criteria. The main difference between the 2005 and 2010 McDonald criteria was the inclusion of MRI evaluations for proof of dissemination in time and space from one MRI examination only (Polman et al. 2011). This alteration of the diagnostic criteria may have reduced the time from disease onset to diagnosis. Some patients potentially eligible to the study may therefore not have fulfilled the 2005 criteria, while they would have been identified with the new criteria. In our study, some patients *not* diagnosed with MS according to the 2005 McDonald Criteria may thus have been classified as CIS and were not included in the study. The most probable consequence of the slight change in diagnostic criteria was a longer delay from disease onset to diagnosis in the patients diagnosed according to the 2005 McDonald Critera.

5.4 Clinical assessments

5.4.1 Neurological assessment

In MS EDSS is widely used as a measure of neurological disability (Kurtzke 1983). At the lower end of the scale, i.e. from EDSS 0-4, the scale is a sum score of neurological deficits. At higher EDSS scores (4.5-10) the scale is essentially a measure of mobility (Kurtzke 1983). Gait disabilities are important in the MS patients' evaluation of bodily functions (Heesen et al. 2008). Still, there is little doubt that other symptoms, like fatigue, depressive symptoms and cognitive difficulties are important and gain too little weight in this scale (Cohen et al. 2012). We used the EDSS as the main descriptive neurological outcome measure in all our papers. The patients included all had an EDSS<4 with no or minor gait impairments, so that the outcome represented a sum score of neurological deficits. To compensate for the underestimation of other neurological outcomes, fatigue, depressive symptoms and cognition, we supplemented the protocol with tests to examine these factors.

Other neurological outcome measures have been proposed to overcome the bias of walking ability in EDSS, of which Multiple Sclerosis Functional Composite (MSFC) is the best known (Rudick et al. 1997; Cutter et al. 1999). MSCF is a composite measure of walking speed, the 25 feet walking test,(25FWT), a measure of hand function, the 9 hole peg test (9HP) and a measure of processing speed and working memory, the Paced Auditory Serial Addition Test 3 seconds version (PASAT) (Gronwall 1977). MSFC has been criticized for the use of the PASAT as the cognitive test of choice. PASAT was originally created to estimate patients' recovery from concussion (Gronwall 1977), and has later been applied to a large range of neurological and psychiatric disorders (Tombaugh 2006). It is often regarded unpleasant to complete both by patients and examiners, it relies on mathematical skills and it is negatively affected by age, IQ and speed of speech (Tombaugh 2006). Further, MSFC has the disadvantage of complicated scoring procedures, as the scale is based on z-scores created for each study, not on standardized norms or cut-off values. New versions of MSFC, possibly with another

cognitive test and easier scoring algorithms, are therefore warranted (Brochet et al. 2008; Drake et al. 2010; Cohen et al. 2012). In Paper I-III we used the raw scores of the tests included in the MSFC. We did, however, also include other cognitive tests, like the SDMT, possibly with better psychometric abilities (Van Schependom et al. 2014a).

5.4.2 Cognitive assessment

Standardized test batteries for the assessment of cognitive impairments in MS patients were introduced in the 1990s. The most well-known batteries are Rao's Brief Repeated Battery (BRB) (Rao et al. 1991) and the Minimal Assessment of Cognitive Function in MS (MACFIMS) (Benedict et al. 2002), with partly overlapping and partly complementary cognitive tests. As we planned this study, an international consensus group published another, even shorter, test battery; the Brief International Cognitive Assessment in Multiple Sclerosis (BICAMS) (Langdon et al. 2011). This 15 minutes battery was meant for use in research and initial non-specialist examinations of cognition in MS, and has the advantage of short administration time, simple procedures and scoring systems, tests of the main domains affected in MS patients, and inclusion of tests that were thought to have good cross-cultural validity (Benedict et al. 2012a; Dusankova et al. 2012). Validation and translation of BICAMS have already been performed in several countries, and more studies are ongoing (Dusankova et al. 2012; Eshaghi et al. 2012; Goretti et al. 2014). We did not perform a thorough validation of this test battery as part of our study. The verbal memory test, CVLT -2 already was thoroughly validated and translated to Norwegian (Lundervold and Sundet 2004). The other tests were non-professionally translated and the symbols used were visually examined and regarded appropriate in a Norwegian population. In paper I we compared the patients' scores with published American norms (Nygaard et al. 2014). This procedure may have led to artificially low scores because of flawed instructions of SDMT and BVMT, or to artificially high scores because of cultural cues overlooked among the symbols in the same tests. However, both SDMT and BVMT require minimally verbal instruction and the symbols used have not been assessed differently in any cross-cultural translation (Benedict et al. 2012a; Dusankova et al. 2012; Eshaghi

et al. 2012). We therefore believe that the administration of these tests were appropriate. For Paper II, III and IV the outcome of interest were either change in test score (Paper II), association with tests of other functions (Paper III) or comparison to a Norwegian healthy control sample (Paper IV), thus the bias was non-differential, if present at all.

For assessments of general ability levels in Paper I we used the Norwegian version of the vocabulary and matrix reasoning of the Wechsler's Abbreviated Scale of Intelligence (WASI) (Wechsler 1999; Brager-Larsen 2001). The Norwegian version of WASI provides very similar intelligence estimates as the original and longer Wechlser Adult Intelligence Scale III (WAIS III) and the Norwegian translation has retained its properties as a robust estimate of general ability levels (Bosnes 2009).

The healthy controls included in Paper I and Paper II had been investigated at baseline with a comprehensive battery of cognitive tests before the patient examinations in our studies. To ensure both comparability with the healthy controls and tests relevant for an MS population, we chose not to adhere strictly to either battery, but to create a test battery that would capture both comparability with the controls and disease-specific relevance (Table 3). Thus we were able to compare patients and controls on certain cognitive domains and to use tests common in MS research to ensure readability and comprehension within the research community.

We encountered an unforeseen methodological problem with the use of BVMT and, most of all, with CVLT. For the latter both patients and healthy controls performed much better than previous published norms (Nygaard et al. 2014). Thus we encountered a possible ceiling effect, where the true abilities of the participants could not be measured, as most of the participants performed at the upper extreme of the test scores. These results may be caused by a selection bias; both patients and controls were recruited from young, well-educated, urban, mostly female persons. The healthy control sample of Paper III and IV was partly recruited from the hospital environment, and remembering daily to-do-lists may be part of their professional work, thus they

Cognitive domain	Test used	Tested in patients	Tested in Healthy Controls in Paper I and II	Tested in Healthy Controls in Paper III and IV
General ability level	WASI vocabulary and matrix reasoning	Yes	Yes	No
Processing speed	SDMT	Yes	No	Yes
Processing speed/working memory	PASAT	Yes	No	No
Verbal memory	CVLT II	Yes	Yes	Yes
Visuospatial memory	BVMT	Yes	No	Yes

Table 3. Cognitive tests used in Paper I-IV. Illustration: Gro Owren Nygaard

could be particularly gifted or trained in this kind of short-term memory, which may represent a selection bias compared to the patients.

We have not published the results of all the tests that the patients and controls have undergone due to excess of collected data. This could have led to a *publishing bias* (Laake et al. 2008), i.e. that we only published positive results. However, which cognitive outcome measures to include in the papers were decided on before the statistical analyses. The patients mainly scored similarly as the controls, also indicating that such a selection of positive results did not take place.

5.4.3 Assessment of evidence of disease activity

In Paper II we evaluated the patients in our cohort after 13 months according to NEDA. Patients with no relapses, no disability progression and no radiological progression at follow-up compared to baseline were classified as NEDA. *A relapse* was defined as any new neurological symptoms, not associated with fever or infection, lasting for at least 24 hours and accompanied by new neurological signs. *Disability progression* was defined as an increase in EDSS≥1 compared to baseline in the absence of a relapse the last six weeks before examination. *Radiological progression* was defined as at least one new or enlarging T2 or FLAIR WML (with or without gadolinium

enhancement on T1) compared to MRI at baseline. Patients with *either* a relapse, *or* disability progression *or* radiological progression were classified as with evidence of disease activity (EDA).

In our definition of NEDA we did not make any distinction between neurological worsening and neurodegenerative disability progression, as recommended in the new guidelines for defining the clinical course in MS (Lublin et al. 2014; Giovannoni et al. 2015). Further, we could not know for sure whether the disability progression sustained, since we performed only one follow-up examination in this study.

An international panel recently has suggested a decision model based on an extended variant of NEDA; The Multiple Sclerosis Decision Model (MSDM) (Stangel et al. 2014). This model has the advantage of including a detailed neurological assessment and assessments of both fatigue and cognition; and of less rigorous dichotomous outcomes. The disadvantages are time consuming assessments and complicated algorithms. Thus the use of MSDM may cause time constraints and require clinical training. In contrast, NEDA is based on a dichotomous MRI evaluation, medical history and neurological assessment; which is manageable also in busy outpatient clinics.

5.4.4 Assessment of fatigue

In our study, we used the Fatigue Severity Scale (FSS) to measure fatigue, a nine item self-report scale for rating fatigue in chronic medical and neurological disorders (Krupp et al. 1989). FSS has the advantages of being well-established, easy to fill in and has shown associations with brain functional and structural changes in MS patients (Filippi et al. 2002; Rocca et al. 2014a). The Norwegian translation and validation has shown good levels of response and the scores are correlated with self-perceived morbidity. The cut-off at four points for categorization into fatigue or non-fatigue has been questioned in a Norwegian validation study, since a large proportion of the participants fulfilled the criteria of fatigue with this cut-off (Lerdal et al. 2005).

A new fatigue scale, Fatigue Scale for Motor and Cognitive Function (FSMC), has shown a better distinction between motor and cognitive fatigue symptoms in MS

(Penner et al. 2009). Such a distinction may improve the structure-versus-symptom relationship in future studies. We therefore supplemented the FSS with FSMC assessments in the follow-up of the patients in our study, though these results are not yet published.

5.4.5 Assessment of depression

In MS research, structured interviews to assess whether the patients fulfil diagnostic criteria for depressive disorders are seldom used. Instead questionnaires of depressive symptoms are used to rate and categorize patients according to self-reported depressive symptoms (Feinstein et al. 2014). We have used the Beck Depressive Inventory-II (BDI) (Beck et al. 1996), a self-report psychometric scale, for the assessment of depression. This has been widely used in MS research. There are some notable shortcomings of this scale; the scale overlaps with symptoms that may be caused by neurological changes in MS, like fatigue, altered sleep patterns, changes in appetite, poor concentration and impaired memory (Feinstein et al. 2014). Alternative scales, like the Beck Depression Inventory -Fast Screen (Benedict et al. 2003) and Hospital Anxiety and Depression Scale (Honarmand and Feinstein 2009) have also been validated for use in MS and may be better at separating depression from disease-related symptoms (Feinstein et al. 2014). Still BDI is the psychometric scale suggested to be used in the evidence based guidelines for assessment and management of psychiatric disorders in individuals with MS by the American Academy of Neurology (Minden et al. 2014). Our choice of BDI was partly pragmatic, as the healthy controls used in Paper I and II had already filled in this questionnaire in their baseline analyses. The choice of this scale may have led to a confounding of disease-related symptoms on the depression rating. One solution to this problem could have been to exclude the questions most vulnerable to such a confounding from the analyses, as has been done in previous studies of MS and depression (Landrø et al. 2004; Smestad et al. 2010). However, to ensure comparability with the healthy controls, to other studies and for ease of the reader, we chose to adhere to the standard version of the scale.

5.4.6 The use of questionnaires

The patients investigated in this thesis were asked to fill in a comprehensive questionnaire at home, including both questions about depression and fatigue and in addition other questionnaires not yet published. We could not control that all patients filled in the questionnaires. Therefore the studies suffer to some extent from incomplete data collection. As we did not control the environment in which the patients filled in the questionnaires, their answers may have been influenced by unobserved differences in environment (like different living situations influencing perceived fatigue or depression, or different levels of fatigue at different times of the day). Patients could also have been influenced by the surge of the moment; if they filled in the questionnaires right after a long and tiresome examination they may have felt more tired or depressed than usual. The questionnaire may also have been difficult to interpret; though the Norwegian translations and validations make that less probable. And a last; the questionnaires were long and time consuming to fill in; and may thus themselves have led to exhaustion or lack of completion. These factors could have led to concerns concerning internal validity. However, most patients returned the questionnaires, completed them correctly and did not complain of the length of the questionnaires, and we therefore believe that most of these limitations were overcome.

5.5 Neuroimaging

There are several possible confounders when using neuroimaging in patient and control samples. First of all, the imaging modality of choice may not envisage all relevant pathology. Second, the quality of the actual images acquired may be poor. Third, the analysis tools may not be reliable. And last, non-disease related factors may confound the results (like age, gender, life habits, genetics or comorbidities) (De Stefano et al. 2014). Each of these points will be discussed below.

5.5.1 Image acquisition

The patients and the controls of Paper I and II underwent MRI examinations in the same 1.5 T Siemens Avanto scanner (Siemens Medical Solutions) with a 12-channel

head coil. This scanner was chosen because the controls had already undergone examinations in this machine. Recent literature has found that stronger magnets not only to detect more MS related lesions, but also to detect more relevant lesions; and the associations between MRI and disability is higher with higher field strengths (Stankiewicz et al. 2011). We regarded the advantages of including a healthy control group scanned in the same machine as more important, but this choice may have contributed to the relatively low association between cortical structure and patient signs and symptoms in Paper I.

The controls were scanned between June 2007 and December 2008 at baseline and between January 2011 and June 2013 at follow-up. The patients were scanned between January 2012 and January 2013 at baseline and between April 2013 and February 2014 at follow-up. The longer interval between the MRI examinations of the controls may have led to changes in field inhomogeneities and other changes in the MRI machine. However, the Magnetization Prepared Rapid Gradient Echo (MP-RAGE) sequences were kept identical between the scanning periods. We used 3-dimensional T1-weighted MP-RAGE sequences for surface-based and volumetric analyses, and combined the MP-RAGE and the Fluid-Attenuated Inversion Recovery (FLAIR) sequence to estimate the white matter (WM) lesion load.

The sequences parameters of the MP-RAGE sequences were: repetition time/ echo time/time to inversion/flip angle = 2400 ms / 3.61 ms / 1000 ms / 8°, matrix 192 × 192, field of view= 240. Each scan lasted 7 min 42 s and consisted of 160 sagittal slices with a voxel size of 1.20 × 1.25 × 1.25 mm. The FLAIR sequence parameters were as follows: repetition time / echo time / time to inversion / flip angle = 6000 ms / 3.33 ms / 2200 ms / variable T2, matrix 256 × 204, field of view= 260. Each scan lasted 7 min 02 s and consisted of 176 slices, with a slice thickness of 1 mm and a voxel size of 1.0 × 1.0 × 1.0 mm.

The ideal voxel size for surface based and volumetric studies is not known. However, a voxel size between 1.0 and 1.25 mm is considered the easiest to work with in the software program we applied (http://surfer.nmr.mgh.harvard.edu/).

Partial voluming, i.e. the phenomenon that a mixture of different tissue within one voxel may lead to misclassification of the tissue and confound the borders of different tissue types in MRI studies, may have influenced the location of the CNS GM-WM borders in the studies. Furthermore, artefacts either caused by field inhomogeneities, by metal implants in the participant or by ghosting during the image acquisition could have led to distorted images. To ensure proper clinical patient follow-up and good quality of the imaging studies, all the scans were evaluated by clinical neuroradiologists and examined for defects and distortions before image analyses, ensuring good imaging quality for all analyses.

5.5.2 Image analyses

For the volumetric and surface-based analyses we used the open source software program FreeSurfer, version 5.1 in Paper I and version 5.3 in Paper II (Figure 16). The analyses were performed in the neuroimaging lab of the Lifespan Changes in Brain and Cognition (LCBC) research group at the Department of Psychology, University of Oslo, with the kind assistance from experienced researchers in the lab. Freesurfer analyses have been applied to MS patient samples also previously (Sailer et al. 2003). Both surface based and volumetric analyses can be performed with this software, both of which are highly relevant for MS research. Furthermore, the longitudinal pipeline in FreeSurfer enables good quality longitudinal analyses (Reuter et al. 2012). The analyses can be run in an automated mode to limit analysis time. However visual inspection is necessary and manual edits may be performed. The long analysis time makes it unfeasible to use this software in everyday clinical practice, but is a good tool in research when large nodes of computational power are available (Vrenken et al. 2012).

The difference in MRI intensity between gray and white matter varies with location in the CNS. This variance is caused partly by local variance in the histology of

cortical gray matter and the subjacent white matter and partly by acquisition artifacts, such as variable penetration of the radio frequency pulse, magnetic field inhomogeneities and local differences in receiver coil sensitivity. (Cortical gray matter in motor cortex is brighter than in frontal cortex, because of its higher myelin content). Furthermore, the highly folded structure of the cerebral surface and the thin fingerlike protrusions of white matter in the cerebral gyri complicate linear approaches to surface reconstruction. These factors have previously led to difficulties in imaging processing of the cerebral surface (Fischl 2012). FreeSurfer has provided solutions to the above-mentioned difficulties. The identification of GM-WM-boundaries is based on local differences in intensity. Non-linear registration is applied instead of approximating the surface to a plane. Furthermore, a method where the surface of the cortical boundaries are inflated to a sphere before registration to a common atlas is utilized (Fischl et al. 1999a, 1999b).

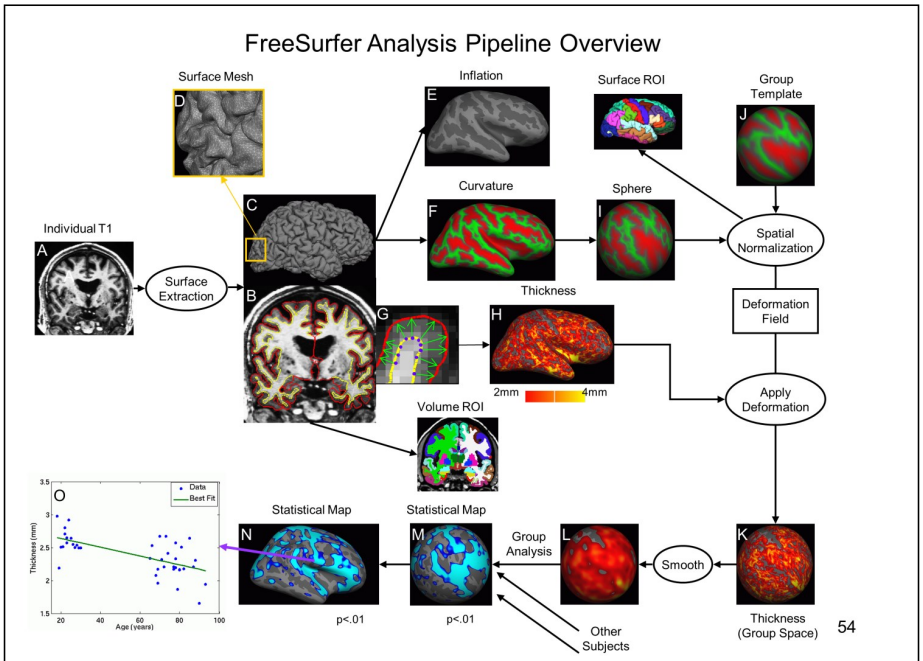

Figure 16. Freesurfer pipeline. Printed with permission from https://surfer.nmr.mgh.harvard.edu.

The reliability and accuracy of this method have been tested by comparisons to histological measures of cortical thickness (Rosas et al. 2002) and by within-subject test-retest studies, as well as by comparison with previous studies of cross-subject regional cortical thickness (Fischl and Dale 2000). Furthermore, comparisons have been made between FreeSurfer estimates and manual measures of cortical thickness in disease and control populations (Kuperberg et al. 2003) and estimation of the stability of the thickness measures with respect to scanner platform, field strength and sequence type have been performed (Han et al. 2006). Finally, the correlations identified between cortical structure and cognitive measures are stable across scan session, scanner manufacturer and field strengths (Dickerson et al. 2008).

Still, there are several possible processing failures when using the FreeSurfer software. These failures may be divided into hard and soft failures (Fischl 2014). Hard failures are failures in the software programming, leading the programs to end before the processing is complete. These failures may be avoided by an ordered structure of directories, by using well-documented commands and by good data quality. Soft failures are errors during the analysis process, like skull strip errors, segmentation errors, intensity normalization errors, pial surface misplacement or errors creating topological brain defects, like holes and handles on the cortical surface. In the case of these errors, the processing of the structural images is completed, but the result may need manual modifications (Fischl 2014). Manual modifications, on the other hand, may lead to biases, because the operator is not blinded to the identity of the participant or because the operator does not treat the material equally for other reasons. As far as possible, we have tried to control for these errors, by structured analysis procedures, visual evaluation of the segmentation and blinded modification or exclusion of wrongly segmented images.

FreeSurfer is constructed for the analysis of 3D T1 weighted images of about 1 mm^3. A recent study comparing 3T and 7T images of healthy individuals concluded that both this software and other image analysis software may overestimate human cerebral cortical thickness from analyses of images from 1.5 or 3T MRI machines,

because of partial voluming (Lüsebrink et al. 2013). This overestimation is, however, hard to overcome with the available MRI platforms and image analysis tools available today.

FreeSurfer has been used in MS research for more than a decade. One of the first studies identifying regional cortical thickness in MS patients was performed using this technique (Sailer et al. 2003). Recent studies have provided evidence that this method can be used in MS multicenter-studies (Narayana et al. 2012) and longitudinal analyses (Rinaldi et al. 2012). The impact of juxtacortical lesions on the segmentation of the GM/WM border has specific interest in MS research. Recent research has suggested that lesion filling, i.e. replacing juxtacortical WMLs with the signal intensity of surrounding WM to improve GM/WM separation, could improve MRI analyses in MS samples (Battaglini et al. 2012). We did not utilize this method and may have underestimated the annual GM atrophy in our analyses (see Paper II). However, we do not believe that this limitation impacts on our main results.

Estimates of white matter lesion load (WMLL) for analyses in Paper I and III were performed using an automatic pipeline for WMLL estimates (http://ki.se/en/nvs/cascade) (Damangir et al. 2012). This method has also been used

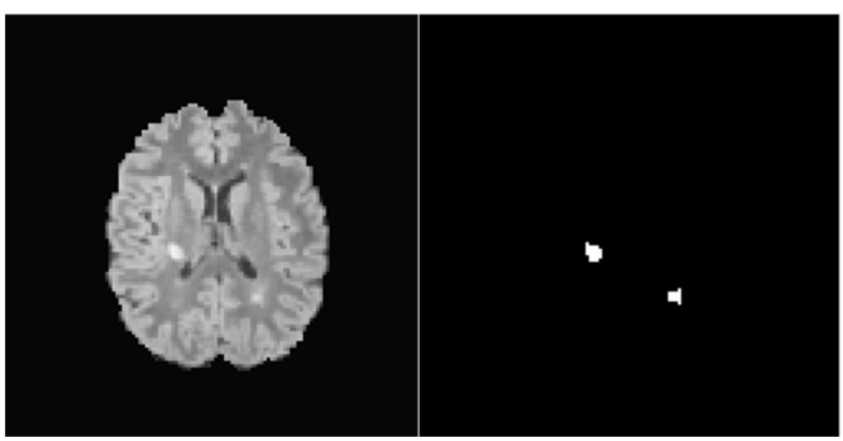

Figure 17. WM lesions on FLAIR image of MS patients and delineation of WM lesion using Cascade.
Illustration: Piotr Sowa/Gro Owren Nygaard

in the estimation of WMLL of patients with coronary heart disease and Alzheimer's disease (Vuorinen et al. 2014a, 2014b). The method achieved high sensitivity (90%) and specificity (99.5%) when compared to manual delineation of WML (Figure 17) (Damangir et al. 2012).

Taken together, we regard the image analysis tools used in this study well validated both in healthy participants and in the MS population, and well suited for the purposes in this study.

5.6 Assessment of vision, retinal nerve layer and visual evoked potentials

The decrements to the visual pathway may confound the results of tests of eye movements and pupillary responses. We therefore controlled for the results of these tests in Paper III and IV. These tests are discussed briefly below.

5.6.1 The link between neuroimaging and brain structure

Several studies support an association between MRI results and pathological findings in MS (Filippi et al. 2012; Rocca et al. 2015). We therefore chose to use MRI to assess CNS differences between patients and controls and to assess longitudinal volumetric changes.

Figure 18. The Early Treatment Diabetic Retinopathy Study (ETDRS) standardized viewer. Model ESV 3000, printed with permission from Good-Lite Co., Elgin, IL).

5.6.2 Assessment of vision

In Paper III and IV assessments of vision of patients and controls were performed at the Department of Ophthalmology by an experienced ophthalmologist and co-worker, MD Sigrid Aune de Rodez Benavent (SADRB), and by experienced research opticians at the department. Visual acuity was

tested at four meter using an Early Treatment Diabetic Retinopathy Study (ETDRS) standardized viewer (Figure 18) (model ESV 3000; Good-Lite Co., Elgin, IL) (Ferris et al. 1982). The spherical equivalents of the participants' prescription, a measure of the curvature of the cornea, were reported (Millidot 2009).

5.6.3 Assessment of retinal nerve fiber layer

Optic coherence tomography (OCT) offers a unique possibility to non-invasively and directly assess a part of the CNS, the retinal nerve fiber layer (RNFL) (Balcer et al. 2014). In Paper IV, where our main aim was to examine task-related pupillary responses in early MS patients, we controlled for the possible effect of differences in RNFL between the groups. Retinal imaging was performed by the same trained ophthalmologist (SADRB) with the spectral domain RS-3000 OCT Retina Scan (Nidek Inc., CA, USA). RNFL data were obtained with the Disc Circle protocol with a scan width of 3.45 mm and a scanning speed of 53 000 A-scans/s, centered on the optic nerve (Figure 19). All scans included had a signal strength of 8/10 or better.

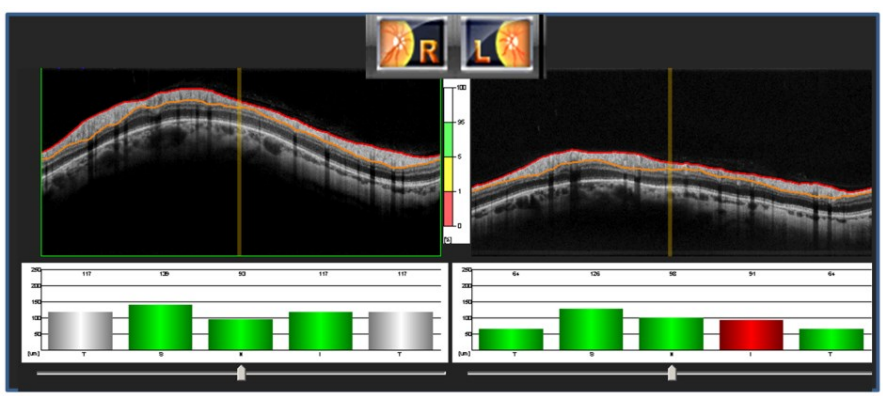

Figure 19. OCT of MS patient with a history of ON on her left eye. RNFL is delineated by the orange and red lines. Illustration: Sigrid Aune des Rodez Benavent/Gro Owren Nygaard

5.6.4 Assessment of visual evoked potentials

Assessments of visual evoked potentials to p100 (VEP) estimates the time for the neural impulses caused by a checker board stimuli to reach from the retina via the optic nerve to the visual cortex (Figure 20) (Sand et al. 2013). VEP is a sensitive marker of previous ON, and can be used when a history of ON is a deciding factor for a MS diagnosis (Polman et al. 2011; Sand et al. 2013). It is frequently pathological in MS patients, also in patients without a history of clinical ON, and prolonged VEPs are associated with disability (Di Maggio et al. 2014). VEPs were obtained and analyzed at the Department of Clinical Neurophysiology, OUH, Ullevål. The examinations were performed with dimmed light. The screen was placed 100 cm from the eyes of the patients, with a Dantec Keypoint Focus system with checkerboard patterns (check size 65') presented at 2 Hz with a 16" cathode ray tube screen. Three hundred responses were averaged from the mid-occipital lobe to the mid-frontal lobe. The VEP results were evaluated by two experienced clinical neurophysiologists (Kristian Bernhard Nilsen and Lars Etholm). VEP was regarded pathological with VEP delay > 110 ms and/or with a delay at least 6 ms greater than compared to the contralateral eye.

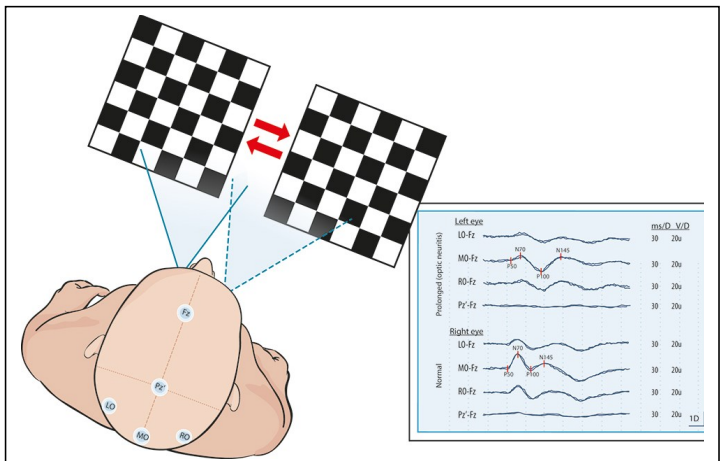

Figure 20. Assessment of visual evoked potentials. Printed with permission from (Sand et al. 2013), Tidsskriftet for den Norske Legeforening and J. Engqvist.

5.7 Eye-tracker measurements

We used a SMI (SensoMotoric Instruments, Teltow, Germany) R.E.D. eye-tracking device with a remote unit attached below a 22 inch computer screen (Dell, P2210) for the assessment of eye movements and the task-related pupillary responses in Paper III and IV. Measurements were conducted throughout the day for both groups.

We did not use a head fixator or chin rest during the experiments. This rendered the experiments more sensitive to confounders like disease-related head tremor (White and Fielding 2012). To control for this, all participants went through a calibration procedure, where a five-point pattern was displayed to the participants before running the eye-tracking sessions. The participants who did not reach the calibration criteria were excluded from the analyses.

Blinking temporarily disrupts the pupillometric measurements, but we did not observe any pathological blinking in the MS patients examined. We would therefore expect these disruptions to affect patients and controls similarly. Any disruption would then

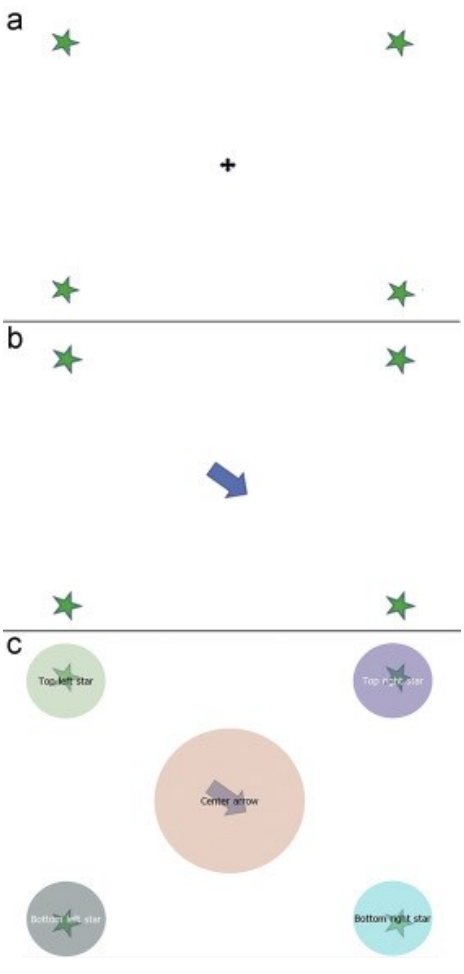

Figure 21. The experimental setup for assessment of saccadic initiation time. Illustration: Sigrid Aune des Rodez Benavent/Gro Owren Nygaard

lead to a non-differential measurement bias, and not cause any false differences between the groups.

Data was recorded at a sampling rate of 60 Hz, i.e. new measurements every 16 ms. Higher sampling rates are possible and would have captured more details of rapid eye movements, minute pupillary changes and, possibly, more noise. Fielding and coworkers have performed similar experiments with a sampling rate of 1000 Hz (Fielding et al. 2009). The variations in measurements of delay to saccadic onset were similar in magnitude in Fielding's study and ours (Fielding et al. 2009). Therefore, for our main outcome, saccadic initiation (SI) time, we believe that our sampling rate was sufficient.

Our experimental setup was designed and the stimuli presentation was implemented using SMI Experiment Center®, which synchronized eye-movement recordings to the presentation of the stimuli. As can be seen from the illustration (Figure 21), the participants were instructed to fixate at the center of the screen and then move their gaze towards the star in the appropriate corner with the appearance of a central arrow. Some cognitive processing was necessary to decide on the direction of the arrow. This time spent to evaluate the direction of the arrow was included in the SI time. In everyday situations one would often have to evaluate a cue before a saccadic initiation. Therefore we our experimental design was realistic. However, as we do not separate the cognitive operation of choosing direction and the saccadic eye movement our results are not readily comparable to previous studies (e.g. (Fielding et al. 2009).

5.8 Statistical considerations

5.8.1 Sample size

The *reference population* is the population from which the participants of the studies were drawn. If not all the participants in the reference population are included in a study, the study will potentially suffer from *sampling variation*, i.e. variations in the

variables measured because of differences between the chosen sample and the reference population (Kirkwood and Sterne 2003). Because of sampling variation we cannot rule out the possibility that the size of the effect observed in the study will be different from the true effect. In other words we can never guarantee that a study will be able to detect an effect however large we make it. However, we can increase the probability of a true result by increasing the sample size. This is called the *power* of the study. Ideally, we would perform power calculations, i.e. calculations of the sample size necessary to have a certain probability of capturing a real effect in our study, before the initiation of a study (Kirkwood and Sterne 2003). However, as our study was restricted by patient and MRI availability we did not perform such analyses.

5.8.2 Statistical tests

We used IBM SPSS Statistics v 22 (SPSS, Chicago, IL) for statistical analyses. We visually inspected histograms and Q-Q plots of the data to assess whether the data was normally distributed. All data satisfied this normality check. We then tested for difference between patients and controls, between the patients at different time points and between subgroups (e.g. EDA, NEDA and HC) with appropriate tests. Independent samples t-tests, paired samples t-tests, χ^2 –tests and one-way between-group analyses of variance (ANOVAs) with Bonferroni-corrected post-hoc tests were applied. The χ^2 -tests performed on categorical variables with two values only (e.g. gender) were corrected for possible overestimation with Yate's continuity correction.

To control for differences in age and gender between the patient groups (for example EDA and NEDA) we performed one-way between-group analyses of covariance (ANCOVAs) where appropriate with the dependent variable of interest (for example volumetric measurements and atrophy rates), group as a fixed factor, and age and gender as covariates.

In Paper II the scan interval was longer for HC than patients, therefore annual percent change of the cortical and subcortical volumes were estimated as described in FreeSurfer version 5.3 (Reuter et al. 2010).

5.8.3 Type 1 and Type 2 errors

When performing a hypothesis test, we get one of four outcomes, as illustrated in Table 4. We may correctly sustain or discard the null-hypothesis. However, two mistakes can be made. First, we may discard the null-hypothesis even though it is true. This is called a *Type 1 error*. Second, we may accept the null-hypothesis even though it is wrong. This is called a *Type 2 error* (Kirkwood and Sterne 2003; Laake et al. 2008). We can reduce the probability of Type 1 errors by using a lower significance level, and Type 2 errors by increasing the power of the study, either by a higher sample size or an increased precision of the measurements.

All results in this thesis were reported based on a *significance level* of 0.05. The significance level, or p-value, equals the probability of the occurrence of a result as extreme as, or even more extreme, than the one observed, even though the null hypothesis is true (Kirkwood and Sterne 2003). When we reported that we found a difference in disability between NEDA and EDA patients at follow-up in Paper II, the probability of the observed difference occurring by chance, even though it was not true, was below 5 %.

	Reality	
Conclusion of significance test	Null hypothesis is true	Null hypothesis is false
Reject null hypothesis	Type 1 error	Correct conclusion
Do not reject null hypothesis	Correct conclusion	Type 2 error

Table 4. Type 1 and type 2 errors. Based on (Kirkwood and Sterne 2003). Illustration: Gro Owren Nygaard

When performing multiple testing, we corrected for this, either using Monte Carlo simulations provided in Freesurfer (Paper I) or by Bonferroni corrections (Paper II and IV), to keep the chance of type I errors low. Further, we sought to use methods with good precision, to reduce the chance of type II errors. However, as our studies were restricted by MRI and patient availability, we did not have the opportunity to increase power by increasing sample size. In Paper IV the large variation in task-related pupillary responses (Peak pupillary dilation was 15.8 % (SD 7.4) in patients and 15.7 % (SD 7.9) in controls) and relatively small sample size (41 patients and 43 controls) may have led to a type II error; there may be differences between patients and controls not identified with our tests.

5.9 Ethics

5.9.1 Ethical approvals

This project was approved by the Ethical Committee of the South-Eastern Region of Norway. All participants received oral and written information and gave written consent to participate in the study.

5.9.2 Ethical considerations of clinical and cognitive assessments

The main inconvenience to the participants in this study was the possible exhaustion caused by the time consuming examinations. The patients may have felt obliged to participate in the study because of loyalty to the treating institution or because of personal knowledge of any of the clinicians involved in the study. Furthermore the patients may have become more aware of the large variety of possible symptoms in MS because of the different tests, and may thus have been unnecessarily worried.

To avoid exhaustion we always made sure that the patients wanted to proceed during the examinations and adjusted the test situations when necessary. Whenever the patients were worried about the test results we provided follow-up consultations to give feedback and explain the results of the tests. And, finally, the positive responses

throughout the examinations ensured us that participation in the study was mostly a positive experience for both patients and controls.

5.9.3 Ethical considerations of MRI assessments

MRI assessments are generally regarded safe, even though there are several minor and possibly major risk factors involved, mainly caused by the strong magnetic field in the magnet (Westbrook et al. 2011). Magnetic objects or implants may cause damage to the participants if the safety requirements are not followed, and the radio frequency pulse leads to a transient heating of the patient, possibly inducing the heat-related Uthoff's phenomenon, causing reversible neurological symptoms in MS patients. Furthermore, the noise of the MRI machine and the long period of immobility may lead to reversible discomfort or to fear in participants with claustrophobia (Westbrook et al. 2011).

The intravenous injection of Gadolinium to evaluate blood-brain-barrier leakage is also considered safe to patients without renal failure (Westbrook et al. 2011). However, recent studies have indicated that Gadolinium may in fact accumulate in the brain of patients after several injections (Kanda et al. 2015). The pathophysiological consequence of this accumulation is not known. Gadolinium is, however, a lethal agent and Gadolinium chelates should not be administered unless there are medical indications. In this study we performed MRIs with Gadolinium chelate-injections as part of clinical follow-up. However, because the patients in the study were scanned and clinically followed at two locations of the hospital physically and technologically separated, we were not able to completely avoid that some patients underwent more examinations than strictly necessary.

6. General discussion

6.1 Structure of the cerebral cortex in early MS patients

Gray matter atrophy early in the disease course has been associated with long-term disability in MS (Filippi et al. 2013a; Popescu et al. 2013) and is considered an important target in new treatment studies (Filippi et al. 2014). In the first paper of this thesis we confirmed that the cerebral cortex of MS patients with short disease duration (mean 26 months) was thinner than in healthy controls. These results were in line with previous research (De Stefano et al. 2003; Sailer et al. 2003), and showed that cortical thinning was present also in patients from our population-based cohort.

While cortical thickness has been thoroughly examined in MS, cortical surface area has barely been studied. Previous studies have shown that cortical surface area is reduced in developmental disorders like Williams syndrome (Meda et al. 2012) and microcephaly (Rimol et al. 2010). In other neurological disorders, like Parkinson's disease, an increased cortical surface area compared to controls has been identified (Jubault et al. 2011). A small study of patients with Alzheimer's disease did not reveal differences in surface area between patients and controls in the temporal lobe (Dickerson et al. 2009). We did not identify any reductions in cortical surface area in MS patients compared to healthy controls in Paper I. Another recent study, predominantly studying cerebral curvature and white matter volume in CIS and MS patients, also found preserved cortical surface area in MS patients using Freesurfer software (Deppe et al. 2014). Other studies have found a reduced cortical surface area in MS patients compared to controls (Hier and Wang 2007; Gorgoraptis et al. 2010). This difference may in part be caused by differences in disease duration and procedure used in the studies. Compared to the first study (Hier and Wang 2007), we included more patients and used a robust method. Compared to Gorgoraptis and colleagues (Gorgoraptis et al. 2010) our patients had a lower age and lower disability, otherwise our methods were comparable.

Pathophysiologically the cortical surface area is an interesting measurement, because meningeal inflammation may play an important role in MS pathogenesis. Ectopic B-cell follicle-like structures in the deep sulci of the brain have been identified in around 40% of patients with secondary progressive MS. The presence of such inflammation was associated with a more severe disease course (Howell et al. 2011). Such inflammation could potentially cause a reduction of the cortical surface area. Our study indicated that if cortical inflammation was present early in the disease, it did not alter the cortical surface area. Taken together, ours and other's research may therefore indicate that the cortical surface area is affected differently during the course of the disease. Longitudinal studies of changes in the structure of the cerebral cortex in MS are lacking, but may provide further insight into the pathophysiological events in early MS and the clinical impact of these changes.

6.2 Association between cortical structure and specific symptoms – indicators of emotional brain reserve in MS patients

In Paper I we found that higher disability in MS patients was associated with a regionally thinner cortex and an overall higher lesion load (r=0.3), similar to other studies (De Stefano et al. 2003; Sailer et al. 2003; Narayana et al. 2012). This gap between disability and proof of structural brain damage by imaging, termed the "clinico-radiological paradox" (Barkhof 2002), may partly be explained by the lack of specificity of the disability score EDSS (Cohen et al. 2012) and by the fact that not all structural brain damage is visible on standard MRI sequences (Filippi et al. 2012). In addition functional brain changes may occur as a response to the disease (Rocca et al. 2003).

We also identified an association between fatigue and cortical volume of the right fronto-parietal region, similar to previous studies of MS patients with fatigue (Calabrese et al. 2010a; Pellicano et al. 2010; Gobbi et al. 2014). These results fit with the hypothesis that there is a thalamo-striato-cortical determinant to fatigue (Engström et al. 2013). Furthermore, they support the concept that fatigue in MS is related, at

least in part, to characteristics of frontal and parietal cortical areas, known to be involved in cognitive and attention processing, even from the early stages of disease.

We further identified an association between depression and cortical surface area, mainly in the frontal and parietal lobes, as well as an association between depression and a smaller cortical volume in the same regions. Associations between depression and regional cerebral volume changes in MS patients have also been identified previously (Bakshi et al. 2000a; Gobbi et al. 2014). We found a negative association between cortical surface area and depressive symptoms in cerebral regions with no reduced surface area in the patient group compared to the controls. This may possibly indicate the presence of an emotional “brain reserve”; a larger surface area in certain regions may be related to less depressive symptoms when presented with a neurological disease. This hypothesis corresponds to the hypothesis of a cognitive “brain reserve" in MS, where a larger life-time brain volume is associated with less cognitive decline (Sumowski et al. 2014).

6.3 Cognitive function and cognitive reserve in early MS

We identified no cognitive impairment in our patient group compared to published norms. This result is in contrast to most previous studies of early RRMS patients (Rao et al. 1991; Amato et al. 1995, 2010; Achiron and Barak 2003). The first studies of cognition in early MS patients diagnosed patients according to more stringent diagnostic criteria than we used (Amato et al. 1995). Thus the other patients may have had a longer disease duration or a more severe disease than our patients. Further, many studies of cognition in MS have been clinic-based, contributing to a possible selection of more severely ill patients (Achiron and Barak 2003).

The combination of large regional thickness differences between patients and controls, as well as the sparse associations between cognitive performance and cortical morphology in our patient sample, fits with the cognitive reserve hypothesis, i.e. that premorbid education, leisure or other activities may protect against, conceal or

postpone cognitive decline (Sumowski et al. 2009, 2010, 2014; Amato et al. 2013). Most of our patients had high general ability levels and high levels of education. They were either students or working, confirming that this was a well-functioning patient group, and that they participated in "reserve-building" activities (Schwartz et al. 2015). It must, however, be kept in mind that the tests applied here did not capture all aspects of cognition that may be affected by the disease.

Even in the absence of evidence of cognitive decline in our patients, we identified associations between test performance and cortical structure in the patient group. For verbal memory, we identified an association between good performance and a larger cortical surface area in parts of the left occipital and temporal cortex. For visuospatial memory we identified an association between performance and right temporo-parietal volume. These results were similar to studies of regional lobar cortical thickness of MS patients with a longer disease duration (Benedict et al. 2002). We found no association between regional cortical measures and SDMT results in the patient group. This may be explained both by the good performance of the patients and by the nature of the test, further investigated in Paper III.

6.4 Evidence of disease activity in early MS and relation to treatment

With the emergence of new disease modifying treatment (DMT) options, "disease activity free status" (Havrdova et al. 2009; Bevan and Cree 2014) or "no evidence of disease activity" (NEDA) (Rotstein et al. 2014) has been introduced as an ambitious goal of multiple sclerosis (MS) therapy. The rationale for this concept is that MS treatment should aim for no signs of disease activity; neither new relapses, disability progression nor new or enlarging white matter (WM) lesions. In Paper II we evaluated the disease activity in our early RRMS cohort 13 months after baseline and found that 46 % showed evidence of disease activity in spite of treatment according to national guidelines. Thus 54 % of our patients were categorized as NEDA. This proportion of patients with no evidence of disease activity was comparable to a recent cohort study, which found that 46 % of their patients fulfilled the NEDA criteria after

one year (Rotstein et al. 2014). Lower proportions of NEDA have been observed in most clinical trials, both for patients receiving DMTs and placebo (Havrdova et al. 2009; Giovannoni et al. 2011; Lublin et al. 2013). In contrast, a recent interim report on the effect of AHCT of MS patients reported 78 % NEDA after 3 years (Nash et al. 2015). In our study patients were included irrespective of disease activity and treatment, while most clinical trials include patients with active disease only. Furthermore, the patients in our cohort were assigned to treatment by their neurologist, not randomized, and all patients in our cohort had disease duration ≤ 3 years. Current literature shows that we are still far from the goal of no evidence of disease activity in MS patients. The low proportion of NEDA among the patients receiving first line DMTs (40 %) in our study is of particular interest. Long term effects of first line DMTs in registry studies remain uncertain (Shirani et al. 2012; Tedeholm et al. 2013). Our study supports that these drugs may not give sufficient protection against disease activity in early MS.

6.5 Disability progression, cognition and gray matter atrophy in early MS

Changes in disability, as measured by EDSS, is the most common outcome studied in MS populations (Cohen et al. 2012). In Paper II we found that the NEDA patients improved in disability. A disability improvement has also been reported in other studies, but the observation is rare (Tremlett et al. 2012; Nash et al. 2015). Such an improvement may reflect tissue repair in the absence of inflammation. A disability improvement in the absence of evidence of disease activity makes NEDA a highly relevant goal for future treatment of MS patients. Such data bears promise that adequate treatment may not just halt the disease progression; it may even lead to improvements in function.

In the NEDA group, there was a trend towards an improvement also in processing speed, probably the main cognitive domain affected in MS (Van Schependom et al. 2014b). Even though the observation period of our study was short, this result may have been a consequence of disease stability. The EDA patients caught up with the NEDA group on verbal learning at follow-up. This may have been caused by

a combination of practice effects and because they had not yet reached the ceiling of the test score at baseline. Fatigue and depressive symptoms were similar between the patient groups at baseline and at follow-up, indicating that neither of these factors can predict EDA, nor are they the direct consequence of EDA in a one-year perspective.

In line with previous studies, the patients in our study showed both a thinner cerebral cortex and a smaller subcortical volume compared to controls, and annual subcortical GM atrophy rates were larger in patients than controls (Geurts et al. 2012). The subcortical GM atrophy rates between the EDA and NEDA patients differed numerically, but were not significantly distinguishable in our sample. However, the subcortical atrophy rates of the patients with disease activity (EDA) were significantly higher than in the healthy controls. We therefore hypothesized that pathological neurodegeneration in the EDA patient group drives the increased atrophy rates of the RRMS patients.

6.6 Saccadic initiation time and hand motor speed in early MS

Early features of RRMS may include eye motor disturbances (Reulen et al. 1983; Frohman et al. 2005; Graves and Balcer 2010), fine motor control of the hand (Cutter et al. 1999) or cognitive dysfunction (Amato and Ponziani 2001; Amato et al. 2010). The Symbol Digit Modalities Test (SDMT) (Smith Aron 1982) is a widely used test of processing speed, recently suggested as sentinel test for cognitive impairment in MS (Van Schependom et al. 2014a). It is part of several test batteries used in the assessment of cognitive impairment in MS patients (Benedict et al. 2002; Langdon et al. 2011) and is suggested for use in clinical trials (Benedict et al. 2012b). Because of the wide use of the SDMT (Benedict et al. 2004; Drake et al. 2010; Langdon et al. 2011), it is important to identify whether decrements in motor function could lead to input or output level problems related to the procedure of the test.

In Paper III we tested Saccadic initiation time (SI time), i.e. the time from a central visual cue appears to the onset of an appropriate saccade in patients and

controls. We found that SI time was increased in patients with MS compared to healthy controls. We also tested motor hand function of the patients with 9HP. We found that both hand function (9HP), and SI time were associated with the test results of the wSDMT. These motor functions were not associated with the auditory and oral test of the same functional domains, PASAT. The fact that SDMT was associated with both saccadic initiation time and hand motor speed implies that the clinician should keep in mind that eye movement disorders and hand motor difficulties could confound the results of this test. These confounders may not affect the real-life value of this test, but in diseases with motor difficulties, like MS, input and output problems may contribute to false low cognitive test scores.

6.7 Pupillary responses to problem solving in MS patients and controls

Cognitive dysfunction and fatigue are common and troublesome symptoms, often present early in the disease course of MS (Krupp et al. 1988; Amato et al. 2010). The cause of these symptoms are only partly accounted for by functional and structural changes visible on MRI (Bakshi et al. 1999; Genova et al. 2013; Rocca et al. 2014a, 2015). Whether there is a connection between autonomic disturbances, fatigue and cognitive difficulties in these patients is currently unknown. In Paper IV we tested whether measures of pupil size during problem-solving in early MS patients could detect early functional brain changes associated with fatigue and cognition.

Pupillometry has been used in psychological research as a marker of *processing load*, i.e. the intensity of mental activity (Laeng et al. 2012). The task-evoked pupillary response provides a reliable and sensitive indicator of within-patient variations in processing load in memory, language, reasoning and perception tasks, and it is sensitive to between-group differences in intelligence (Beatty 1982).The task-evoked pupillary response is associated with activation of locus coeruleus (LC) and noradrenergic activation of large brain regions, including sympathetic and parasympathetic nuclei. Thus the pupillary response mirrors both brain activation (e.g. cognitive load) and central autonomic changes.

We found that patients and controls showed similar pupillary responses to cognitive tasks at a group level. This result indicated that most patients and controls had a similar and normal LC response to cognitive tasks. Thus most patients showed a well-functioning task-related allocation of attention that facilitated efficient cognitive processing. This result fits with recent fMRI results of cognitive processing of early MS patients, where normal activation patterns were found in the cognitively preserved patients (Rocca et al. 2014b).

6.8 Pupillary responses to problem solving in patients with different symptoms

As expected from the literature, we found that controls with a low cognitive score (LCS) had an increased pupillary response to simple cognitive tasks compared to controls with normal cognitive scores (Hess and Polt 1964; Beatty 1982). We did, however, not identify any increased pupillary response in the LCS-MS patients. This lack of response could indicate an altered LC activation during cognitive tasks in these MS patients. There was a trend towards a smaller pupillary response in patients with fatigue and depression, but the group differences did not reach significance.

From the clinical impression that MS patients may experience symptom relief with tricyclic antidepressants (TCA) and l-dopa treatment, the hypothesis that MS may be caused by altered noradrenergic (NA) transmission was raised (Berne-Fromell et al. 1987). Even though this hypothesis has little support, there are an increasing number of studies indicating that the LC/CNS NA-system is altered in MS patients. In experimental autoimmune encephalitis (EAE) mice and in autopsies of chronic MS patients, reduction in CNS NA levels and damage to LC neurons have been described (Polak et al. 2011). The same research group found that LC damage increases the symptom severity in EAE, and that increasing NA and NA precursor levels reverse this effect (Simonini et al. 2010).Treatment of EAE mice with a vincamine derivate called vindeburnol, which temporary depletes CNS NA and leads to an upregulation of LC NA levels and metabolism, leads to a reduction in EAE symptoms (Polak et al. 2012). A

randomized controlled trial of treatment to normalize NA levels of the CNS (lofepramine, phenylalanine and B12) in 69 MS patients in different stages of the disease, led to a reduction in MS symptoms in the treated patients (Wade et al. 2002). In a small subgroup of patients, a reduction in T1 lesions and a slower atrophy rate on MRI was observed (Puri et al. 2001). Thus, there are several indicators that the LC/CNS NA-system is altered in MS patients and that this alteration has clinical and pathological consequences.

In line with this, modafinil, a sympathomimetic used in the treatment of attention deficit and hyperactivity disorders, has been suggested as a treatment for fatigue in MS patients (Niepel et al. 2013). The autonomic disturbances present in the fatigued patients prior to treatment were alleviated by the treatment, and it was suggested that the effect of modafinil partly was an indirect dopaminergic effect on the LC (Niepel et al. 2013). The hypothesis that dopaminergic disturbancies may be a key in MS fatigue has also recently been proposed (Dobryakova et al. 2015). Our results indicated that these pathways are mostly preserved in early MS patients, but we propose that pupillary responses in patients with cognitive impairment and fatigue with long disease duration should be investigated further.

6.9 Pupillary responses to problem solving in patients with a history of ON or brainstem lesions

The diversity of the symptoms and the dissemination of lesions and non-lesion pathology in the CNS of MS patients make the isolation of specific causes of different symptoms in MS patients difficult. In particular, altered pupillary responses could be caused by both disruptions of the visual pathways, altered activation of the involved nuclei and of the pathways from these nuclei to the pupillary muscles. Prior to this study it was not known whether the disruption of the visual pathways caused by ON or brainstem lesions would lead to an altered task-related pupillary response in MS patients. In Paper IV we were able to control for both brainstem lesions and a history of ON. Neither brainstem lesions nor ON affected the pupillary response found in patients

with preserved vision. This result is important for future studies of the task-related pupillary response in MS patients.

7. Theoretical and clinical implications of the thesis

In this thesis we have examined cognition, disease activity and structural MRI changes in a cohort of early RRMS patients. The results discussed in this thesis have several theoretical and clinical implications.

Damage to cerebral gray matter and a rapid increase in disability early in the disease course are associated with worse long term outcome in MS patients (Degenhardt et al. 2009; Scalfari et al. 2010, 2014; Popescu et al. 2013). The absence of disease activity measured by NEDA identified in Paper II was associated with disability regression and normal GM atrophy rates compared to healthy controls. Thus this thesis supports the use of NEDA as treatment goal for MS patients.

The large proportion of patients on first line DMTs with evidence of disease activity after just one year identified in Paper II (46 %) implies that we did not reach the goal of NEDA in these patients. Considering the potential long term consequences of early disease activity, this study supports a more liberal use of second line DMTs in early MS patients.

After the completion of this study new DMTs have become available and are part of the new treatment guidelines for MS in Norway (under revision) (Hartung et al. 2014; Thomas and Wakefield 2015). Future studies should focus on the effect on disease activity, disability, cognition and GM changes in patients on these treatments as well.

The good and stable performance of the patients on cognitive tests in Paper I-IV results has several implications. Previous studies have found conflicting effects of cognitive reserve on cognition in MS patients as the disease has progressed (Amato et

al. 2013; Sumowski et al. 2014). Our patient group seemed quite robust to cognitive changes early in the disease process. Further investigations of cognitive decline in population-based MS cohorts diagnosed according to the new diagnostic criteria are warranted. Our study was not designed as an epidemiological study, but the cohort was drawn from an unselected patient pool. We therefore expect the disease related symptoms and signs reported in this thesis to resemble those of RRMS patients drawn from similar populations. According to our results, early RRMS patients are mostly cognitively well-functioning, which is relevant for the clinical approach to patient information and care.

We identified associations between depressive symptoms and cortical surface area and volume, and between fatigue and cortical volume in Paper I. These brain regions were not different between patients and controls. A possible implication of these results is that larger cortical surface area and higher cortical volume in certain brain regions could be protective against depressive and energy-related symptoms. Our results indicate that premorbid brain resources may be relevant not only in cognition, but also in the mental health of MS patients. This result should trigger further research into potential “mental brain reserve”.

In Paper III we found that wSDMT was associated with both saccadic initiation time and hand motor speed. Thus in diseases with motor difficulties, like MS, input and output problems may contribute to false low cognitive test scores. The study pointed to a problem potentially present when performing testing of any kind: other factors than the one we want to assess may confound the results. Paying attention to the potential pitfalls of confounders in the assessment of patients may help ensuring correct diagnosis and treatment; this holds true both for patients with MS and other neurological disorders.

In Paper IV we found that most patients tested had normal pupillary responses to cognitive tasks, indicating that this method did not identify LC or central autonomic changes on a group level in early MS. We have shown that this method may be used in

MS studies, since pupillary responses were preserved in both patients with a history of ON and brainstem lesions on MRI. The patients with low cognitive scores did not show the expected large pupillary responses in our study. This is an indicator that LC activation may be altered in CI MS patients. Future studies of pupillary responses in CI MS patients may contribute to a better understanding of the mechanisms of cognitive impairment in MS.

Schoonheim and coworker have suggested that cognitive impairment is a result of network collapse, induced by structural brain damage and network inefficiency (Schoonheim et al. 2015). Studies of cognitive reserve have shown that education or other intellectual stimuli prior to disease onset may modulate the effect of structural brain damage on cognitive function in MS patients (Sumowski et al. 2009). It is currently not known whether functional changes in cerebral networks are delayed in

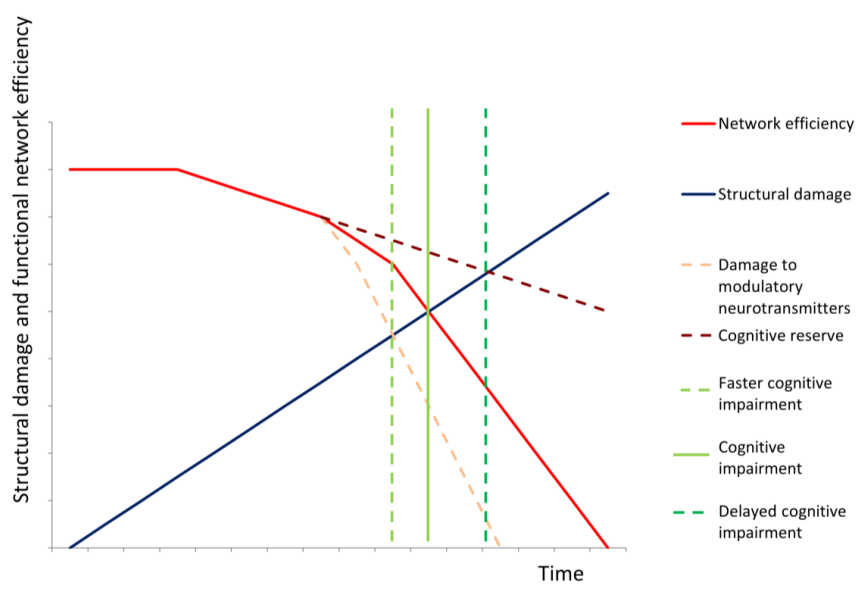

Figure 22. Model of factors contributing to the onset of cognitive decline in MS. The model is based on hypotheses of network collapse, cognitive reserve and the role of modulatory neurotransmitters in the development of cognitive decline in MS (Sumowski et al. 2009; Hanken et al. 2014; Schoonheim et al. 2015). Illustration: Gro Owren Nygaard

MS patients with cognitive reserve. The lack of LC activation, illustrated as the low pupillary response in LCS-MS patients could contribute to such a network collapse. From the combined results from literature and the papers included in this thesis I propose a model to explain the mechanisms contributing to the onset of cognitive impairment in MS patients (FIgure 22). I hypothesize that cognitive reserve would stabilize functional brain networks and delay the diminishing network efficiency, as illustrated with the dark red dotted line. LC alterations or other alterations of modulatory neurotransmitters in the CNS, on the other hand, could contribute to a faster network collapse, as illustrated by the pink line, and thus contribute to an earlier onset of cognitive impairment in these patients.

8. Future perspectives

This thesis includes baseline and one-year follow-up data of a cohort of early MS patients. Longitudinal studies, at least with small drop-out rates, are valuable in studying the disease evolution. This study, with its well-defined population and large data collection at baseline, may provide a valuable basis for a long-term assessment of cognition, disease activity and MRI changes in RRMS patients. Such studies could lead to a better understanding of the associations between cognition, brain structure and functional brain changes in MS and improve future treatment of this patient group.

We identified very little cognitive difficulties in our patient sample. However, the tests included here only covered some of the cognitive domains possibly affected in MS. Future studies could utilize other data from this cohort study, not yet analyzed, to study subtle cognitive impairments in these patients in more detail. In particular, analyses of continuous performance tests (Anti-saccade test and Attention Network Test), designed to identify minute differences between groups could elaborate on brainstem involvement and aspects of attention impairments.

Whether GM or WM changes are the first to appear in MS is still unknown. This could be studied in order to understand the first events initializing the disease. It would therefore be interesting to further characterize the gray matter changes of these patients, and contribute to identify new methods evaluating GM changes in early MS.

Through the work on this thesis I have I have realized that proper prevalence and incidence studies of cognitive impairments in MS populations are still scarce. Studies of the magnitude of this problem are still warranted.

Further, this study has taught me a great deal about performing an observational study. It has inspired me to wish for a more comprehensive data collection of MS patients in Oslo, in Norway and internationally. Large collaborations with good inclusion rates may contribute to unveil associations between risk factors,

like genetic susceptibility and environmental exposures, clinical and structural changes in MS.

At last, future research should ask new research questions. We could perhaps amend the pathological processes seen in MS by enhancing the reparative strategies of the CNS**,** e.g. by inducing remyelination in demyelinating lesions. Emerging research holds promise of new treatment strategies with the ambition not only to slow the disease development, but also to improve future function in MS patients.

The next decades of MS research hopefully will lead to better understanding of who acquires the disease, why they acquire it, how we should treat them and how we can prevent others from acquiring this devastating neurological disease.

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10. Errata

Page 9, line 18(the heading of Paper III): …" symbol digit Modalities Test"… corrected to …"*Symbol Digit* Modalities Test"…

Page 22, figure legend: "Figure 9. Definitions of disease course in MS" corrected to "*Figure 10*. Definitions of disease course in MS" because of duplication in the manuscript. All the following figures are re-numbered accordingly.

Page 38, line 2: …" the modulation of cytocike production"… corrected to …" the modulation of *cytokine* production"…

Page 49, line 28: …" diagnosis in the patients diagnosed according to 2005"… corrected to …" diagnosis in the patients diagnosed according to *the 2005 McDonald Critera*"…

Page 54, line 14: …" may case time constraints and require clinical training"… corrected to …" may *cause* time constraints and require clinical training

Page 78, line 22: …" experimental automimmune encephalitis (EAE)"… corrected to … "experimental *autoimmune* encephalitis (EAE)"…

Page 82, figure 21 (new 22): y-axis legend "Structural and functional damage" corrected to "Structural damage and functional network efficiency"

Paper IV, page 28, line 649, figure caption: "SI Fig 2. Fig 5." corrected to "SI Fig 2."