UNIVERSITÀ VITA-SALUTE SAN RAFFAELE

CORSO DI DOTTORATO DI RICERCA INTERNAZIONALE IN MEDICINA MOLECOLARE

Curriculum in Neuroscienze e Neurologia Sperimentale

FUNCTIONAL AND STRUCTURAL MRI CORRELATES OF COGNITIVE DYSFUNCTION IN PEDIATRIC MS AND A COMPARISON OF COGNITIVE RESILIENCE WITH ADULT-ONSET MS

DoS: Prof.ssa Maria Assunta Rocca

Second Supervisor: Prof.ssa Kyla Mckay

Tesi di DOTTORATO DI RICERCA di: Damiano Mistri

Matr. 020166

Ciclo di dottorato: XXVII

SSD: MED 26/ MED 37/ MED 39

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- The present work "Structural and functional imaging features of cognitive phenotypes in pediatric multiple sclerosis" (Section 3 and Section 7) was performed by Damiano Mistri. The present work has already been published in Annals of Clinical and Translational Neurology (PMID: 38804116, DOI: 10.1002/acn3.52090).
- The present work "Validation and standardization of the Consecutive-letter Substitution Test in an Italian population sample" (Section 6) was performed by Damiano Mistri. The study protocol has been published on ClinicalTrials.gov (https://clinicaltrials.gov/study/NCT06066281?term=consecutive-letter%20substitution%20task&rank=1).
- The present work "The association between cognition and motor performance is beyond structural damage in relapsing-remitting multiple sclerosis" (Section 9.1) was performed by Damiano Mistri. The present work has already been published in Journal of Neurology (PMID: 35278104, DOI: 10.1007/s00415-022-11044-8).
- The present work "Cognitive function in primary and secondary progressive multiple sclerosis: A multiparametric magnetic resonance imaging study" (Section 9.2) was performed by Damiano Mistri. The present work has already been published in European Journal of Neurology (PMID: 37246467, DOI: 10.1111/ene.15900).

- The present work “Cognitive phenotypes in multiple sclerosis: mapping the spectrum of impairment” (Section 9.3) was performed by Damiano Mistri. The present work has already been published in Journal of Neurology (PMID: 38007408, DOI: 10.1007/s00415-023-12102-5).
- The present work “Monoaminergic network dysfunction and development of depression in multiple sclerosis: a longitudinal investigation” (Section 9.4) was performed by Damiano Mistri. The present work has already been published in Journal of Neurology (PMID: 38112782, DOI: 10.1007/s00415-023-12138-7).

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Abstract

During my PhD, I focused on the study of the MRI substrates of cognition in pediatric-onset (PO) multiple sclerosis (MS) patients and on the validation of a novel paper-pencil cognitive test, the Consecutive-letter Substitution Test (CST), to assess information processing speed (IPS).

By applying unsupervised machine learning, we identified three cognitive phenotypes in pediatric MS patients: normal functioning, mild verbal memory and semantic fluency deficits, and global impairment, each associated with different clinical features, as well as structural and functional MRI abnormalities. In a longitudinal setting, I tested the association between consolidated resilience, inter-network functional connectivity changes and cognitive decline in different age groups of MS patients. Results showed that the protective role of consolidated resilience on cognition was mediated by inter-network connectivity of the frontoparietal network (FPN) in pediatric MS patients and by the inter-network connectivity of the salience and executive networks in adult MS patients. These findings suggest that the association between higher consolidated resilience and better cognitive outcomes in MS patients is moderated by specific RS FC patterns between cognitive networks, that change with age. Using DTI, we explored the maturational processes of the superficial white matter (SWM) in POMS and identified widespread reductions in myelination level compared to age-expected values, also demonstrated their association with IPS and visuospatial abilities. Increased cortical thinning and decreased cortical volume were found across the entire cortical mantle. Finally, we provided preliminary evidence of the validity and reliability of the CST, an IPS test that, compared to the Symbol Digit Modalities Test (SDMT), aims to be less influenced by visual scanning abilities and visuospatial memory. The CST showed strong convergent validity with measures of working memory, attention and IPS. It provided a more focused measure of cognitive speed compared to the SDMT, which was also influenced by verbal memory and verbal fluency abilities.

In conclusion, in this PhD project we used conventional and advanced neuroimaging techniques to elucidate the substrates of cognitive decline in POMS, and introduced the CST, a reliable test for assessing IPS.

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Acronyms and abbreviations

9-HPT	Nine-hole peg test
aCompCor	Anatomical component-based noise correction
ADS	Acute demyelinating syndrome
ANOVA	Analysis of variance
AOMS	Adult-onset multiple sclerosis
BASC-II	Behavior Assessment System for Children Second Edition
BD	Block design
BOLD	Blood oxygenation level-dependent
BRB-N	Brief Repeatable Battery of Neuropsychological Tests
BVMTR	Brief Visuospatial Memory Test-Revised
CC	Corpus callosum
CDI	Children Depression Inventory
CELF-3	Clinical Evaluation of Language Fundamentals 3rd edition
CFI	Comparative fit index
CI	Confidence interval
CIS	Clinically isolated syndrome
CL	Cortical lesion
cltr	Consistent long-term retrieval
CNS	Central nervous system
CNT	Contingency Naming Test
COWAT	Controlled Oral Word Association Test
CPT	Continuous Performance Test
CSF	Cerebrospinal fluid
CST	Consecutive-Letter Substitution Test
CVLT	California Verbal Learning Test
CVLTC	California Verbal Learning Test for Children
DE-TSE	Dual-echo turbo spin echo
DKEFS	Delis–Kaplan Executive Function System
DMN	Default-mode network
D-MS	Multiple sclerosis patients who developed depressive symptoms at follow-up
DSC	Digit Symbol Coding

DSM-5	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
DT	Diffurion tensor
DTI	Diffusion tensor imaging
EBV	Epstein-Barr virus
EDSS	Expanded disability status scale
EOWPVT	Expressive One Word Picture Vocabulary Test
EPI	Echo planar imaging
ETL	Echo train length
FA	Fractional anisotropy
FC	Functional connectivity
FDR	False discovery rate
FDT	FMRIB's Diffusion Toolbox
FLAIR	Fluid attenuated inversion recovery
fMRI	Funtional magnetic resonance imaging
FOV	Field of view
FPN	Frontoparietal network
GM	Gray matter
HCs	Healthy controls
HLA	Human leukocyte antigen
ICA	Independent component analysis
ICD-10	International Statistical Classification of Diseases and Related Health Problems 10th Revision
IPS	Information processing speed
IQ	Intelligence quotient
IQR	Interquartile range
KMO	Kaiser–Meyer–Olkin
K-SADS-PL	Kiddie Schedule for Affective Disorders and Schizophrenia for School-Age Children - Present and Lifetime Version
lts	Long term storage
LV	Lesion volume
MADRS	Montgomery-Åsberg Depression Rating Scale
MCST	Modified Card Sorting Test
MD	Mean diffusivity
MI	Multidomain involvement
MMD	Mild Multi-Domain Impairment
MNI	Montreal Neurologic Institute
MRI	Magnetic resonance imaging
MS	Multiple sclerosis
MSD	Mild Single-Domain Impairment

MVS	Mild verbal learning and memory/semantic fluency involvement
NA	Normal appearing
NBV	Normalized brain volume
NCD	Neurocognitive disorder
NCGMV	Normalized cortical grai matter volume
NDGMV	Normalized deep gray matter volume
ND-MS	Multiple sclerosis patients who did not develop depressive symptoms at follow-up
NeMo	Network modification
NGMV	Normalized gray matter volume
NWMV	Normalized white matter volume
OR	Odds ratio
PBVC	Percentage brain volume change
PC	Preserved cognition
PCNB	Penn Computerized Neurocognitive Battery
PedsQL-MFS	Paediatric Quality of Life Inventory Multidimensional Fatigue Scale
PET	Positron emission tomography
PLS	Partial least squares
PMS	Progressive multiple sclerosis
POMS	Pediatric-onset multiple sclerosis
PP	Primary progressive
RCI	Reliable change index
RMSEA	Root mean square error of approximation
RR	Relapsing-remitting
RS	Resting state
SD	Standard deviation
SDMT	Symbol Digit Modalities Test
SMD	Significant Multi-Domain Impairment
SP	Secondary progressive
SPART	10/36 Spatial Recall Test
SRH	San Raffaele Hospital
SRMR	Standardized root mean square residual
SRT	Selective Reminding Test
SSD	Significant Single-Domain Impairment
T25FW	Timed 25-Foot Walk test
TBSS	Tract based spatial statistic
TE	Echo time
TI	Inversion time
TLI	Tucker-Lewis index

TMT	Trail Making Test
TR	Repetition time
VBM	Voxel-based morphometry
VLMT	Verbaler Lern-und Merkfähigkeitstest
WAIS	Wechsler Adult Intelligence Scale
WASI	Wechsler Abbreviated Scale of Intelligence
WCST	Wisconsin Card Sorting Test
WIAT	Wechsler Individual Achievement Test
WISC	Wechsler Intelligence Scale for Children
WM	White matter
WRAML	Wide Range Assessment of Memory and Learning
WSR	Word Selective Reminding test

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1. Introduction

1.1 Background and general overview

Multiple sclerosis (MS) is a chronic, inflammatory disease of the central nervous system (CNS) that leads to both demyelination and neurodegeneration (Filippi *et al*, 2018). It is most commonly diagnosed in young adults between the ages of 20 and 40 years but it can also occur in children and adolescent. Pediatric-onset MS (POMS) is defined as the onset of the disease before the age of 18 years representing 2 - 10% of individuals with MS (Boiko *et al*, 2002; Charvet *et al*, 2014; Margoni *et al*, 2022a; Renoux *et al*, 2007).

The relapsing-remitting (RR) disease course is the initial presentation of the disease in 98% of pediatric patients, the primary progressive form is rare (Waldman *et al*, 2016). Possibly due to better myelin repair capabilities, POMS patients have higher disease activity that adult-onset MS (AOMS) patients but show better resilience to demyelinating attacks (Chitnis *et al*, 2020; Ghassemi *et al*, 2015). Pediatric MS patients take longer time to reach disability milestones and transition to the secondary progressive (SP) stage compared to their adult counterpart (Harding *et al*, 2013; Renoux *et al.*, 2007). However, due to the onset of MS during such a critical period of life, MS patients with an onset during childhood become disabled at a younger age than AOMS patients (Waldman *et al.*, 2016). Additionally, compared to AOMS, POMS patients exhibit a steeper cognitive decline that leads to a diagnosis of cognitive impairment at a relatively young age in almost a third of cases (Amato *et al*, 2016; McKay *et al*, 2019).

Cognitive impairment is present in 30 - 50% of POMS patients and in 40 - 60% of patients of AOMS patients (Margoni *et al.*, 2022a; Portaccio *et al*, 2021b). In both cases memory, attention, and information processing speed (IPS) are frequently involved, while language and general intelligence are more affected in POMS than in AOMS (Margoni *et al.*, 2022a; Portaccio *et al.*, 2021b).

MRI studies demonstrated that pediatreic MS is associated with a progressive loss of brain integrity due to disruptions in tissue microstructure (Longoni *et al*, 2017). It impacts brain volume and gray matter (GM) volume development relative to age expectations (Banwell *et al*, 2016; De Meo *et al*, 2019b), cognitive functioning and

mood. Moreover, the quality of life of parents and family members can also be affected (O'Mahony *et al*, 2018). The following chapters will summarize the current knowledge of the epidemiology, clinical symptoms, neuropsychological features and MRI characteristics of pediatric MS patients.

1.2 Epidemiology

Recent meta-analysis of epidemiological studies on pediatric MS, report a rate of global incidence of 0.87 (95% confidence interval [CI] = 0.35 - 1.40) per 100,000 children annually. These rates range from 0.05 (95% CI = 0.03 – 0.08) per 100,000 children annually in Tunisia, to 2.85 (95% CI = 2.83 – 2.86) per 100,000 children annually in Sardinia (Jeong *et al*, 2019; Yan *et al*, 2020). Global prevalence is reported to be 8.11 (95% CI = 2.28 - 13.93) per 100,000 children, ranging from 0.69 (95% CI = 0.58 – 0.80) per 100,000 children in Japan to 26.92 (95% CI = 26.61 – 27.23) per 100,000 children in Sardinia (Jeong *et al.*, 2019; Yan *et al.*, 2020).

In 2016, a global epidemiological study (Wallin *et al*, 2019) identified a positive correlation between MS prevalence and the distance from the Equator in both the Northern and Southern hemispheres, suggesting that prevalence and incidence of pediatric MS is influenced by geographic location and socioeconomic factors (Wallin *et al.*, 2019).

Multiple studies pointed out that the incidence of pediatric MS increases with age. The incidence in subjects aged 12 years and over ranges from 2.60 per 100,000 person-years (Denmark) to 6.41 per 100,000 person-years (Sardinia). The reported incidence in subjects aged 12 years or younger ranges from 0.10 (95% CI = 0.05 - 0.18) per 100,000 children annually in Israel to 0.42 per 100,000 children annually in Sardinia. The incidence in people aged up to 10 years is between 0.04 per 100,000 children annually (Denmark) and 0.1 per 100,000 children annually (Germany) (Jeong *et al.*, 2019).

The female:male ratio in adult MS patients is approximately 3:1, and a similar female predominance is also evident in pediatric patients up to 12 years of age. The reported gender ratios range from 1.24:1 in Germany to 3.4:1 in Slovenia (Jeong *et al.*, 2019).

1.3 Risk factors

Despite the causes of MS are still unclear, widely recognized risk factors are: genetic haplotypes, specific single nucleotide polymorphisms, smoking (or exposure to second-hand smoke), vitamin D deficiency, obesity (particularly during adolescence), and specific viral exposures (Otallah & Banwell, 2018).

1.3.1 Genetic factors

Human leukocyte antigen (HLA)-DRB1*15:01 and specific non-HLA single nucleotide polymorphisms are well-known genetic risk factors for children and adults (Disanto *et al*, 2011; Otallah & Banwell, 2018). The results of a cohort study of 188 pediatric patients with acute demyelinating syndrome (ADS) and 466 AOMS patients, showed that the combined effect of 57 single nucleotide polymorphisms had a greater impact on MS risk than HLA-DRB1*15:01 alone (Otallah & Banwell, 2018; van Pelt *et al*, 2013). A subsequent investigation found an association between 28 single nucleotide polymorphisms and POMS (Gianfrancesco *et al*, 2017b; Otallah & Banwell, 2018).

1.3.2 Smoking

A cohort study of 919 MS patients and more than 3000 healthy controls (HCs) demonstrated that exposure to second-hand smoke between the ages of 10 and 19 years is associated with a higher risk of developing MS later in life. The Authors reported an odds ratio [OR] of 1.232 in men and an OR of 1.432 in women (Oturai *et al*, 2020). The exposure to passive smoke can increase the susceptibility to MS especially in combination with the presence of HLA-DRB1*15:01 (Lavery *et al*, 2018).

1.3.3 Low Vitamin D levels

Multiple studies suggest that low levels of vitamin D are associated with higher risk of developing MS and greater disease activity (Filippi *et al.*, 2018; Olsson *et al*, 2016; Pierrot-Deseilligny & Souberbielle, 2017). Although the exact mechanisms remain unclear, some studies have pointed out that the active form of vitamin D, 1,25-dihydroxycholecalciferol may exert an effect on immune system regulation (Filippi *et al.*, 2018; Olsson *et al.*, 2016; Pierrot-Deseilligny & Souberbielle, 2017). Vitamin D

status is influenced by sunlight exposure, which is why inadequate sun exposure has been proposed as a risk factor for vitamin D deficiency. In line with this notion, multiple epidemiological studies have observed a higher prevalence of MS in northernmost countries, where the sun exposure is lower, compared to countries closer to the equator, where the sun exposure is higher (Filippi *et al.*, 2018; Olsson *et al.*, 2016; Pierrot-Deseilligny & Souberbielle, 2017).

Mowry et al. (Mowry *et al*, 2010) performed a retrospective study of 110 POMS cases, and results showed that every 10 ng/ml increase in serum 25-hydroxyvitamin D_3 levels was associated with a 34% reduction in the risk of future relapses. A subsequent meta-analysis including 569 POMS patients and more than 16,000 HCs (Gianfrancesco *et al*, 2017a) reported evidence for a causal relationship between high body mass index (BMI), lower vitamin D levels and increased susceptibility to POMS.

1.3.4 Obesity

Multiple studies suggest that childhood obesity, especially in girls, is associated with higher risk of developing MS (Gianfrancesco & Barcellos, 2016; Langer-Gould *et al*, 2013). Obesity induces a chronic, low-grade inflammation that may influence MS pathogenesis. In addition, individuals with high body fat have reduced vitamin D metabolites, and lower vitamin D levels may constitute an additional risk factor for MS. Therefore, overweight adults and children may be at particularly high risk of developing MS compared to people of normal weight. However, the mechanisms underlaying the association between obesity, vitamin D deficiency and development of MS are still unknown (Gianfrancesco & Barcellos, 2016).

1.3.5 Infections

Epstein-Barr virus (EBV) has been associated with increased risk of both POMS and AOMS (Filippi *et al.*, 2018; Otallah & Banwell, 2018), and multiple epidemiological investigations showed that up to 100% of MS patients are seropositive for EBV (Filippi *et al.*, 2018; Olsson *et al.*, 2016; Thomas *et al*, 2023). Although the exact biological mechanism that link MS to EBV are still unclear the main hypotheses include possible altered immune response to EBV and molecular mimicry (Bjornevik *et al*, 2023). No

significant evidence was found on the association between MS and other herpes viruses (Leibovitch *et al*, 2018; Otallah & Banwell, 2018).

1.3.6 Gut microbiota

A case-control study including 18 pediatric MS cases and 17 HCs, showed that, similar to other inflammatory diseases, POMS patients had 2.5 (95% CI = 1.3 – 4.9) times the abundance of Actinobacteria compared to healthy children (Tremlett *et al*, 2016b). Another investigation of 17 pediatric patients with MS demonstrated an association between depletion of Fusobacteria and increased risk of relapses (hazard ratio = 3.2, CI = 1.2 – 9.0) (Tremlett *et al*, 2016a; Tremlett & Waubant, 2018).

1.4 Pathophysiology

Compared to AOMS, POMS patients show unique characteristics despite strong evidence suggest that most of the pathophysiological mechanisms that drive MS development and progression is independent by the age of onset (Bar-Or *et al*, 2016; Margoni *et al.*, 2022a). Pediatric MS patients tend to exhibit a higher frequency of clinical relapses and MRI lesion formation than adult patients. However, they usually show better motor recovery following acute relapses, and take longer to reach significant disability milestones. On the other hand, cognitive decline is steeper in POMS than in AOMS (McKay *et al.*, 2019). There are two main hypotheses that try explain these differences: incomplete maturation of the immune and nervous systems in children, and differences in CNS resilience and repair abilities between pediatric and adult MS patients (Margoni *et al.*, 2022a).

The hallmark of MS is the presence of focal white matter (WM) lesions characterized by demyelination, inflammation, gliosis, and axonal degeneration (Bar-Or *et al.*, 2016; Margoni *et al.*, 2022a). Demyelination can also occur in the cortex, cerebellum and deep GM (Margoni *et al.*, 2022a). Although pathological investigations are scarce in pediatric MS, one study (Pfeifenbring *et al*, 2015) compared axonal pathology between 19 pediatric MS patients and 12 adult MS patients. The Authors reported a significantly higher extent of acute axonal degeneration in POMS than AOMS. In addition, age of

the patients was negatively correlated with the extent of acute axonal damage (Pfeifenbring *et al.*, 2015).

1.5 Symptoms and clinical course

The first clinical manifestations of MS in pediatric patients are predominantly optic neuritis, isolated transverse myelitis and monofocal brainstem or polyfocal symptoms (Bigi & Banwell, 2012).

Optic neuritis is the initial presentation of the disease in 14% to 35% of pediatric MS cases, and can manifest as impaired vision, diminished colour perception, pain with eye movement and central scotoma (Bigi & Banwell, 2012; Venkateswaran & Banwell, 2010). Mild or unilateral visual loss may be underreported in young children since they can be able to compensate well for this deficit or may not notice the change (Venkateswaran & Banwell, 2010). In a study of 36 pediatric patients suffering from an acute episode of optic neuritis (unilateral in 58% of the cases and bilateral in 42%), 83% of subjects showed an excellent recovery returning to a visual acuity of 20/40 or better. Compared to unilateral optic neuritis, bilateral optic neuritis resulted associated with a higher risk of developing MS (Wilejto *et al*, 2006).

Isolated transverse myelitis is typically a monophasic event associated with negative brain imaging findings. It can result in motor, sensory and autonomic dysfunction, representing the first clinical manifestation of MS in 10% of the pediatric cases (Bigi & Banwell, 2012).

Huppke and colleagues (Huppke *et al*, 2013) conducted a retrospective analysis of 88 POMS cases to identify the most frequent manifestations of MS in pre-pubertal (onset of MS before 11 years of age) and post-pubertal patients (MS onset between ages 14 and 16 years). Pre-pubertal children had more frequently a polysymptomatic onset (49%) compared to post-pubertal patients (37%), characterized by motor symptoms (45%) and brainstem symptoms (42%), including diplopia and facial weakness. In addition, seizures (6%) and sphincter dysfunction (6%) were observed exclusively in younger children. Post-pubertal children presented more commonly with sensory symptoms (46%) and optic neuritis (32%). More than 25% of pre-pubertal children experienced cognitive problems over the first two years of disease, including attention

deficits (66%), reduced academic performance (42%) and psychiatric symptoms (42%) (Huppke *et al.*, 2013).

POMS typically follows a relapsing-remitting clinical course with a higher relapse rate than AOMS (1.13 vs 0.40) in the initial phase of the disease (Gorman *et al*, 2009). Between 25 and 40% of children experience a second attack within a year of MS onset, between 50% and 60% within two years, and 66% within three years (Ruggieri *et al*, 2004). However, due to the high repair capabilities of the CNS in children, complete symptoms remission is usually observed early in the disease (Renoux *et al.*, 2007). RRMS is the predominant phenotype of MS in pediatric patients (95 - 98%), occurring at higher rates than in adults (85 - 90%). Primary progressive MS is reported in less than 3% of pediatric patients, while in adult MS patients the proportion increases at 10 – 15% (Brola & Steinborn, 2020).

1.6 Diagnosis

The MS diagnostic criteria in adults have been refined in 2017, and such a revision is applied also to patients aged 11 and over (Brola & Steinborn, 2020; Margoni *et al.*, 2022a; Thompson *et al*, 2018b). The diagnosis of MS relies on the demonstration of disease dissemination in space (i.e., detection of lesions in multiple regions of the CNS) and time (i.e., detection of lesions at different points in time). In addition, the presence of clinical symptoms associated with MS attack, along with lack of evidence of MS-like disease, are required for a definite diagnosis of MS (Thompson *et al.*, 2018b).

In a prospective cohort study of 324 patients with incidental acquired demyelinating syndrome, the 2017 McDonald criteria for the diagnosis of MS were able to identify pediatric MS patients with a sensitivity of 0.71 (95% CI = 0.56 – 0.83) and a specificity of 0.96 (95% CI = 0.90 – 0.98). The performance of the 2017 McDonald criteria was higher than the 2010 McDonald criteria and the 2016 MAGNIMS criteria, showing a significant increase in sensitivity (Fadda *et al*, 2018).

1.7 Cognitive deficits and neuropsychological assessment

The onset of MS during a critical period of life for maturation of WM pathways, development of cognitive networks and acquisition of cognitive abilities, may expose

pediatric patients to cognitive dysfunction and negatively affect academic attainment (Amato *et al*, 2008). Pediatric MS patients often require special education services (37%) including reading (8%) and math assistance (8%), with 16% needing personalized education plans at school (Aaen *et al*, 2019).

POMS detrimentally affects IPS, sustained and focused attention, visuomotor and visuospatial abilities, memory, language abilities, executive function and general intelligence (Lysenko *et al*, 2022; Portaccio *et al.*, 2021b). Cognitive impairment is a broad term that refers to any difficulty an individual may experience with cognitive functions including reasoning, thinking, attention, learning and memory (Roy, 2013). In pediatric MS, cognitive impairment has been often defined operationally as failure of at least three tests of a comprehensive neuropsychological battery (Amato *et al*, 2014; Amato *et al.*, 2008; Goretti *et al*, 2011; Öztürk *et al*, 2020; Storm van's Gravesande *et al*, 2019b; Till *et al*, 2011b). The threshold for test failure was typically set at 1.5 standard deviations (SD) below the normative value (Storm van's Gravesande *et al.*, 2019b; Till *et al.*, 2011b) or a score under the 5th percentile of the reference population (Amato *et al.*, 2014; Amato *et al.*, 2008; Goretti *et al.*, 2011; Öztürk *et al.*, 2020). Other studies have considered POMS patients cognitively impaired based on an abnormal performance on at least one (Krupp *et al*, 2022), two (MacAllister *et al*, 2005; Rocca *et al*, 2014c), or four tests (Barlow-Krelina *et al*, 2021). Another approach has defined cognitive impairment as failure on one third (Charvet *et al*, 2016; Charvet *et al.*, 2014; Julian *et al*, 2012), or 20% (Weisbrot *et al*, 2013) of the administered neuropsychological tests. Despite the use of different neuropsychological batteries and impairment criteria, multiple studies suggest that cognitive impairment is common in pediatric MS, with prevalence rates ranging from 29 to 36% (Amato *et al.*, 2008; Julian *et al.*, 2012; MacAllister *et al.*, 2005; Rocca *et al.*, 2014c; Till *et al.*, 2011b).

The exact trajectory of cognitive evolution in POMS has not yet been determined due to lack of long-term studies. Over short periods of time after MS onset, pediatric patients rarely exhibit cognitive changes and may even show improvements in cognitive functioning. However, cognitive decline becomes more evident after approximately five years, with rates of cognitive deterioration reaching up to 56% (Portaccio *et al.*, 2021b).

1.7.1 Learning and memory

Learning is defined as the process of acquiring new information, while memory refers to the faculty of storing and retrieving what has been learned (Brem *et al*, 2013; Johansson & Wahlin, 1998). Learning and memory problems have been consistently reported in POMS patients (Portaccio *et al.*, 2021b).

In a previous cross-sectional study (Wuerfel *et al*, 2018), significant lower scores at tests measuring short-term and long-term verbal memory (Verbaler Lern-und Merkfähigkeitstest [VLMT]) (Helmstaedter et al, 2001) and short-term visuospatial memory (Battery for Assessment in Children-Memory and Retentiveness Test) (Lepach & Petermann, 2008) were detected in 40 pediatric MS patients compared to 37 age- and sex-matched HCs (all $p \leq 0.011$) (Wuerfel *et al.*, 2018). Difficulties in short-term and long-term visuospatial memory were also found by an investigation comparing the performance at the Brief Visuospatial Memory Test-Revised (BVMTR) between 43 POMS patients and 45 HCs (all $p < 0.001$) (Smerbeck *et al*, 2011a).

A recent investigation (Storm van's Gravesande *et al.*, 2019b) found no significant differences between 106 POMS patients and 210 HCs in tests measuring short-term and long-term verbal memory (VLMT) (Helmstaedter *et al.*, 2001) and visuospatial long-term memory (Rey-Osterrieth Complex Figure test - delayed recall) (Strauss *et al*, 2006). However POMS patients exhibited poorer performance than controls on one test (Digit span forward) measuring short-term memory span ($p < 0.001$) (Härting *et al*, 2000).

One of the largest investigations of cognitive function in POMS included 187 pediatric RRMS patients and 44 pediatric patients with clinically isolated syndrome (CIS) (Julian *et al.*, 2012). Verbal memory assessment was conducted using the California Verbal Learning Test (CVLT) version II (Delis *et al*, 2000) or Child version (Delis *et al*, 1994), according to the age of the participant. Mild impairment was defined as performing at least 1 SD below the normative mean, while the threshold for moderate impairment was 2 SDs. The results showed that the overall prevalence of verbal memory impairment ranged from 13 to 29% (mild impairment: 9 – 25%; moderate impairment 4 – 9%), depending on the CVLT subscore.

In a multicenter study including 61 pediatric MS patients (Amato *et al.*, 2008), verbal learning and memory were assessed using the Selective Reminding Test (SRT) from the Brief Neuropsychological Battery for Children (Portaccio *et al*, 2009b), while

visuospatial learning and memory were measured with the 10/36 Spatial Recall Test (SPART) from the same battery (Portaccio *et al.*, 2009b). Test failure was defined as performing below the 5th percentile of HCs performance. Of the 32 cognitively impaired patients, 53% failed the SRT-long term storage (lts), 42% the SRT-consistent long-term retrieval (cltr), 39% the SRT-delayed recall, 56% the SPART and 18% the SPART-delayed recall. The same group conducted a longitudinal investigation on 48 pediatric MS patients (Pastò *et al*, 2016). Although no significant differences between pediatric patients and HCs were found in tests of verbal and visuospatial memory at baseline, after a mean follow-up of 4.7 years the patients group performed worse than HCs on the SRT-lts ($p = 0.011$), SRT-cltr ($p = 0.001$), SRT-delayed recall ($p = 0.004$), SPART ($p = 0.001$) and SPART-delayed recall ($p = 0.008$).

In a longitudinal study, MacAllister et al. (MacAllister *et al*, 2007) used the Wide Range Assessment of Memory and Learning (WRAML) (Sheslow & Adams, 1990) to evaluate verbal and visuospatial memory in 12 POMS patients. For each test, the Authors defined impairment as a performance that fell 1.5 SDs below the population norms. At baseline, no patient failed the WRAML – short-term verbal memory subtest, 5/12 (42%) patients failed the WRAML – long-term verbal memory subtest, 4/12 (33%) failed the WRAML – short-term visuospatial memory subtest, and 2/12 (17%) failed the WRAML – long-term visuospatial memory subtest. After a mean follow-up of 1.8 years, no patient failed the WRAML – short-term verbal memory subtest, 4/12 (33%) patients failed the WRAML – long-term verbal memory subtest, 1/12 (8%) failed the WRAML – short-term visuospatial memory subtest, and 3/12 (25%) failed the WRAML – long-term visuospatial memory subtest.

Till and colleagues (Till *et al.*, 2011b) assessed verbal memory of 35 POMS patients using the Word Selective Reminding test (WSR) from the Test of Memory and Learning 2nd edition (Reynolds & Voress, 2007). The threshold for test failure was set at 1.5 SDs below the normative value. A total of 4/35 (11%) POMS patients failed the WSR – immediate recall, but no participant failed the WSR – delayed recall.

1.7.2 Complex attention

Complex attention is the ability of process information by directing, relocating and dividing attentional focus (Daffner *et al*, 2015). It includes sustained attention, divided

attention, selective attention and IPS (Sachdev *et al*, 2014). Complex attention, and IPS in particular, is the most frequently involved cognitive function in adult MS patients and have therefore also been extensively studied in POMS (Benedict *et al*, 2020).

In a multicentre international longitudinal study, Akbar and colleagues (Akbar *et al*, 2017) analyzed the performance of 82 pediatric MS individuals on the Symbol Digit Modalities Test (SDMT) (Portaccio *et al.*, 2009b; Smith, 1982) to track IPS decline. The results showed that increasing age until to the end of adolescence was associated with improved performance on the SDMT. Once early adulthood was reached, SDMT scores tended to remain stable and then gradually decline (Akbar *et al.*, 2017). In line with these findings, Pastò et al. (Pastò *et al.*, 2016) found no significant differences in SDMT (Portaccio *et al.*, 2009b) and Trail Making Test (TMT) – A (a measure of focused attention, visual scanning and psychomotor speed) (Portaccio *et al.*, 2009b) scores between POMS and HCs at baseline. However, compared to HCs, POMS patients had lower scores on TMT-B (a measure of divided attention) ($p < 0.001$). At follow-up, the POMS patients group showed significantly worse attentional performance than HCs (SDMT: $p = 0.007$; TMT-A: $p = 0.015$; TMT-B: $p = 0.037$).

No significant differences between POMS patients and HCs on SDMT scores were detected in four other studies (Barlow-Krelina *et al.*, 2021; Bethune *et al*, 2011; Charvet *et al*, 2017; Storm van's Gravesande *et al.*, 2019b). One investigation (Storm van's Gravesande *et al.*, 2019b) also evaluated the performance on TMT (Strauss *et al.*, 2006) and found lower scores in pediatric MS patients vs controls on TMT-A ($p < 0.001$), but not on TMT-B.

On the other hand, Smerbeck and colleagues (Smerbeck *et al.*, 2011a) did found lower SDMT (Rao, 1991) scores in pediatric MS patients vs HCs ($p = 0.035$). Wuerfel et al. (Wuerfel *et al.*, 2018), used the d2-R test (a paper-and-pencil test in which the examinee is asked to identify a target stimulus among distractors within a limited time to assess attention and concentration) (Brickenkamp, 2002), the SDMT (Smith, 1982), the Coding subtest from the Wechsler Adult Intelligence Scale-IV (WAIS-IV)/ Wechsler Intelligence Scale for Children-IV (WISC-IV) (Petermann, 2008; Petermann & Petermann, 2007), the Symbol Search subtest from the WAIS-IV/WISC-IV and the Cancellation subtest from the WAIS-IV/WISC-IV, to assess sustained attention and IPS. The results showed poorer performance of pediatric MS patients compared to

control group on the SDMT-oral version ($p = 0.015$), SDMT-written version ($p = 0.001$), Coding subtest ($p = 0.005$), d2-R processed objects ($p = 0.010$) and d2-R concentration ($p = 0.018$); while no differences were found between groups in Symbol Search and Cancellation Test scores (all $p > 0.26$).

A nationwide multicentre study (Wallach *et al*, 2019) investigated the presence of IPS deficit in 500 POMS patients and 116 CIS patients with disease onset before 18 years of age, using the SDMT (Smerbeck *et al*, 2011b). Age- and sex- adjusted SDMT scores (Smerbeck *et al.*, 2011b) were converted to z-scores. Test failure was defined as a z-score $\leq$ -1.0. Impaired IPS was found in 23% of POMS patients and 16% of CIS patients. After a mean follow-up of 1.8 years, both CIS and POMS patients exhibited decreased performance on the SDMT in 14% of cases.

MacAllister et al. (MacAllister *et al.*, 2007) used the TMT (1994) to evaluate focused attention, visual scanning and divided attention. At baseline, 4/12 (33%) patients failed the TMT-A and 5/12 (42%) failed the TMT-B. After a mean follow-up of 1.8 years, 4/12 (33%) patients failed the TMT-A and 9/12 (75%) failed the TMT-B.

In a previous cross-sectional study (Julian *et al.*, 2012) IPS assessment was performed using the Coding subtest from the WAIS-IV/WISC-IV, and the results showed that the prevalence of mild impairment was 24%, while moderate impairment occurred in 11% of patients.

Till and colleagues (Till *et al.*, 2011b) used the TMT-A (Reitan, 1959) to assess focused attention, the SDMT to evaluate IPS (Smith, 1982) and the Continuous Performance Test (CPT), to assess sustained attention (Conners *et al*, 2003). A total of 13/34 (38%), POMS patients failed the TMT-A, 4/33 (12%) failed the SDMT and 2/33 (6%) failed the CPT.

A recent cross-sectional study (Cacciaguerra *et al*, 2024) of 76 pediatric MS patients assessed IPS with the Coding subtest form the WAIS-IV/WISC-IV and SDMT (Orsini & Pezzuti, 2013; Orsini *et al*, 2012; Portaccio *et al*, 2009a), and attentional performance using the TMT (Portaccio *et al.*, 2009a). For each test, the Authors defined impairment as a score falling below the 5th percentile of the normative reference. Results showed that 21% of patients failed the Coding subtest, 9% failed the SDMT, 10% failed the TMT-A and 16% failed the TMT-B.

In a multicenter study (Amato *et al.*, 2008), IPS was assessed using the SDMT from the Brief Neuropsychological Battery for Children (Portaccio *et al.*, 2009b). Of the 18 cognitively impaired patients who were administered the SDMT, 5 (28%) failed the test.

1.7.3 Language

Language is a system of conventional signs (spoken, manual or written) by means of which human beings code and decode information. It presents different aspects: receptive language, expression and fluency, each of which can be affected by brain pathology (Benson, 1996; Roby-Brami *et al*, 2012; Sachdev *et al.*, 2014). Language abilities are rarely affected in AOMS, but language involvement is frequently reported in pediatric patients, possibly because the onset of the disease during a critical age for acquisition of complex linguistic abilities may affect their development (Suppiej & Cainelli, 2014).

In a previous cross-sectional study (Wuerfel *et al.*, 2018), significant lower scores at tests measuring word knowledge (Vocabulary subtest from the WAIS-IV/WISC-IV) (Petermann, 2008; Petermann & Petermann, 2007), verbal concept formation and verbal reasoning (Comprehension subtest from the WAIS-IV/WISC-IV) (Petermann, 2008; Petermann & Petermann, 2007) were detected in pediatric MS patients compared to HCs (all $p \leq 0.003$). However, no significant differences were found in tests measuring verbal fluency (Regensburger Word Fluency Test) (Aschenbrenner *et al*, 2000) and word finding (K-NEK: Word search) (Melchers & Schürmann, 2004).

Storm van's Gravesande and colleagues (Storm van's Gravesande *et al.*, 2019b) found lower phonemic ($p < 0.001$), but not semantic fluency scores (Regensburger Word Fluency Test) (Aschenbrenner *et al.*, 2000) in pediatric MS patients compared to HCs.

In a longitudinal investigation (Pastò *et al.*, 2016), pediatric MS patients and HCs were administered with an extensive neuropsychological battery (Portaccio *et al.*, 2009b) to assess receptive and expressive language abilities. At baseline, no significant differences with HCs were found in any measure, while after a median follow-up of 4.7 years, the patients group performed worse than HCs on the Phrase Comprehension Test ($p = 0.05$), the Phonemic Verbal Fluency Test ($p < 0.001$) and Semantic Verbal Fluency Test ($p < 0.001$). No significant differences were detected between pediatric patients and HCs in Indication of Pictures Test, Token Test and Oral Denomination Test.

In a previous longitudinal study, MacAllister et al. (MacAllister *et al.*, 2007) used the Controlled Oral Word Association Test (COWAT) (Spreen & Benton, 1977) to assess phonemic fluency, the Boston Naming Test (Kaplan *et al*, 1983) to evaluate naming ability and the Listening to Paragraphs subtest of the Clinical Evaluation of Language Fundamentals 3rd edition (CELF-3) to examine receptive language (Semel *et al*, 1995), in POMS patients. At baseline, 1/12 (8%) patient failed the COWAT, 3/12 (25%) failed the Boston Naming Test and 2/12 (17%) failed the CELF-3 Paragraphs subtest. After a mean follow-up of 1.8 years, 3/12 (25%) patients failed the COWAT, 4/12 (33%) failed the Boston Naming Test and 2/12 (17%) failed the CELF-3 Paragraphs subtest (MacAllister *et al.*, 2007).

In a large cross-sectional study (Julian *et al.*, 2012) language assessment was conducted using the Vocabulary subtest from the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999) to evaluate word knowledge, the Pseudoword Decoding test from the Wechsler Individual Achievement Test - II (WIAT-II) (Wechsler, 2005) to explore phonetic decoding skills, and the Expressive One Word Picture Vocabulary Test (EOWPVT) to evaluate expressive vocabulary (Gardner, 1979). The prevalence of reduced word knowledge was 13% (mild impairment 10%; moderate impairment 3%), impaired phonetic decoding skills was 11% (mild impairment 10%; moderate impairment 1%) and impaired expressive abilities was 9% (mild impairment 9%) (Julian *et al.*, 2012).

A recent cross-sectional study (Cacciaguerra *et al.*, 2024) evaluated pediatric MS patients with the Phonemic and Semantic Verbal Fluency Tests (Portaccio *et al.*, 2009b). The results showed that 7% of the patients failed the phonemic fluency test and approximately 1% failed the semantic fluency test (Cacciaguerra *et al.*, 2024). Higher rates of impairment were found by Öztürk and colleagues (Öztürk *et al.*, 2020), who administered the KAS-Animal Test (a measure of phonemic and semantic verbal fluency) (Öktem, 1994) to 46 pediatric MS patients. Test failure was defined as a score below the 5th percentile for age. The results showed that 9/46 (20%) pediatric patients exhibited verbal fluency deficit. This finding was confirmed at the two-year follow-up examination.

Till and colleagues (Till *et al.*, 2011b) used the Verbal Fluency subtest from the Delis–Kaplan Executive Function System (DKEFS) (Delis *et al*, 2001) to assess

phonemic fluency and Vocabulary subtest from the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1999) to evaluate word knowledge. A total of 2/31 (6%), POMS patients failed the phonemic fluency test and 3/31 (10%) failed the Vocabulary subtest.

In a multicenter study (Amato *et al.*, 2008) receptive and expressive language abilities were extensively assessed (Portaccio *et al.*, 2009b). Among cognitively impaired patients 22% failed the Semantic Verbal Fluency Test (expressive language), 17% the Phonemic Verbal Fluency Test (expressive language), 11% the Indication of Pictures Test (receptive language), 28% the Phrase Comprehension Test (receptive language), 39% the Token Test (receptive language), while none failed the Oral Denomination Test (expressive language).

1.7.4 Executive function

Executive function is an umbrella term that refers to a group of mental abilities (planning, decision-making, working memory, responding to feedback, inhibition and cognitive flexibility) that a person requires to predict, plan, initiate, monitor, adjust, and conclude goal-directed behaviour (Mezzacappa, 2011; Sachdev *et al.*, 2014). Executive function emerges during first few years of life (Best & Miller, 2010), following a non-linear trajectory rapidly develops in late childhood/adolescence (from 10 to 15 years of age) and stabilizes in late-adolescence/early adulthood (from 18 to 20 years of age) (Tervo-Clemmens *et al*, 2023). Executive deficits are frequently noted in AOMS (Marchesi *et al*, 2022) and growing evidence suggest that similar impairments may also be present in pediatric patients (Amato *et al.*, 2008; Barlow-Krelina *et al.*, 2021; Julian *et al.*, 2012; Öztürk *et al.*, 2020; Storm van's Gravesande *et al.*, 2019b; Till *et al.*, 2011b; Wuerfel *et al.*, 2018).

Wuerfel and colleagues (Wuerfel *et al.*, 2018) detected significantly lower scores in pediatric MS patients vs HCs on the Arithmetic subtest from the WAIS-IV/WISC-IV (Petermann, 2008; Petermann & Petermann, 2007) (measuring calculation abilities and working memory; $p = 0.002$), the Corsi Block Tapping Task (Farrell Pagulayan *et al*, 2007) (measuring working memory; $p = 0.003$), the Letter-Number-Sequencing subtest from the WAIS-IV/WISC-IV (Petermann, 2008; Petermann & Petermann, 2007) (measuring working memory; $p = 0.030$) and the D-KEFS Design Fluency test (Delis *et*

al., 2001) (measuring problem-solving; p = 0.003); but not on the D-KEFS-Colour Word Interference test (Delis *et al.,* 2001) (measuring inhibition and cognitive flexibility; p = 0.36) and the Digit Span subtest from the WAIS-IV/WISC-IV (Petermann, 2008; Petermann & Petermann, 2007) (measuring short-term memory and working memory; p = 0.14). Similarly, a recent investigation (Storm van's Gravesande *et al.*, 2019b) found no significant differences between POMS patients and HCs in tests measuring working memory (Digit span backward) (Härting *et al.*, 2000) and inhibition (MUSIC Stroop Interference Test) (Storm van's Gravesande *et al.*, 2019b).

A cross-sectional study (Barlow-Krelina *et al.*, 2021) explored executive function in POMS patients using the Penn Computerized Neurocognitive Battery (PCNB) (Gur *et al*, 2012). For each test, impairment was defined as a z-score ≤ -1.5. A total of 22/62 (35%) patients showed working memory impairment on the Letter N-Back test, and 17/65 (26%) exhibited an inhibitory control deficit on the Go-No-Go Test. However, POMS patients and HCs had a similar performance on the Penn Conditional Exclusion Test, which assesses cognitive flexibility (p = 0.70).

Julian and colleagues (Julian *et al.*, 2012) assessed cognitive flexibility and working memory using the Contingency Naming Test (CNT) (Anderson *et al*, 2000), while short-term memory/working memory were evaluated with the Digit Span subtest from the WAIS-IV/WISC-IV. The results showed that the prevalence of impairment on the CNT – Time was 23% (10% mild; 13% moderate), on the CNT – Efficiency was 31% (27% mild; 4% moderate) and on the Digit Span subtest was 27% (22% mild; 5% moderate).

In a longitudinal study (Öztürk *et al.*, 2020), cognitive inhibition was measured with the Stroop Test (Bekci & Karakas, 2009). The results showed that 15/46 (33%) pediatric patients exhibited cognitive inhibition deficit. At the two-year follow-up examination, a similar prevalence of the deficit was observed with 14/46 (30%) pediatric patients failing the Stroop Test.

Till and colleagues (Till *et al.*, 2011b) used the Wisconsin Card Sorting Test (WCST) (Heaton *et al*, 1993a) to assess cognitive flexibility. A total of 3/32 (9%) failed the test. Similarly, in a multicenter study (Amato *et al.*, 2008) cognitive flexibility was evaluated with the Modified Card Sorting Test (MCST) (Portaccio *et al.*, 2009b).

Among cognitively impaired patients, 41% failed the MCST - category, 29% the MCST – perseverative errors and 12% the MCST – non perseverative errors.

1.7.5 General intelligence

Intelligence can be defined as the capacity to act intentionally, think rationally and adapt to the environment (Hindes *et al*, 2011; Wechsler, 1958). The intelligence quotient (IQ) is a numerical representation of a standardized intelligence test result and is generally used to compare individual intelligence to that of other people with similar demographic features. Therefore, the IQ can only be interpreted as "low" or "high" in comparison to the average value of people belonging to the same age group (Norstokke *et al*, 2011).

The IQ scores of pediatric MS patients generally fall within the normal range, however multiple studies reported poorer performance on intelligence tests than HCs (Carroll *et al*, 2019; Green *et al*, 2018; Lysenko *et al.*, 2022; Pastò *et al.*, 2016; Till *et al.*, 2011b; Wuerfel *et al.*, 2018).

In a group of 32 POMS patients and 30 HCs who underwent intelligence assessment with the WASI (Wechsler, 1999), Green et al. (Green *et al.*, 2018) detected an average IQ ten points lower than in controls ($p < 0.001$).

Till and colleagues (Till *et al.*, 2011b) administered the WASI (Wechsler, 1999) to 35 POMS patients and 33 age- and sex-matched HCs, and found lower Full Scale IQ scores ($p = 0.001$) and Verbal IQ scores ($p < 0.001$) in the patients group compared to controls, while no significant differences were found in Performance IQ scores ($p = 0.08$). Moreover, lower Full Scale IQ scores in POMS patients resulted correlated with poorer performance on a wide range of cognitive measures.

In a cross-sectional study including 30 pediatric MS patients and 30 HCs (Carroll et al., 2019), IQ was measured with the WAIS-IV/WISC-IV (Wechsler, 2003, 2014). Full Scale IQ ($p < 0.001$), Verbal Comprehension Index ($p = 0.003$), Perceptual Reasoning Index ($p = 0.004$) and Processing Speed Index ($p = 0.003$) were significantly reduced in pediatric MS patients vs HCs. No differences were reported in Working Memory Index scores ($p = 0.053$). Similarly Wuerfel et al. (Wuerfel *et al.*, 2018) found lower Full Scale IQ (Petermann, 2008; Petermann & Petermann, 2007) ($p = 0.003$), Verbal

Comprehension Index (p = 0.001), and Processing Speed Index (p = 0.017) in 37 pediatric MS patients compared to 37 HCs. The Authors found no significant differences in Perceptual Reasoning Index (p = 0.097) and Working Memory Index (p = 0.187).

In another cross-sectional study (Smerbeck *et al.*, 2011a) of 43 pediatric MS patients and 45 HCs, MS patients and controls had comparable WASI outcomes (Wechsler, 1999).

Low IQ is usually defined as a score < 70 on the WAIS-IV/WISC-IV with an estimated prevalence of 2.2% in the general population (Orsini & Pezzuti, 2013; Orsini *et al.*, 2012). To date, only a few studies explored the prevalence of pediatric MS patients who meet this criterion. In a recent study, Cacciaguerra and colleagues found that 4% of the pediatric MS patients who underwent intelligence assessment (Orsini & Pezzuti, 2013; Orsini *et al.*, 2012) obtained a WAIS-IV/WISC-IV score below 70. In a multicenter study including 61 pediatric MS patients (Amato *et al.*, 2008) IQ was evaluated with the Wechsler Intelligence Scale for Children–Revised (WISC-R) (Wechsler, 1974), and results showed that 5/61 (8%) patients had an IQ score < 70.

In healthy subjects, psychometric intelligence is the sum of multiple higher-order mental abilities that are strongly associated with processing speed and working memory capacity (Fry & Hale, 2000). Whether IQ reduction in POMS is due to IPS and working memory deficits is still debated. A recent study (Kapanci *et al*, 2019) which requires further confirmation due to the small sample size (21 pediatric MS patients) suggested that the low IQ scores observed in pediatric MS patients are mainly related to slower reaction times and more error-prone information processing due to impaired WM integrity.

High IQ is associated with better outcomes in numerous neurological (Makin *et al*, 2018; Mathias & Wheaton, 2015) and psychiatric (Keyes *et al*, 2017) conditions. Its protective effect against cognitive impairment is attributed to an increased distributed processing capacity despite the presence of structural or functional brain alterations (Santarnecchi *et al*, 2015). In light of this, IQ is frequently considered a proxy of cognitive reserve (Nogueira *et al*, 2022). In an Italian longitudinal study (Pastò *et al.*, 2016) of 48 POMS cases, higher IQ levels in cognitively preserved patients predicted

stable or even improved cognitive performance after a mean follow-up of 4.7 years (OR = 1.11; 95% CI = 1.03 - 1.20; p = 0.006).

1.8 Fatigue and psychiatric symptoms

Fatigue can be described as a subjective and pervasive lack of physical and/or mental energy that limits participation in everyday life activities (Freal *et al*, 1984). Up to 76% of POMS patients experience fatigue, which can be assessed with self-report or parent-report questionnaires (Carroll *et al*, 2016). One study (Amato *et al.*, 2014) employed the Fatigue Severity Scale (Krupp *et al*, 1989), a commonly used self-report measure in adult subjects, and showed a 20% prevalence of fatigue in a sample of 48 POMS patients. Higher rates of fatigue were found in two studies (MacAllister *et al.*, 2005; Till *et al*, 2012b) that asked a yes-or-no question about whether the subject experienced fatigue, with chronic fatigue reported in 44 – 49% of the cases. The most widely used questionnaire is the Pediatric Quality of Life Inventory Multidimensional Fatigue Scale (PedsQL-MFS) (Varni *et al*, 2004), which evaluates general fatigue, cognitive fatigue, and sleep quality, and can be administered to both pediatric patients and parents. Despite one study (Zafar *et al*, 2011) reported no differences when comparing the PedsQL-MFS self-reported measure of general fatigue between 30 pediatric patients and 52 HCs, multiple evidence (Goretti *et al.*, 2011; MacAllister *et al*, 2009; Parrish *et al*, 2012) suggest that POMS patients are more fatigued than healthy peers with rates of chronic fatigue ranging from 14 to 56%. The lack of energy can also be observed by the parents who generally notice higher levels of fatigue and a greater need for rest in their offspring compared to the parents of healthy children (Carroll *et al.*, 2016; Goretti *et al.*, 2011; Parrish *et al.*, 2012). Relationship between fatigue and cognitive functioning was explored in two studies of POMS, with results reporting a negative correlation with problem-solving skills, verbal learning, sustained attention, divided attention and verbal comprehension (Carroll *et al.*, 2016; Goretti *et al.*, 2011; Holland *et al*, 2012). Fatigue and depressive symptoms often overlap and co-occur in patients with POMS, negatively impacting quality of life (Goretti *et al.*, 2011; Storm van's Gravesande *et al*, 2019a). In a cohort study of 106 POMS patients, fatigue assessed with PedsQL-MFS resulted associated with female gender and the presence of depressive symptoms evaluated with

Beck Depression Inventory II (Hautzinger *et al*, 2006) or Depressionstest für Kinder (Rossmann, 2005). In addition, fatigue and depressive symptoms together were able to explain 68% of the variance of Health-Related Quality of Life scores in the patients group.

Psychiatric comorbidities can also be present in patients with POMS. Multiple investigations (Amato *et al.*, 2014; Destan Bunul *et al*, 2024; Goretti *et al.*, 2011) reported higher scores in POMS patients vs HC on the Children Depression Inventory (CDI) (Kovacs, 1985), with approximately 8 – 30% of the subjects exhibiting significative depressive symptomatology. The Behaviour Assessment System for Children Second Edition (BASC-II) (Reynolds & Kamphaus, 2004) was administered to POMS patients and/or their parents in four studies (Charvet *et al.*, 2016; Holland *et al.*, 2012; Parrish *et al.*, 2012; Till *et al*, 2013) that detected elevations on scales assessing depression, anxiety, hyperactivity, attention problems, withdrawal, atypical behaviours, and somatization. A national study of 201 pediatric MS patients in the United Kingdom showed an increased rate of psychotic disorders (International Statistical Classification of Diseases and Related Health Problems 10th Revision [ICD-10] code: F20 - F29), mood disorders (ICD-10 code: F30 - F39), and intellectual disability (ICD-10 code: F70 – F79) compared to the reference cohort. Two studies (Goretti *et al.*, 2011; Weisbrot *et al.*, 2013) used the Kiddie Schedule for Affective Disorders and Schizophrenia for School-Age Children - Present and Lifetime Version (K-SADS-PL) (Kaufman *et al*, 1997), a semi-structured psychiatric diagnostic interview, to assess a wide range of psychiatric manifestations in POMS. The most common diagnoses, according to the Diagnostic and Statistical Manual of Mental Disorders – IV (DSM-IV), were anxiety disorders (33%), attention deficit hyperactivity disorder (26%), and mood disorders (15 – 24%). Similarly to fatigue, psychiatric symptoms are often associated with lower cognitive performance in pediatric MS patients. Consequently, the co-occurrence of cognitive impairment, fatigue and psychiatric comorbidities in POMS can make these manifestations difficult to disentangle.

1.9 Neuroimaging in pediatric MS

The use of MRI in pediatric MS is essential for diagnosis, monitoring disease progression, and evaluating treatment response (Banwell *et al.*, 2016). However, the clinical manifestations of MS show only a weak association with conventional MRI measures obtained from dual-echo, T1-weighted, and post-contrast T1-weighted images (Filippi & Rocca, 2007; Fox *et al*, 2011). Advanced MRI techniques, such as diffusion tensor imaging (DTI) and functional MRI (fMRI), have provided clearer insight into the substrates of cognitive impairment and physical disability in this population (Barlow-Krelina *et al*, 2019; Cirillo *et al*, 2016; De Meo *et al*, 2017; Rocca *et al*, 2014a; Rocca *et al*, 2010a; Till *et al*, 2011a; Yap *et al*, 2016).

1.9.1 White matter damage

On MRI, POMS patients are characterized by the presence of multiple WM lesions. Like adults, these lesions in children and adolescents are typically asymmetric and located in the periventricular and juxtacortical WM (Banwell *et al.*, 2016; Filippi *et al*, 2019). A more frequent involvement of infratentorial areas has been described in pediatric MS patients compared to the adult counterpart (Ghassemi *et al.*, 2015; Margoni *et al.*, 2022a; Waubant *et al*, 2009). This finding may be related to developmental differences, because myelination follows a caudo-rostral gradient and MS lesions usually affect highly myelinated WM regions (Margoni *et al.*, 2022a). Pediatric MS patients typically accrue higher volumes of brain T2-hyperintense and T1-hypointense WM lesions than adults (Ghassemi *et al*, 2014b; Yeh *et al*, 2009) with a faster accumulation of new T2 lesions after the first demyelinating attack (Verhey *et al*, 2013). On the other hand, younger children show better repair capabilities than adults since WM lesions tend to decrease in volume over time at a higher rate (Ghassemi *et al*, 2014a). T2-hyperintese lesions often show ill-defined borders, are usually large, confluent and tend to diminish in volume or disappear over time (Chabas *et al*, 2008; Margoni *et al.*, 2022a). Multiple studies demonstrated that pediatric patients have high remyelination abilities, which diminish over the years. This reduction in remyelination capacity observed in late adolescence may be related to the completion of primary

myelination or other developmental mechanisms (Brown *et al*, 2014; Ghassemi *et al.*, 2014b).

The integrity of normal appearing (NA) WM can be affected by inflammation, demyelination, gliosis, axonal degeneration as well as impaired maturation of WM tracts (Longoni *et al.*, 2017; Margoni *et al.*, 2022a; Margoni *et al*, 2022b; Rocca *et al*, 2015f; Vishwas *et al*, 2013; Yap *et al.*, 2016). Conventional MRI techniques are not able to detect these microstructural alterations, possibly explaining the weak correlation between focal WM lesions and neurologic deficits in MS (Filippi, 2015). DTI is an advanced MRI technique that provides in vivo measurement of water diffusion to assess microstructural abnormalities. Tissue integrity, myelin content and axonal damage can be indexed with fractional anisotropy (FA) and mean diffusivity (MD). FA is a measure of directional selectivity of water molecules diffusion, ranging from 0 (isotropic diffusion) to 1 (highly anisotropic diffusion). Tissues such as myelinated axons hinder water diffusion due to their structure and myelin sheath and are characterized by high FA values. Conversely, isotropic motion of water molecules, as observed in cerebrospinal fluid (CSF), corresponds to low FA values (Preziosa *et al*, 2011; Valsasina *et al*, 2005). MD represents the average diffusion of the water molecules across the three orthogonal directions, and therefore higher MD values are associated with lower WM integrity (Preziosa *et al.*, 2011). Alterations in NAWM, as indicated by abnormal FA and MD values, have been found in AOMS and POMS patients by multiple studies (Filippi *et al*, 2016; Rocca *et al.*, 2010a; Rocca *et al.*, 2015f; Till *et al.*, 2011a; Vishwas *et al.*, 2013) and have been associated with higher T2 lesion volume (LV), suggesting the role of Wallerian neurodegeneration in determining NAWM damage (Absinta *et al*, 2010). One study suggested that compared to AOMS, POMS patients exhibit more diffuse NAWM abnormalities independent of disease duration and age, indicating that pediatric patients are more susceptible to NAWM damage (Aliotta *et al*, 2014), possibly because the physiological maturation of WM tracts is completed only around the age of 20 (Lebel *et al*, 2008; Margoni *et al.*, 2022a). However, Absinta et al., (Absinta *et al.*, 2010) by comparing DTI metrics of 48 patients with pediatric MS and 30 adult MS patients, found a higher lesion MD in the latter, suggesting that a "younger" brain may have better repair capabilities.

In the initial phases of the disease, POMS patients are more resilient to MS-related damage than AOMS patients (Bonacchi *et al*, 2021). However, with disease progression, POMS patients exhibit more severe NAWM microstructural abnormalities due to failure of compensatory mechanisms (Bonacchi *et al.*, 2021). A DTI study of 22 MS patients with disease onset before the age of 12 (Rocca *et al.*, 2015f) investigated microstructural damage in the main interhemispheric, intrahemispheric, and projection tracts. Results showed that compared to healthy children, pediatric MS patients had lower FA and increased MD in corpus callosum (CC) and bilateral superior longitudinal fasciculus. Notably, only microstructural abnormalities in the CC were associated with T2-hyperintese LV, suggesting that the main WM tracts have different susceptibility to MS pathological changes. In addition, HCs showed a linear increase in FA and decrease in MD with age, while pediatric MS patients did not, indicating a failure in WM maturational processes. In line with this, another DTI study (Longoni *et al.*, 2017) demonstrated that pediatric MS patients and children with a single demyelinating attack show impaired maturation of WM tracts and progressive loss of WM integrity.

1.9.2 Gray matter damage

Several MRI sequences can be employed to detect focal cortical lesions (CLs) in MS patients, Double inversion recovery (DIR) sequences (Geurts *et al*, 2011). POMS patients develop CLs in 8 – 34% of the cases, which is lower than the occurrence observed in AOMS (~ 66%) (Absinta *et al*, 2011; Calabrese *et al*, 2012; Rocca *et al*, 2015c). One possible explanation is that different stages of GM maturation are associated with different susceptibility to MS-related pathology. In line with this hypothesis, the CLs detected in pediatric patients are usually found at the WM/GM boundary, consistently with myelin proliferation into the peripheral cortical neurophil occurring in this area during developmental years (Absinta *et al.*, 2011).

Similar to AOMS, POMS is characterized by early involvement of the thalamus and basal ganglia (Amiri *et al*, 2023; De Meo *et al*, 2021b; Eshaghi *et al*, 2018) with 25 – 46% of pediatric MS patients exhibiting lesions in these regions at the first MRI assessment (Dale *et al*, 2000; Mikaeloff *et al*, 2004). Using Voxel-based morphometry (VBM), Mesaros et al. (Mesaros *et al*, 2008) detected isolated thalamic atrophy in 28

pediatric MS patients. Thalamic tissue loss resulted associated with higher T2 LV and the Authors suggested that thalamic involvement was at least partially secondary to axonal transection due to WM lesions. This hypothesis was also supported by a subsequent study with similar findings (Aubert-Broche *et al*, 2011). An additional mechanism has been suggested by Fadda et al. (Fadda *et al*, 2019), who demonstrated an MS-specific surface-in pattern of thalamic atrophy in children with MS, possibly related to the presence of toxic soluble factors in the CSF. In line with this, a recent multiparametric MRI study (De Meo *et al.*, 2021b) detected microstructural abnormalities in thalamic regions close to the CSF and in those near the WM, that correlated with reduced cortical thickness and WM lesion volume, respectively. These findings demonstrate the presence of two distinct pathological mechanisms: microstructural damage likely due to immune cytotoxic factors in the CSF and upstream neuronal degeneration resulting from focal WM lesions (De Meo *et al.*, 2021b). A previous cross-sectional investigation (Rocca *et al*, 2015d) detected significant hippocampal atrophy in pediatric MS patients, which resulted associated with reduced brain and GM volume, but not with T2 LV. Moreover, radial mapping analysis revealed a higher vulnerability to damage of the cornu ammonis and subiculum compared to other hippocampal subregions. GM structural damage in terms of cortical atrophy was analyzed in a longitudinal study (Calabrese *et al.*, 2012) that compared changes in GM fraction between pediatric and adult MS patients over three years. Cortical atrophy progressed at similar rates in both patients' groups, independently from age and WM pathology, suggesting that reduced GM volume in MS is largely unrelated to WM inflammation.

Failure of sex- and age-expected GM development was observed by a recent longitudinal study of 68 POMS (De Meo *et al.*, 2019b) which detected impaired cortical, cerebellar, thalamic and caudate growth. The influence of focal WM lesions on the volume of closely integrated brain regions and late-developing areas supports the hypothesis of an age-dependent regional vulnerability to MS, as observed in WM (Rocca *et al.*, 2015f). However, the lack of association between deviation from age- and sex-expected volumes and WM focal lesion in several cortical areas confirms the existence of a primary involvement of GM from the early phases of MS.

Compared to AOMS, POMS patients are less vulnerable to MS-related GM damage, however over time POMS patients exhibit more severe GM atrophy due to failure of compensatory mechanisms (Bonacchi *et al.*, 2021). Consistently, longitudinal studies have reported a more severe accumulation of physical disability (Bonacchi *et al.*, 2021) and a steeper cognitive decline (McKay *et al.*, 2019) over time in POMS compared to AOMS.

1.9.3 Functional MRI

The application of structural MRI techniques over the past decades has proven that MS-related brain structural changes are not limited to WM focal lesions but include NAWM and GM microstructural damage; and that GM atrophy can be detected early in the disease and progresses at least partially independently of demyelination and inflammation (Filippi & Rocca, 2009). Although axonal degeneration is strongly correlated with cognitive impairment, this clinical symptoms can only be partially explained by the extent of brain tissue injury. This may be attributed to intra- and inter-subject variability in the capability to recover from structural brain damage. Brain plasticity involves the interplay of different mechanisms including increased axonal sodium channels expression, rearrangements of synapses, increased activation of parallel pre-existing connection or "latent" pathways, and reorganization of distant brain areas (Filippi & Rocca, 2009; Tomassini *et al*, 2012). Brain plasticity phenomena can be investigated with fMRI which detects neural activity by measuring changes in the blood oxygenation level-dependent (BOLD) signal. fMRI studies demonstrated that cortical reorganization may occur as result of WM damage, proportionally to the extent of the injury, limiting the functional consequences of structural abnormalities. However, once the adaptive abilities fail or become exhausted over time, the cerebral cortex is no longer able to compensate for tissue injury leading to the accrual of irreversible neurological deficits (Rocca *et al*, 2016; Rocca & Filippi, 2006, 2007). fMRI has been widely used to study neural activities during the performance of specific tasks or during task-free resting state (RS) conditions (Zhang *et al*, 2016). The latter has been used primarily in the MS field to investigate the brain's compensatory strategies in response to structural damage (Rocca *et al.*, 2016; Rocca & Filippi, 2006, 2007).

Functional connectivity (FC) is a measure of temporal synchronization of spatially remote spontaneous neuronal activities (Linnman *et al*, 2012). The early stages of AOMS are characterized by enhanced FC (Faivre *et al*, 2012), as disease progresses, FC usually plateaus or diminishes (Schoonheim *et al*, 2010), leading to development of irreversible cognitive dysfunction (Rocca *et al*, 2010b). Although it has not been definitively confirmed whether these dynamic changes also occur in pediatric MS patients, a broad spectrum of functional abnormalities has been reported in POMS (Akbar *et al*, 2015b; Cirillo *et al*, 2015; Rocca *et al.*, 2014a; Rocca *et al.*, 2014c; Rocca *et al.*, 2010b; Yap *et al.*, 2016).

This section focuses exclusively on cognitive networks. The default-mode network (DMN) comprises highly interconnected brain areas (posterior cingulate cortex, precuneus, medial prefrontal cortex and bilateral temporoparietal junction) that become inactive when attention is focused on external stimuli. While the individual is not performing cognitive tasks, DMN increases its connectivity supporting internal thought processes, such as mind wandering, daydreaming, introspection, remembering past events, and prospective thinking. Moreover, the DMN is highly integrated with other control networks such as the frontoparietal network (FPN) and salience network, working in concert to enable context- and task-specific network switching. DMN nodes serve as points of convergence with other cognitive networks and are differentially involved in cognitive functions such as visual imagery, episodic memory formation, spatial memory, semantic memory, semantic judgments, internal speech monitoring, and emotion regulation (Menon, 2023). Although some studies reported increased FC of the DMN following microstructural WM damage in POMS (Akbar *et al.*, 2015b; Yap *et al.*, 2016), others have found reduced connectivity of the DMN, particularly in posterior regions (Rocca *et al.*, 2014a; Rocca *et al.*, 2014c). A previous fMRI study (Akbar *et al.*, 2015b) found higher FC in the FPN in POMS patients compared to HCs, while another investigation (Rocca *et al.*, 2014c) observed decreased FC of multiple nodes of the secondary visual, sensorimotor, executive control and bilateral working memory networks in pediatric patients with MS. In addition, pediatric MS patients had increased FC of the right medial frontal gyrus of the attention network, in association with higher T2 LV. Cirillo and colleagues (Cirillo *et al.*, 2015) investigated cerebellar RS FC of 48 pediatric MS patients and 27 HCs. The results showed that, compared to controls,

pediatric patients had decreased FC between the cerebellum and the thalamus as well as the caudate nuclei. In contrast, they exhibited increased connectivity between the cerebellum and left frontal regions.

1.10 MRI substrates of cognitive dysfunction

A growing body of literature has explored the neural mechanisms underlying cognitive deficits in POMS. Cognitively impaired pediatric MS patients typically exhibit higher T2 LV than those cognitively normal (Rocca *et al.*, 2014a; Till *et al*, 2012a). In particular, higher infratentorial lesion burden has been associated with poorer linguistic abilities (Weier *et al*, 2015). Conversely, although evidence is limited to one study (Rocca *et al.*, 2015c), CLs do not seem to play a key role in cognitive dysfunction in pediatric MS.

The application of DTI revealed that abnormal WM microstructure is associated with various cognitive manifestations in POMS. Low FA values in the CC and in right frontal and parietal regions have been correlated with poor arithmetic abilities (Till *et al.*, 2011a). A cross-sectional study (Rocca *et al.*, 2014a) found a higher degree of microstructural damage of the posterior CC, cingulum and bilateral parieto-occipital tracts in pediatric MS patients with cognitive impairment compared to those with preserved cognitive function. In addition, Bethune et al. (Bethune *et al.*, 2011) demonstrated an association between reduced FA in the CC and poorer performance on tests assessing IPS in children and adolescents with MS.

Of volumetric MRI measures, thalamic atrophy has been shown to contribute significantly to cognitive deficits in pediatric MS. It has been correlated with lower IQ and poorer performance on tests assessing, IPS, working memory, planning, problem-solving, visual memory and expressive verbal abilities (Fuentes *et al*, 2012; Till *et al.*, 2011b; Till *et al.*, 2012a). Indeed, the thalamus is involved in several cognitive functions as it actively modulates the signals transmitted to the cognitive cortical areas (Saalmann & Kastner, 2015).

Hippocampal structural damage in pediatric MS patients was found to be associated with impaired verbal learning and memory (Fuentes *et al.*, 2012). Using radial mapping analysis, a previous study (Rocca *et al.*, 2015d) found that cognitively impaired

pediatric MS patients exhibited reduced volumes of the dentate gyrus and subiculum of the right hippocampus compared to those with preserved cognition. Reduced volume of the subiculum and cornu ammonis 1 of the right hippocampus as well as atrophy of the dentate gyrus of the left hippocampus has been associated with poor receptive and expressive language abilities. Interestingly, higher scores on tests measuring attention and verbal comprehension have been correlated with an increased volume of bilateral dentate gyrus, indicating that hypertrophy of this hippocampal region might delay the development of cognitive deficits.

Reduced amygdala volumes in children and adolescents with MS has been associated with lower levels of communication abilities and socialization. Moreover, the right amygdala volume has been positively correlated with visuospatial memory, while the left amygdala has been linked to the pediatric MS patients' social skills (Green *et al.*, 2018). The amygdala is notably involved in emotional responses and formation of emotionally based memories. However, its connections with the hippocampus and other subcortical regions could make the amygdala a node of an extended network dedicated to encoding, storing, and recalling visual information (Green *et al.*, 2018; Richter-Levin & Akirav, 2000).

Pediatric MS patients with cognitive impairment were found to have more severe atrophy of the right precuneus and left middle temporal gyrus than cognitively normal patients (Rocca *et al.*, 2014a). Indeed, the precuneus plays a role in self-centered mental imagery strategies and recollection of past experiences (Cavanna & Trimble, 2006), while the middle temporal gyrus is involved in verbal comprehension and semantic cognition (Briggs *et al*, 2021).

Atrophy of the posterior lobe of the cerebellum adversely affects the performance on tests assessing IPS and verbal abilities in POMS patients (Weier *et al.*, 2015). The cerebellum is highly connected to the spinal cord, the thalamus and several cortical associative regions, playing a key role in multiple networks serving motor coordination and cognitive-behavioral functions (Timmann *et al*, 2010; Wang *et al*, 2014; Weier *et al.*, 2015).

In addition to structural MRI studies, fMRI research has provided deeper insight into the impact of POMS on cognitive networks. Three task-based fMRI studies have been conducted in pediatric MS patients (Akbar *et al*, 2015a; Barlow-Krelina *et al.*, 2019; De

Meo *et al.*, 2017). The first study revealed that, during the execution of a sustained attention active fMRI task, cognitively impaired patients showed reduced activation in parietal, occipital and cerebellar areas, as well as abnormal deactivation of the anterior cingulate cortex, due to microstructural alterations in the WM tracts connecting these regions (De Meo *et al.*, 2017). In the second study (Barlow-Krelina *et al.*, 2019), cognitively preserved POMS patients exhibited higher activation than controls in frontal and parietal areas during the execution of a working-memory task, indicating that increased brain activity in POMS patients may represent a compensatory mechanism to maintain cognitive performance. In line with this hypothesis, Akbar and colleagues (Akbar *et al.*, 2015a) found an association between a faster response on the fMRI version of the SDMT and increased activation of the left superior occipital region in POMS patients.

Cognitively impaired pediatric MS patients are characterized by RS FC abnormalities in large-scale brain networks that appear to be different from those observed in adults. The latter typically exhibit reduced connectivity in the anterior regions of the DMN and increased RS FC in the posterior areas (Bonavita *et al*, 2011; Roosendaal *et al*, 2010). In contrast, pediatric MS patients show reduced connectivity of the precuneus in association with cognitive impairment (Rocca *et al.*, 2014a; Rocca *et al.*, 2014c), whereas increased RS FC of frontal areas has been observed in patients with preserved cognitive performance (Akbar *et al.*, 2015b; Rocca *et al.*, 2014a). The posterior brain functional alterations in pediatric MS patients mirror the pattern of structural abnormalities (focal T2 lesions, GM and WM structural alterations and NAWM microstructural damage), indicating that RS FC reduction is likely a result of axonal degeneration (Rocca *et al.*, 2014a). Conversely, the greater recruitment of frontal functional resources may represent a compensatory mechanism to sustain cognitive performance in response to POMS-related structural damage (Akbar *et al.*, 2015a; De Meo *et al.*, 2017) . Multiple studies (Portaccio *et al.*, 2021b; Rocca *et al.*, 2014a; Rocca *et al.*, 2014c) have hypothesized that the maturation of CNS is associated with changes in the functional organization of the brain architecture, possibly explaining the differences observed between pediatric and adult MS patients. Indeed, the tracts connecting the posterior cingulate cortex and the anterior prefrontal cortex, which are involved in various cognitive functions (Paus, 2005), mature with age (Fair *et al*, 2007;

Rocca *et al.*, 2014a; Supekar *et al*, 2010). A recent study (Cacciaguerra *et al.*, 2024) found that poorer attentional performance in younger pediatric MS patients (< 16 years of age) was correlated with decreased connectivity in the sensorimotor, basal ganglia and language networks. Conversely, for patients aged 16 years or older, it resulted associated with increased RS FC in the sensorimotor, basal ganglia, executive, default-mode and language networks, confirming that the RS FC substrates of cognitive impairment change with age in POMS. The cerebellum is another critical region for understanding the cognitive manifestations of pediatric MS patients. As mentioned above, structural MRI studies have demonstrated that cerebellar atrophy contribute to cognitive impairment in POMS (Weier *et al.*, 2015). Moreover, focal lesions can disrupt its FC leading to reduced connectivity with frontal, temporal and parietal cortical regions further contributing to cognitive dysfunction (Cirillo *et al.*, 2015). Finally, the only study (Rocca *et al.*, 2014c) that explored the influence of inter-network RS FC on the cognitive performance of pediatric MS patients, found that higher connectivity between the DMN and the sensorimotor network, as well as between the attention network and the left-working memory network was associated with cognitive impairment, indicating that the hierarchical organization of brain network is also a critical determinant of cognitive status of these patients.

2. Aims of the work

The use of a multiparametric approach, combining structural and functional MRI techniques, represents a rewarding strategy to better understand the mechanisms associated with cognitive deficit in both POMS and AOMS. Previous studies in POMS were mainly performed on registry data, with several limitations, including the availability of only a few cognitive tests and absence of accompanying MRI data. In this project, we will combine multiparametric MRI techniques with clinical and neuropsychological measures to define the functional and structural substrates of cognitive dysfunction in POMS patients. Then, we will apply these tools to longitudinal data to track their evolution over time, and to identify neuropsychological, clinical and MRI substrates of consolidated resilience and predictors of cognitive decline.

The specific aims of the present PhD project are:

1. To identify cognitive phenotypes in pediatric MS patients and to compare their demographic, clinical, structural and functional MRI features, in order to characterize the mechanisms underlying the different cognitive manifestations in POMS;

2. To compare the degree of consolidated resilience between POMS and AOMS, and to explore the different functional mechanisms supporting the maintenance of cognitive status in both groups;

3. To characterize the age-expected patterns of maturation of superficial WM, cortical thickness and GM volume in POMS patients and their deviations in comparison to matched HCs, and to evaluate the association of these neural substrates with physical disability and cognitive performance;

4. To validate a new neuropsychological tool that can overcome the limitations of the SDMT, providing additional information about cognitive processing speed in adult subjects.

3. Structural and functional imaging features of cognitive phenotypes in pediatric multiple sclerosis

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RESEARCH ARTICLE

Structural and functional imaging features of cognitive phenotypes in pediatric multiple sclerosis

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Abstract

Objective: The present study aimed to identify the clinical and MRI features of the distinct cognitive phenotypes in pediatric multiple sclerosis (pedMS). **Methods:** PedMS patients (n = 73) and healthy controls (n = 30) underwent clinical examination and 3.0T MRI. All patients completed neuropsychological testing, and cognitive phenotypes were identified by performing K-means clustering on cognitive scores. MRI metrics included brain T2-hyperintese lesion volume and normalized brain volumes. Within seven cognitively relevant cortical networks, structural disconnectivity (i.e., the mean percentage of streamlines connecting each pair of cortical regions passing through a lesion) and resting-state (RS) functional connectivity (FC) were estimated. **Results:** Three cognitive phenotypes emerged: Preserved cognition (PC; n = 27, 37%), mild verbal learning and memory/semantic fluency involvement (MVS; n = 28, 38%), and multidomain involvement (MI; n = 18, 25%). Age, sex, and disease duration did not differ among groups. Compared with healthy subjects, PC patients had decreased RS FC within the default mode network (p = 0.045); MVS patients exhibited lower cortical volume and reduced RS FC within the frontoparietal network (all p = 0.045); and MI patients showed decreased volumes in all brain compartments except the hippocampus, and reduced RS FC within the frontoparietal network (all $p \leq 0.045$). Compared to PC, MI patients had more severe disability and higher structural disconnectivity within four cortical networks (all $p \leq 0.045$). Compared to PC and MVS, MI patients had lower intelligence quotient (all $p \leq 0.005$). **Interpretation:** We identified three cognitive phenotypes in pedMS that demonstrate the existence of a spectrum of impairment. Such phenotypes showed distinct clinical and MRI characteristics that contributed to explain their cognitive profiles.

Introduction

Pediatric MS accounts for approximately 2-10% of all MS cases (Margoni *et al.*, 2022a). Despite having higher relapse rate than the adult counterparts, pediatric patients exhibit a slower transition to SP MS and a more gradual development of physical disability (Margoni *et al.*, 2022a). However they tend to experience a steeper cognitive decline, leading to cognitive dysfunction at a significantly younger age in more than 30% of cases (Amato *et al.*, 2008; Margoni *et al.*, 2022a). Processing speed, complex aspects of attention and memory are the most commonly impaired cognitive functions. Unlike adults, language abilities and general intelligence may also be affected (Portaccio *et al*, 2021a).

MRI studies in pediatric MS have shown that a higher frequency of focal WM lesions in the corpus callosum, thalamus, and parieto-occipital regions (Rocca *et al*, 2014b), as well as more severe atrophy of the these regions (Till *et al.*, 2011b) and the hippocampus (Rocca *et al.*, 2015d) contribute to cognitive dysfunction. Moreover, patients with cognitive impairment exhibit reduced RS FC of the precuneus (Rocca *et al.*, 2014b; Rocca *et al.*, 2014c) and dentate nucleus with basal ganglia, frontal, temporal and parietal regions, compared to cognitively preserved patients (Cirillo *et al.*, 2016).

Most previous investigations classified cognitive functioning according to a dichotomous view, namely, preserved *vs* impaired, overlooking the heterogeneity of the cognitive manifestations in pediatric MS (De Meo *et al*, 2021a). Recent studies in adult MS patients (De Meo *et al.*, 2021a; Podda *et al*, 2021) deployed machine learning models to identify the recurring patterns of cognitive deficit and demonstrated the existence of four to five cognitive phenotypes ranging from intact cognition to severe and widespread impairments in cognitive functioning (De Meo *et al.*, 2021a; Podda *et al.*, 2021). Work extending this cognitive phenotypes framework beyond AOMS is lacking. A detailed description of the different cognitive profiles in children and adolescents with MS could offer a deeper understanding of the neural substrates of pediatric MS-related cognitive abnormalities, potentially providing the basis for more tailored interventions.

Against this background, in this study we employed an unsupervised machine learning technique to determine whether unique cognitive phenotypes exist in pediatric

MS. Then, we investigated the association between these cognitive phenotypes and specific demographic, clinical, and MRI variables, encompassing brain T2-hyperintese LV, normalized brain volumes, as well as structural disconnectivity (i.e., the mean proportion of connecting streamlines passing through a lesion estimated using the Network Modification [NeMo] Tool) (Kuceyeski *et al*, 2013; Tozlu *et al*, 2020) and RS FC strength within seven main cortical networks (visual network, somatomotor network, dorsal attention network, ventral attention network, limbic network, FPN and DMN) (Thomas Yeo *et al*, 2011).

Methods

Participants. This retrospective cross-sectional study included data from 73 RR (Thompson *et al.*, 2018b) pediatric MS patients and 30 age-, sex-and education-matched HCs at the IRCCS San Raffaele Scientific Institute in Milan, Italy. Inclusion criteria were: no previous history of major systemic, psychiatric or neurological disorders (other than MS); no concomitant therapy with antidepressants or psychoactive drugs; right-handedness (Oldfield, 1971). In addition, pediatric MS patients were required to be relapse- and steroid-free for at least one month before the clinical and MRI examination; and to have a score < 20 on the CDI, which represents the clinical threshold for depressive symptoms (Poli *et al*, 2003). Appropriate testing was performed as necessary to rule out leukodystrophies and myelin oligodendrocyte glycoprotein antibody-associated disorders.

Clinical and neuropsychological assessment. On the day of the MRI, all patients underwent neurological examination with Expanded Disability Status Scale (EDSS) (Kurtzke, 1983) assessment and record of ongoing disease-modifying treatments. Experienced neuropsychologists administered the Brief Neuropsychological Battery for Children (Portaccio *et al.*, 2009a) which assesses: verbal learning and memory (using the SRT), visuospatial learning and memory (through the SPART), attention and information processing speed (using the TMT and the SDMT), and expressive language (through a Semantic verbal fluency test and a Phonemic verbal fluency test). Z-scores were calculated for each cognitive test according to normative data (Portaccio *et al.*, 2009a). A z-score for each cognitive domain was then obtained by averaging the z-

scores of the corresponding tests (Sepulcre *et al*, 2006). For patients in the age range between 6 and 15 years, the intelligence quotient was assessed using the WISC-IV (Orsini *et al.*, 2012), while for patients with age ≥16 years it was measured with the WAIS-IV (Orsini & Pezzuti, 2013). Fatigue was also evaluated through the FSS (Krupp *et al.*, 1989).

MRI acquisition. Brain images were acquired from all subjects using two 3.0 T MRI scanners (first scanner: Achieva [n = 21 HCs and n = 55 pediatric MS patients], second scanner: Ingenia [n = 9 HCs and n = 17 pediatric MS patients] Philips Medical Systems, Eindhoven, The Netherlands). Images acquired using the Achieva scanner included: 1) T2*-weighted single shot echo planar imaging (EPI) sequence for RS fMRI (repetition time [TR] = 3,000 ms, echo time [TE] = 35 ms, field of view [FOV] = 240 mm^2, matrix size = 128 × 128, flip angle = 90°, 30 contiguous axial slices, 4 mm thick); 2) dual-echo turbo spin echo (TR = 2599, TE = 16 - 80 ms, FOV = 240 mm^2, FA = 90°, echo train length [ETL] = 6, matrix = 256 × 256, 44 contiguous axial slices, 3 mm thick); and 3) 3D T1-weighted fast field echo (TR = 25, TE = 4.6 ms, FOV = 230 mm^2, flip angle = 30°, matrix size = 256 × 256, 220 contiguous axial slices, 0.8 mm thick). Images acquired using the Ingenia scanner included: 1) T2*-weighted EPI sequence for RS fMRI, with the same parameters used for the Achieva scanner (TR = 3000 ms, TE = 35 ms, matrix size = 128 × 128, flip angle = 90°, FOV = 240 mm^2, 30 contiguous axial slices, 4 mm thick); 2) 3D fluid attenuated inversion recovery (FLAIR; TR = 4800 ms, inversion time [TI] = 1650 ms, TE = 270 ms, matrix size = 256 × 256, FOV = 256 × 256 mm^2, ETL = 167, 192 contiguous sagittal slices, 1 mm thick); 3) 3D T2-weighted sequence (TR = 2500 ms, TE = 330 ms, ETL = 117, FOV = 256 × 256 mm^2, matrix size = 256 × 256, 192 contiguous sagittal slices, 1 mm thick); and 4) 3D T1-weighted turbo field echo (TR = 7 ms, TI = 1000 ms, flip angle = 8°, TE = 3.2 ms, FOV = 256 × 256 mm^2, matrix size = 256 × 256, 204 contiguous sagittal slices, 1 mm thick).

Conventional MRI analysis. An experienced observer manually outlined T2-hyperintense WM lesions from the dual-echo scans of pediatric MS patients acquired with the Achieva scanner. LV was then calculated using a semi-automated local thresholding segmentation technique (Jim 7.0, Xinapse Systems Ltd, Colchester, UK). For patients acquired on the Ingenia scanner, FLAIR images were resampled at the same resolution of dual-echo images, lesions were manually segmented as described

above, and total T2-LV was measured. On both scanners, normalized brain (NBV), WM (NWMV), and cortical GM (NCGMV) volumes were calculated using FSL SIENAx software on lesion-filled 3D T1-weighted images (Battaglini *et al*, 2012). Normalized volumes of bilateral thalamus and bilateral hippocampus were also measured with the FIRST tool (Patenaude *et al*, 2011a).

Structural disconnection analysis. Lesion masks were transformed onto the Montreal Neurologic Institute (MNI) space using the non-linear transformation that had been previously calculated for the SIENAx software. The spatially transformed lesion maps were uploaded to the NeMo tool (https://kuceyeski-wcm-web.s3.us-east-1.amazonaws.com/upload.html) (Kuceyeski *et al.*, 2013), a stand-alone web application based on a set of diffusion-weighted image scans of 420 healthy subjects from the 7T Human Connectome Project. We selected the following options: constrained spherical-deconvolution informed filtering of tractograms (Smith *et al*, 2015); the CocoYeo243-subj parcellation with 243-regions obtained from Schaefer200 (200 cortical), aseg (16 subcortical) and SUIT (27 cerebellar) (Schaefer *et al*, 2018). The NeMo tool computed the average pairwise disconnection (i.e., Change in Connectivity score, which is the proportion of WM streamlines that intersect the volume occupied by a lesion; 0 = no disconnection. 1 = complete disconnection) (Kuceyeski *et al.*, 2013) across the reference sample and displayed the results into a symmetrical N×N matrix, where N represents the number of atlas regions. Finally, we calculated disconnection between regions of seven cortical brain networks (visual network, somatomotor network, dorsal attention network, ventral attention network, limbic network, FPN and DMN, according to the Yeo atlas) (Thomas Yeo *et al.*, 2011) and derived an average disconnection value for each network.

RS FC network pre-processing. The CONN toolbox (Whitfield-Gabrieli & Nieto-Castanon, 2012) was utilized to process RS fMRI data. Using a rigid-body transformation, images were first realigned to the mean of each session to correct for head movements. The mean framewise displacement was calculated as a measure of motion. Subsequently, RS fMRI images were registered to the lesion filled 3D T1-weighted scan, normalized to the MNI space using a non-linear transformation, and smoothed with a 6-mm^3 Gaussian filter. Denoising was performed using the first five cerebrospinal fluid and WM principal components as nuisance covariates through the

anatomical component-based noise correction method (Behzadi *et al*, 2007). The six rigid movement parameters and their first temporal derivatives were regressed out from the data, along with any outliers and spurious effects from the first two time-points identified by the ART toolbox. Finally, the RS fMRI time series were linearly detrended and band-pass filtered between 0.01 and 0.1 Hz.

RS FC network analysis. After RS fMRI pre-processing, the brain was parcelled into 200 cortical regions of interest (ROI) according to the parcellation proposed by Schaefer et al. (Schaefer *et al.*, 2018) RS fMRI time series were extracted from each region by calculating the mean signal of all voxels within each ROI. Bivariate correlations between each pair of ROI, representing the RS FC strengths between cortical regions, were calculated with the Pearson's correlation coefficient between ROI time courses. Correlation matrices were obtained from all participants and were thresholded at correlation threshold $\tau = 0$ into weighted connectivity matrices. As measure of RS FC strength, we used the degree, i.e., the weighted sum of connections for each node (i.e., cortical ROI). Finally, the average degree within seven cortical networks (according to the parcellation proposed by Yeo et al.(Thomas Yeo *et al.*, 2011)) was calculated.

Statistical analysis. Demographic and clinical characteristics were compared between HC and pediatric MS patients using the Mann-Whitney U test and Chi-squared test, as appropriate. For each participant, normalized brain volumes z-scores and RS FC degrees z-scores were calculated by subtracting the mean and dividing by the standard deviation of the HCs scanned on the same scanner. Brain T2 LVs were log-transformed and then standardized into z-scores according to the overall distribution observed in pediatric MS patients.

A K-means cluster analysis was conducted on z-scores of neuropsychological tests to detect homogeneous cognitive phenotypes in pediatric MS patients. The K-means algorithm partitions the data into distinct groups to minimize within-cluster variance (Hartigan & Wong, 1979). To increase the likelihood of obtaining a reliable clustering solution, the procedure was iterated 50 times, thus ensuring greater robustness and reducing bias associated with the random selection of centroids at the start of the algorithm. The NBclust R package (Charrad *et al*, 2014) was used to determine the

optimal number of clusters through a consensus voting approach across 23 different indices.

Demographic, clinical, and neuropsychological differences between cognitive phenotypes were examined using the Chi-squared test, Mann-Whitney U test, or linear models. Benjamini-Hochberg false discovery rate (FDR) correction was applied to account for multiple testing. Comparisons of MRI variables between HC and cognitive phenotypes were performed using age- and sex-adjusted linear models, applying FDR correction.

Statistical significance was set at $p < 0.05$. R-4.2.2 and SPSS version 26.0 (IBM, Armonk, NY, USA) software were used for computations.

Results

Demographic, clinical and conventional MRI measures. Participants' demographics are summarized in Table 1. Compared with HCs, pediatric MS patients had reduced NBV ($p = 0.002$), NCGMV ($p = 0.002$) and normalized thalamic volume ($p = 0.024$) (Table 1).

Table 3.1.1. Main demographic, clinical and conventional MRI characteristics of the subjects enrolled in the study. Bold text indicates a statistically significant result.

	HC (n=30)	**pedMS patients (n=73)**	**p**
Girls, No (%)	15 (50)	49 (67)	0.16[a]
Age, median (IQR), y	15.2 (12.0 to 18.0)	15.9 (14.2 to 17.0)	0.63[b]
Education, median (IQR), y	8.0 (7.0 to 11.0)	9.0 (8.0 to 11.0)	0.64[b]
EDSS, median (IQR)	NA	1.0 (1.0 to 1.5)	NA
Disease duration, median (IQR), y	NA	1.4 (0.7 to 2.6)	NA
CDI, median (IQR)	NA	6.0 (3.0 to 10.0)	NA
FSS, mean (SD)	NA	2.7 (1.1)	NA
Patients receiving DMTs: No treatment, 1st line, 2nd line, No. (%)†		11 (15)/ 37 (51)/ 25 (34)	NA
Subjects scanned with: S1, S2, No (%)	21(70)/ 9 (30)	55 (75)/ 17 (25)	0.67[a]
z-T2 Lesion Volume, mean (SD)	NA	0.0 (1.0)	NA
z-N Brain Volume, mean (SD)	**0.0 (1.0)**	**-0.8 (1.1)**	**0.002[c]**

z-N WM Volume, mean (SD)	0.0 (1.0)	-0.3 (1.4)	0.37[c]
z-N Cortical GM Volume, mean (SD)	**0.0 (1.0)**	**-0.6 (0.9)**	**0.002[c]**
z-N Hippocampal Volume, mean (SD)	0.0 (1.0)	-0.1 (1.0)	0.26[c]
z-N Thalamic Volume, mean (SD)	**0.0 (1.0)**	**-0.7 (1.3)**	**0.024[c]**

[a]Chi-square test; [b]Mann-Whitney U test; [c]Age- and sex-adjusted linear model.

†Classification of DMTs: first line=interferon beta and glatiramer acetate; second line=natalizumab, rituximab, fingolimod, cyclophosphamide and mitoxantrone.

Abbreviations: CDI=Children's Depression Inventory; DMT=disease modifying treatment; EDSS=Expanded Disability Status Scale; GM=gray matter; FSS=Fatigue Severity Scale; HC=healthy controls; IQR=interquartile range; N=normalized; pedMS= pediatric multiple sclerosis; S=scanner; SD=standard deviation; WM=white matter; z=z-score.

Cognitive phenotypes. The optimal number of clusters was three as suggested by the majority rule (13 out of 23 indices) (Charrad *et al.*, 2014). Pediatric MS patients were then subdivided into three cognitive phenotypes by K-means clustering analysis (Figure 1). The first cluster "Preserved cognition" (PC) included 27 patients (37%) who showed a pattern of average or above-average functioning across cognitive domains and a median intelligence quotient of 104 (interquartile range [IQR], 100 to 118). The second group "Mild verbal learning and memory/semantic fluency involvement" (MVS) comprised 28 patients (38%). The median IQ was 100 (IQR, 92 to 117). The third group was named "Multidomain involvement" (MI) and included 18 patients (25%) who had below-average to exceptionally low scores on all cognitive tests. The intelligence quotient was lower in MI (median [IQR] IQ, 92 [76 to 96]; FDR-p $\leq$ 0.005) than in MVS and PC patients (Table 2). No significant differences were found when comparing age, sex, education, disease duration and FSS scores among cognitive phenotypes (FDR-p $\geq$ 0.44) (Figure 2). Patients with MI (median [IQR] EDSS score, 1.5 [1.0 to 2.0]) had more severe physical disability than those with PC (median [IQR] EDSS score, 1.0 [0.0 to 1.5]; FDR-p = 0.006) (Table 2) (Figure 2).

Table 3.1.2. Main demographic, clinical, and neuropsychological features of cognitive phenotypes. Bold text indicates a statistically significant result.

	PC (n=27)	MVS (n=28)	MI (n=18)	FDR-p
Girls, No (%)	21 (78)	17 (61)	11 (61)	≥0.44
Age, median (IQR), y	15.9 (13.7 to 16.8)	16.1 (14.5 to 17.0)	15.7 (14.1 to 17.0)	≥0.99
Education, median (IQR), y	9.0 (8.0 to 11.0)	10.0 (8.0 to 11.0)	9.0 (8.0 to 11.0)	≥0.44
EDSS, median (IQR)	1.0 (0.0 to 1.5)	1.0 (1.0 to 1.5)	1.5 (1.0 to 2.0)	**0.006**[a]
Disease duration, median (IQR), y	1.2 (0.6 to 2.1)	1.4 (0.8 to 2.6)	1.8 (0.5 to 3.6)	≥0.99
CDI, median (IQR)	7.5 (3.0 to 12.0)	5.5 (3.0 to 8.0)	6.0 (1.5 to 11.0)	≥0.90
FSS, mean (SD)	2.7 (0.8)	2.7 (1.2)	2.9 (1.3)	≥0.99
Patients receiving DMTs: No treatment, 1st line, 2nd line, No. (%)†	3 (11)/ 16 (59)/ 8 (30)	5 (18)/ 16 (57)/ 7 (25)	3 (17)/ 5 (28)/ 10 (55)	NA
IQ, median (IQR)	104 (100 to 118)	100 (92 to 117)	92 (76 to 96)	**≤0.005**[a,b]
z-SRT-lts, mean (SD)	1.0 (0.5)	-0.4 (0.8)	-1.2 (1.1)	**≤0.005**[a,b,c]
z-SRT-cltr, mean (SD)	0.7 (0.8)	-0.7 (0.7)	-1.2 (1.0)	**<0.001**[a,c]
z-SRT-recall, mean (SD)	1.0 (0.5)	-0.5 (1.0)	-1.2 (1.3)	**<0.001**[a,c]
z-SPART, mean (SD)	0.4 (1.2)	0.6 (0.8)	-1.8 (2.0)	**<0.001**[a,b]
z-SPART-recall, mean (SD)	0.4 (0.8)	0.5 (0.7)	-2.0 (1.6)	**<0.001**[a,b]
z-TMT-A, mean (SD)	0.2 (0.7)	0.1 (0.7)	-0.9 (1.0)	**<0.001**[a,b]
z-TMT-B, mean (SD)	0.2 (0.7)	0.2 (0.7)	-1.2 (1.5)	**<0.001**[a,b]
z-SDMT, mean (SD)	0.4 (1.0)	0.0 (0.6)	-0.5 (0.6)	**<0.001**[a]
z-Semantic fluency test, mean (SD)	0.4 (0.8)	-0.3 (0.4)	-0.6 (0.5)	**<0.001**[a,c]
z-Phonemic fluency test, mean (SD)	0.3 (1.0)	0.0 (0.7)	-0.2 (1.2)	≥0.12
z-Verbal learning and memory, mean (SD)	0.9 (0.5)	-0.6 (0.7)	-1.2 (1.1)	**≤0.012**[a,b,c]
z-Visuospatial learning and memory, mean (SD)	0.2 (0.6)	0.3 (0.4)	-0.6 (0.8)	**<0.001**[a,b]
z-Attention/processing speed, mean (SD)	0.3 (0.6)	0.1 (0.6)	-0.9 (0.8)	**<0.001**[a,b]
z-Expressive language, mean (SD)	0.4 (0.7)	-0.2 (0.5)	-0.4 (0.7)	**≤0.005**[a,c]

Comparisons performed by Chi-square test (sex), linear models (age, IQ and cognitive z-scores) and Mann-Whitney U test (education, EDSS, disease duration, and CDI). FDR correction was applied to account for the overall number of tests.

Letters indicate significant differences as follows: [a]PC vs MI; [b]MVS vs MI; [c]PC vs MVS.

†Classification of DMTs: first line=interferon beta and glatiramer acetate; second line=natalizumab, rituximab, fingolimod, cyclophosphamide and mitoxantrone.

Abbreviations: CDI=Children's Depression Inventory; cltr=consistent long-term retrieval; EDSS=Expanded Disability Status Scale; FDR=false discovery rate; IQ=intelligence quotient; lts=long term storage; MI=multidomain involvement; MVS=mild verbal learning and memory/semantic fluency involvement; PC=preserved cognition; SD= standard deviation; SDMT=Symbol Digit Modalities Test; SPART=Spatial Recall Test; SRT=Selective Reminding Test; TMT=Trail Making Test; z=z-score.

Figure 3.1.1. Cluster plots of the three cognitive phenotypes identified from cognitive tests z-scores (Mistri et al., 2024). Circles represent pediatric MS patients with Preserved cognition, squares patients with Mild verbal learning and memory/semantic fluency involvement and triangles patients with Multidomain involvement. The big circle, square and triangle represent the centroids for each cluster.

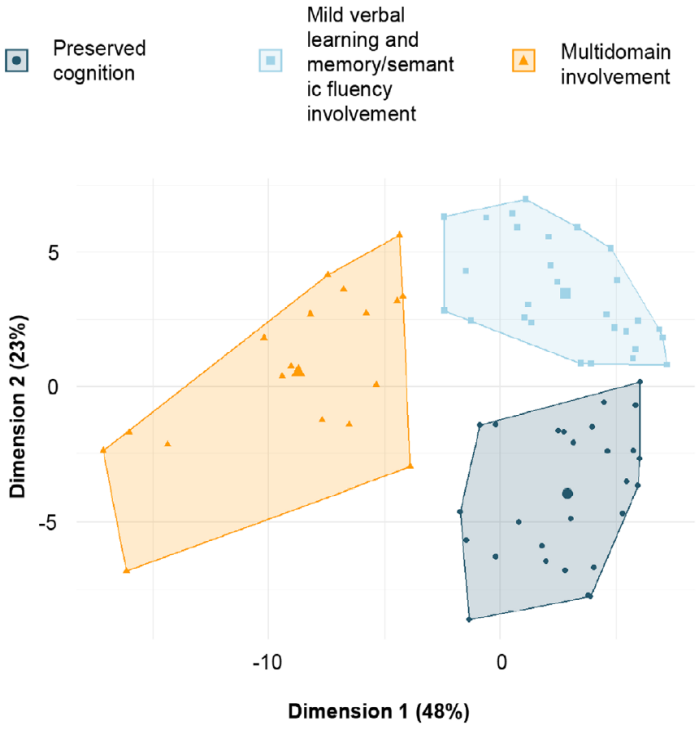
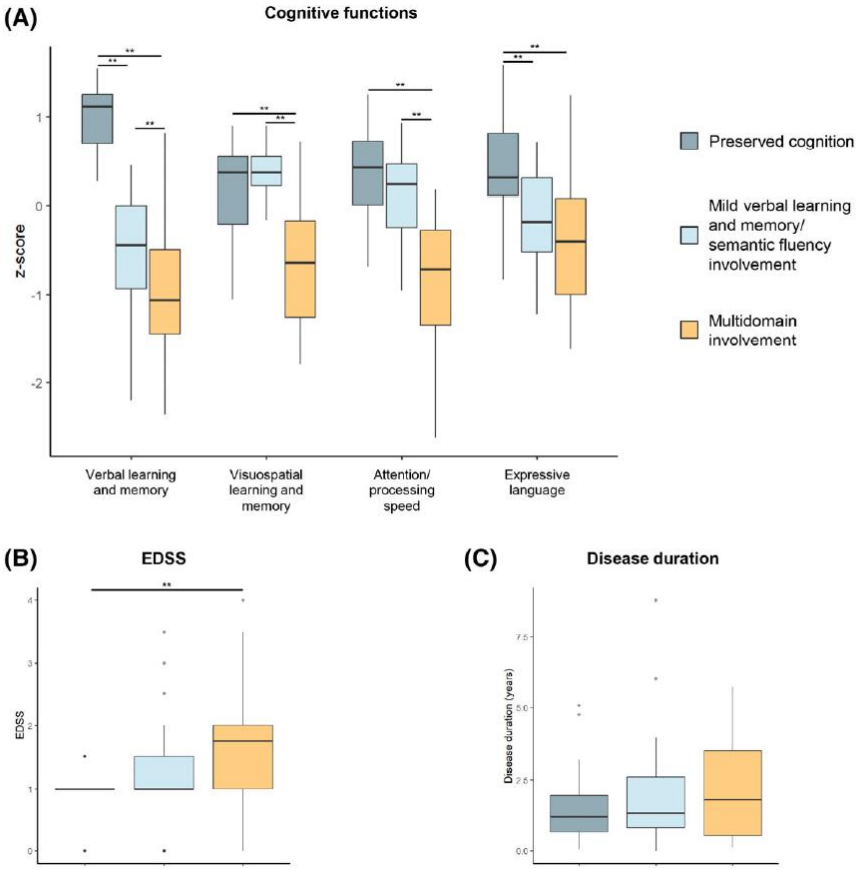


Figure 3.1.2. Comparisons of cognitive and clinical variables between cognitive phenotypes (Mistri et al., 2024). Boxplot of cognitive domain z-scores (A), Expanded Disability Status Scale (EDSS) (B) and disease duration (C) in pediatric MS patients grouped by cognitive phenotype. For each variable the horizontal line represents the median. Asterisks indicate statistically significant differences (** $p < 0.01$; * $p < 0.05$).



MRI features of cognitive phenotypes.

Conventional MRI features. The comparisons of normalized brain volumes between HC and pediatric MS patients with PC revealed no statistically significant differences. Compared with HC, patients with MVS had lower normalized cortical GM volume (FDR-p = 0.045), while those with MI showed lower normalized brain (FDR-p < 0.001), WM (FDR-p = 0.045), cortical GM (FDR-p = 0.010) and thalamic volumes

(FDR-p = 0.041). Compared with patients with PC, those with MI had lower NBV (FDR-p = 0.037). When compared to patients with MVS those with MI were characterized by lower NBV (FDR-p = 0.044) and NWMV (FDR-p = 0.045). No significant differences were found between PC and MVS phenotypes (Table 3).

Table 3.1.3. Differences in estimated marginal means of MRI variables across cognitive phenotypes. Data are presented as estimated marginal means (95% confidence interval). Bold text indicates a statistically significant result.

	HC (n=30)	PC (n=27)	MVS (n=28)	MI (n=18)	FDR-p
z-T2 Lesion Volume	NA	0.3 (-0.1 to 0.7)	0.1 (-0.3 to 0.5)	-0.4 (-0.9 to 0.1)	≥0.12
z-N Brain Volume	-0.1 (-0.4 to 0.3)	-0.4 (-0.8 to -0.1)	-0.6 (-1.0 to -0.3)	-1.4 (-1.8 to -0.9)	**≤0.044**[a,b,c]
z-N WM Volume	0.0 (-0.5 to 0.5)	0.0 (-0.5 to 0.4)	0.0 (-0.4 to 0.5)	-1.1 (-1.7 to -0.5)	**0.045**[a,c]
z-N Cortical GM Volume	0.0 (-0.4 to 0.2)	-0.4 (-0.7 to -0.1)	-0.6 (-0.9 to -0.3)	-1.0 (-1.4 to -0.6)	**≤0.045**[a,d]
z-N Hippocampal Volume	0.0 (-0.4 to 0.4)	-0.1 (-0.5 to 0.3)	-0.2 (-0.6 to 0.2)	-0.5 (-1.0 to 0.0)	≥0.25
z-N Thalamic Volume	0.0 (-0.5 to 0.4)	-0.4 (-0.9 to 0.1)	-0.5 (-1.0 to -0.1)	-1.2 (-1.8 to -0.7)	**0.041**[a]
Structural dysconnectivity					
Visual network	NA	0.2 (0.1 to 0.3)	0.2 (0.1 to 0.3)	0.3 (0.2 to 0.4)	≥0.10
Somatomotor network	NA	0.2 (0.1 to 0.3)	0.2 (0.2 to 0.3)	0.3 (0.3 to 0.4)	**0.041**[b]
Dorsal attention network	NA	0.2 (0.1 to 0.3)	0.2 (0.2 to 0.3)	0.4 (0.3 to 0.5)	**0.045**[b,c]
Ventral attention network	NA	0.2 (0.1 to 0.3)	0.2 (0.2 to 0.3)	0.3 (0.3 to 0.4)	≥0.07
Limbic network	NA	0.1 (0.0 to 0.1)	0.1 (0.0 to 0.1)	0.1 (0.1 to 0.2)	**0.046**[b]
Frontoparietal network	NA	0.2 (0.1 to 0.3)	0.2 (0.2 to 0.3)	0.3 (0.3 to 0.4)	**0.045**[b]
Default-mode network	NA	0.2 (0.1 to 0.3)	0.2 (0.2 to 0.3)	0.3 (0.3 to 0.4)	≥0.07
RS FC z-Degree					
Visual network	0.1 (-0.3 to 0.6)	0.3 (0.0 to 0.7)	0.1 (-0.3 to 0.5)	0.3 (-0.1 to 0.8)	≥0.80
Somatomotor network	0.0 (-0.4 to 0.4)	0.2 (-0.2 to 0.5)	0.1 (-0.3 to 0.5)	0.1 (-0.3 to 0.5)	≥0.89
Dorsal attention network	0.0 (-0.3 to 0.4)	-0.1 (-0.4 to 0.2)	0.0 (-0.3 to 0.3)	-0.1 (-0.5 to 0.3)	≥0.88
Ventral attention network	0.1 (-0.4 to 0.5)	-0.2 (-0.7 to 0.2)	-0.4 (-0.9 to 0.0)	-0.3 (-0.8 to 0.2)	≥0.32
Limbic network	0.0 (-0.3 to 0.5)	0.0 (-0.3 to 0.4)	0.0 (-0.3 to 0.4)	0.0 (-0.5 to 0.4)	≥0.90
Frontoparietal network	0.1 (-0.3 to 0.4)	-0.5 (-0.8 to -0.2)	-0.6 (-0.9 to -0.3)	-0.7 (-1.1 to -0.4)	**0.045**[a,d]

Default-mode network	0.1 (-0.3 to 0.4)	-0.6 (-0.9 to -0.3)	-0.4 (-0.7 to -0.1)	-0.3 (-0.7 to 0.0)	**0.045[e]**

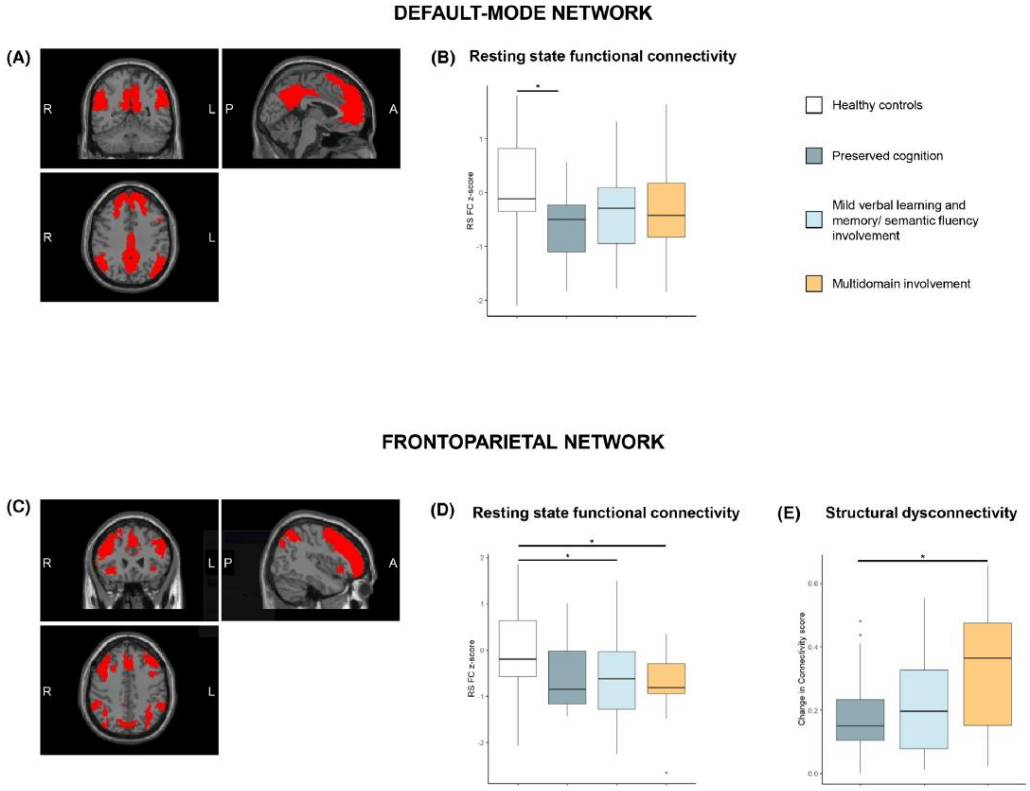
Comparisons performed by age- and sex-adjusted linear models. FDR correction was applied to account for the overall number of tests.

Letters indicate significant differences as follows: [a]HC vs MI; [b]PC vs MI; [c]MVS vs MI; [d]HC vs MVS; [e]HC vs PC.

Abbreviations: HC=healthy controls; FDR=false discovery rate; GM=gray matter; LV=lesion volume; MI=multidomain involvement; MVS=mild verbal learning and memory/semantic fluency involvement; N=normalized; PC=preserved cognition; RS FC=resting state functional connectivity; WM=white matter; z=z-score.

Structural disconnectivity measures. No significant differences in metrics of structural disconnectivity were found between pediatric MS patients with PC and those with MVS. Structural disconnection within the dorsal attention network was significantly more severe in MI patients compared with patients with PC (FDR-p = 0.045) and MVS (FDR-p = 0.045). Additionally, when compared to the PC phenotype, the MI phenotype demonstrated more severe structural disconnectivity within the somatomotor (FDR-p = 0.045), limbic (FDR-p = 0.046) and frontoparietal (FDR-p = 0.045) networks (Table 3) (Figure 3).

Figure 3.13. Differences in structural and functional MRI metrics between cognitive phenotypes (Mistri et al., 2024). (A) Illustrates the defautl-mode network. (B) Boxplot of participants' resting state (RS) functional connectivity (FC) degree within the defautl-mode network. (C) Illustrates the frontoparietal network. Boxplot of participants' RS FC degree (D) and structural dysconnectivity (E) within the frontoparietal network. For each variable the horizontal line represents the median. Asterisks indicate statistically significant differences (** $p < 0.01$; * $p < 0.05$).



RS FC network differences across cognitive phenotypes. Compared with HC, pediatric MS patients with PC exhibited significantly lower RS FC within the DMN (FDR-p = 0.045) (Figure 3), while patients with MVS (FDR-p = 0.045) and MI (FDR-p = 0.045) showed lower mean RS FC z-degree within the FPN (Figure 3). No significant differences in RS FC z-degree were observed among cognitive phenotype in any of the seven networks analyzed (Table 3).

Discussion

By employing the K-means clustering algorithm, we identified three distinct cognitive phenotypes corresponding to different degrees of impairment in patients with pediatric MS. Using such an approach, in addition to patients showing intact cognition or global cognitive decline, we could identify a third cognitive profile characterized by mild deficits in verbal learning and memory and semantic fluency. We analyzed structural and functional MRI network metrics and defined the neural substrates of each

cognitive phenotype. By doing so we provided biological evidence to support our classification.

Previous investigations reported a prevalence of cognitive impairment in pediatric MS ranging from 22% to 35% (Portaccio *et al.*, 2021a), however in this study we found that 63% of patients exhibited cognitive deficit. This result may be related to the presence of patients exhibiting subtle deficits in verbal learning and memory and semantic fluency (n = 28; 38%) that may elude the current classification of cognitive impairment.

We identified three cognitive phenotypes in a group of 73 pediatric MS patients through the application of K-Means cluster analysis. The first cognitive phenotype, PC (37% of the sample), showed the most intact cognitive profile and was characterized by a lower level of physical disability and a higher intelligence quotient than patients with the most impaired neuropsychological profile, namely MI. MRI studies performed on healthy children and adolescents demonstrated a positive correlation between general intelligence and WM integrity (Deary, 2012), as well as higher cortical thickness in the frontal, temporal, parietal and occipital regions (Deary, 2012). Previous research in pediatric MS showed that higher intelligence quotient was associated with preservation of GM volume in the precuneus, cingulate cortex, frontal and temporal regions (De Meo *et al*, 2019a). In line with these results, our investigation revealed no significant differences in normalized brain volumes between HCs and the PC phenotype, suggesting that brain structural abnormalities in these patients are limited. On the other hand, PC patients had a significant reduction of RS FC within the DMN, a result consistent with a previous study showing decreased RS FC between the DMN and the visual network in pediatric MS patients with no or minimal disability (Giorgio *et al*, 2017). Studies on adult MS patients (Rocca *et al*, 2018c) have shown that WM damage may determine reduced RS FC within the DMN in the first phases of the disease, whereas, as disease progresses, RS FC may increase and reflect a maladaptive process potentially contributing to cognitive decline (Meijer *et al*, 2017). In our study, no significant differences in DMN RS FC were observed between HC and pediatric MS patients with MVS and MI. In such cases, it is tempting to speculate that the two effects (decreased DMN connectivity due to MS-related WM damage and increased RS FC, indicating maladaptive processes) outweighed each other, resulting in no detectable

changes. Previous studies in pediatric MS reported an association between cognitive impairment and both increased (Cacciaguerra *et al.*, 2024) and decreased (Rocca *et al.*, 2014b) FC in DMN, therefore our results should be interpreted with caution.

The second cluster included 28 patients (38% of the sample) with mild deficits in verbal learning and semantic fluency. Of note, two large cross-sectional investigations (De Meo *et al.*, 2021a; Podda *et al.*, 2021) identified a similar cognitive phenotype in adult MS patients. One possible explanation for the co-occurrence of these deficits is that the ability to retrieve verbal information from long-term memory, which is required for both verbal fluency and verbal memory tasks (Kave & Sapir-Yogev, 2020), was mildly impaired in patients belonging to this cognitive phenotype. This hypothesis is supported by the findings of a previous study (Delgado-Alvarez *et al*, 2020) in which episodic memory dysfunction emerged as one of the most important predictors of semantic fluency impairment in MS. However, semantic fluency is a multifactorial task that also relies on executive function, attention and language abilities (Baldo *et al*, 2006; Biesbroek *et al*, 2021), and this process can fail due to deficits in any of these cognitive components. Furthermore, while early works on memory impairment in MS posited that difficulty retrieving information from long-term memory was the primary cause of this deficit (Chiaravalloti & DeLuca, 2008), subsequent studies suggested that the primary problem lies in the initial learning of information (Chiaravalloti & DeLuca, 2008).

Verbal fluency develops during childhood and adolescence along with ongoing brain maturation (Gonzalez *et al*, 2021), but research into its neural correlates remains relatively limited in children (Gonzalez *et al.*, 2021). Studies performed in adult patients with acute ischemic stroke consistently reported that impairment in semantic fluency was related to structural damage to frontal, temporal and parietal cortical regions (Biesbroek *et al.*, 2021). Verbal memory relies on the medial temporal lobe (memory formation and consolidation) and the frontal and parietal cortices (memory retrieval) retrieval (Cabeza *et al*, 2008), therefore the neural region overlap may indicate that semantic fluency and verbal memory have a common cortical substrate. In line with this hypothesis, we found that pediatric MS patients belonging to the MVS phenotype showed reduced cortical GM volume compared to HC. In addition, the fMRI analysis revealed decreased RS FC within the FPN in patients belonging to this cognitive phenotype. This result is consistent with previous studies showing reduced RS FC in the

FPN in cognitively impaired adult (Cruz-Gomez *et al*, 2014) and pediatric (Rocca *et al.*, 2014c) patients with MS. The FPN is highly integrated with other networks and serves to quickly instantiate new task states through flexible interaction with other processing and control systems (Marek & Dosenbach, 2018; Marek & Dosenbach, 2019). It plays a crucial role in multiple cognitive abilities, including the retrieval of lexical information from long-term memory (Pakhomov *et al*, 2015).

The third cluster, MI, comprised 18 pediatric MS patients representing 25% of the sample. Compared to patients with PC, patients with MI exhibited lower scores on all cognitive tests except the phonemic fluency test, lower intelligence quotient scores and more severe physical disability. The cognitive and clinical profile of these patients reflects the diffuse brain structural damage observed on MRI, affecting all brain regions examined except the hippocampus. Moreover, patients with MI exhibited a significant deterioration of structural connectivity in four of seven cortical networks (somatomotor network, dorsal attention network, limbic network and FPN) compared to patients with PC. Compared to HC, they exhibited reduced RS FC degree within the FPN. This large-scale network may be particularly susceptible to the pathophysiological effects of MS (Cruz-Gomez *et al.*, 2014) and to the failure of WM maturational changes due to pediatric onset (Longoni *et al.*, 2017), potentially affecting the development of cognitive abilities (Portaccio *et al.*, 2021a). The reduced RS FC within the FPN in both MI and MVS, supports this notion and underscores the key role of FPN in MS-related cognitive impairment (Cruz-Gomez *et al.*, 2014; Meijer *et al.*, 2017; Rocca *et al.*, 2014c).

This study has some limitations. First, the cross-sectional design did not allow us to assess the stability and evolution over time of the cognitive phenotypes. Second, although the cohort of patients enrolled in this study is relatively large considering the rarity of pediatric MS and the difficulty to perform MRI studies, the statistical power of subgroup analysis for each cluster may be limited due to the sample size. For the same reasons, an independent validation cohort was not available. Fourth, brain images were acquired using two different scanners. Although the z-scores of MRI metrics were calculated according to the distributions for each scanner, it is possible that the influence of different imaging parameters was not fully accounted for. To address this issue, we used the same parameters for fMRI sequence. Finally, the current results

depend heavily on the statistical approach. Therefore, future studies should assess the reproducibility of these findings from independent datasets.

Our study provides insights into cognitive phenotypes in pediatric MS as well as associated clinical and MRI markers. We observed that two of the three neuropsychological profiles align with the conventional notions of intact cognition and global cognitive impairment. Of note, a third group, characterized by mild deficits in verbal memory and semantic fluency, introduced a novel cognitive phenotype that challenged the dichotomous view of cognitive function in pediatric MS. The identification of distinct cognitive phenotypes and their underlying neurobiology could contribute to the development of more tailored diagnostic and therapeutic approaches. Cognitive training programs tailored according to the patient's cognitive phenotype may offer a promising way to mitigate future cognitive decline (Margoni *et al*, 2023).

4. Inter-network connectivity moderates the protective effect of consolidated resilience in adult and pediatric multiple sclerosis patients

Abstract

Introduction. Consolidated resilience is a multifactorial measure that combines proxies of cognitive reserve (i.e., an individual's total cognitive resources at a given time) and cognitive resilience (i.e., the difference between observed and predicted cognitive performance based on demographic characteristics and brain integrity). Our aim was to assess whether consolidated resilience moderates the association between inter-network functional connectivity and cognition in different age groups of MS patients.

Methods. A total of 268 MS patients (204 adults and 64 pediatric) and 123 age- and sex-matched HCs underwent 3.0T MRI for assessment of T2 lesion volume (LV) and normalized brain volumes. All MS patients were administered an extensive neuropsychological battery. Consolidated resilience scores were computed using a partial least-squares path model combining proxy-based measures of cognitive reserve (years of education and intelligence quotient [IQ]) with residual-based measures of cognitive resilience. Resting state fMRI scans were acquired from a sub-set of 25 adult and 24 pediatric MS patients at baseline and after a median follow-up of 2.4 years. We identified the main cognitive networks (default-mode network [DMN] I and II, left and right working memory network, basal ganglia network, frontoparietal network [FPN], salience network, executive control network and temporoparietal network) by independent component analysis and calculated inter-network connectivity through pairwise correlations. At each time point, we calculated the degree of each network, i.e., the weighted sum of inter-network correlation coefficients, representing strength of connection of the given network with all remaining ones. Linear mixed models were used to evaluate the effect of consolidated resilience and network degree on cognitive decline.

Results. At baseline, no significant differences were found in sex, disease duration and level of physical disability between pediatric and adult MS patients. Adult and

pediatric MS patients showed similar levels of consolidated resilience. Results from linear mixed models showed significant three-way interactions (consolidated resilience x time x network degree) for verbal memory scores (frontoparietal network [$\beta = 1.42$, $p = 0.010$]) in pediatric MS patients, and for attentional/executive scores (salience network [$\beta = 1.42$, $p = 0.010$]; executive control network [$\beta = -0.70$, $p = 0.008$]) in adult MS patients.

Conclusions. The association between higher consolidated resilience and better cognitive outcomes in MS patients is moderated by specific RS FC patterns between cognitive networks, that change with age.

Introduction

Most previous MRI studies have found a correlation between cognitive deficits in RRMS patients and brain T2-hyperintense focal lesions (Dong *et al*, 2022), reduced NAWM integrity (Eijlers *et al*, 2018; Preziosa *et al*, 2016), GM and thalamic atrophy (Calabrese *et al*, 2009; Eijlers *et al.*, 2018; Preziosa *et al.*, 2016); however, the strength of these associations is heterogeneous (Uher *et al*, 2017). In addition, multiple investigations in RRMS (Fulton *et al*, 1999; Hulst *et al*, 2013; Hynčicová *et al*, 2017; Nourbakhsh *et al*, 2016; Zivadinov *et al*, 2001) have not found a link between these structural MRI metrics and cognitive impairment. Over the years, two constructs have been proposed to explain this variability: cognitive reserve and cognitive resilience. Both terms refer to the ability to perform better than expected given the pathological burden and to have a lower risk of progressing to irreversible cognitive impairment (Elman *et al*, 2022). Although these terms have often been used interchangeably, many efforts have been done recently to provide clearer definitions (Kremen *et al*, 2022; Stern *et al*, 2020). Cognitive reserve represents individual's total cognitive resources at a given time. It is a stable, cumulative ability built over lifetime, and indirect proxies of cognitive reserve are IQ and years of education (Kremen *et al.*, 2022). On the other hand, cognitive resilience reflects the capacity to adapt to adverse brain-related changes including brain and hippocampal atrophy (Kremen *et al.*, 2022) and tau accumulation (Bocancea *et al*, 2021). It is the difference between observed and predicted cognitive performance based on demographic, clinical or structural MRI data such as brain volume, hippocampal volume and WM hyperintensity (Reed *et al*, 2010). Alongside with cognitive reserve and cognitive resilience other related constructs have been proposed to describe different aspects of interindividual variability in maintaining cognitive performance. A recent study (Dobyns *et al*, 2023) introduced a theory-driven, multifactorial "consolidated resilience" measure that combines common proxies of cognitive reserve and measures of cognitive resilience. In a sample of 123 older adults, higher consolidated resilience scores were associated with better performance on memory tests by mitigating the detrimental effect of tau accumulation (Dobyns *et al.*, 2023).

In the context of adult MS, substantial evidence (Amato *et al*, 2013; Artemiadis *et al*, 2020; Sumowski *et al*, 2013) shows that higher educational attainment, IQ, and

engagement in cognitively demanding working and leisure activities attenuate the impact of disease-related pathology on cognitive performance. Conversely, studies in POMS patients have yield more inconsistent results. Three investigations have found an association between proxies of cognitive reserve, such as higher IQ (Pastò *et al.*, 2016; Portaccio *et al*, 2020) or parental education (Till *et al.*, 2013), and the maintenance of cognitive health over time, while three other studies did not (Charvet *et al.*, 2014; Hosseini *et al*, 2014; Wallach *et al.*, 2019).

Research examining the interaction between cognitive reserve and MRI measures in predicting cognitive decline in POMS is still at an early stage. Available evidence suggests that cognitively preserved POMS patients, in response to disease-related structural pathology, exhibit increased activation of cognitive-related cortical areas (Akbar *et al.*, 2015a; Akbar *et al.*, 2015b; Barlow-Krelina *et al.*, 2019). In contrast, previous neuroimaging studies have shown that cognitive reserve in adult MS patients protects against cognitive decline by moderating the effects of focal lesions (Sumowski *et al.*, 2013), GM atrophy (Fuchs *et al*, 2019) and thalamic damage (Rocca *et al*, 2018a). Higher cognitive reserve in these patients has been associated with higher activation of the DMN and decreased recruitment of prefrontal regions, suggesting that adult MS patients with lower lifetime intellectual enrichment may require greater cerebral resources to perform the N-Back task, which is a working memory task (Sumowski *et al*, 2009). Cognitive reserve has also been shown to modulate the intrinsic FC of the salience network, which integrates information from the DMN and the FPN (Bizzo *et al*, 2020). In line with this finding, studies in adult healthy subjects demonstrated that a large-scale brain network, which facilitates the communication between highly integrated cerebral regions, subserves cognitive reserve (Conti *et al*, 2021b; Gonzalez-Burgos *et al*, 2020; Marques *et al*, 2016; Stern *et al*, 2007). Cognitive control processes may be involved in the mechanisms of cognitive reserve and cognitive resilience and therefore these constructs may reflect the ability to utilize brain resources in an efficient way (Marques *et al.*, 2016; Stern *et al.*, 2007). Interestingly, the functional activation patterns associated with cognitive reserve seem to change across the lifespan (Habeck *et al*, 2022). In young adults, higher neuropsychological task performance is linked to minimal, yet efficient use of brain resources, while in older adults it requires flexible

and adaptive recruitment of cortical regions to compensate for age-related structural brain changes (Habeck *et al.*, 2022).

Against this background, we aimed to calculate consolidated resilience scores in both adult and pediatric MS patients and to test their association with cognitive changes over time. We also explored the functional mechanisms underlying consolidated resilience by assessing its interaction with measures of inter-network RS FC in predicting cognitive function changes in both pediatric and adult MS patients.

Methods

Participants. This retrospective study included data from a cohort of 440 MS (Thompson *et al*, 2018a) patients and 123 HCs enrolled at the IRCCS San Raffaele Scientific Institute in Milan, Italy. Inclusion criteria were (1) no major systemic, psychiatric, or neurologic disorders (other than MS), (2) no substance abuse, and (3) Italian native speaker. MS patients had to be relapse-free and steroid-free for at least three months, and on stable disease-modifying treatment for at least six months before MRI.

MRI acquisition. At baseline, a total of 268 (203 adult and 65 pediatric MS patients; MS imaging cohort) out of 440 MS patients had complete neuropsychological data and 123 age-, sex-, and education-matched HCs (88 adult HCs and 35 pediatric HCs; HC imaging cohort) underwent brain MRI using two 3.0 T scanners (Achieva [127 MS patients and 47 HCs] and Ingenia [141 MS patients and 76 HCs], Philips Medical Systems, Eindhoven, The Netherlands) within two days of clinical examination. Using the same scanner and protocols, 24 pediatric RRMS patients (age < 18 years) and 25 sex- and disease duration-matched adult RRMS patients (age ≥ 18 years) were assessed again after a median follow-up of 2.4 years (Achieva scanner 14 adult and 20 pediatric MS patients, Ingenia scanner 11 adult and 4 pediatric MS patients; longitudinal cohort). The following acquisitions were obtained using the Achieva scanner: dual-echo turbo spin echo (TR = 2599 ms, TE = 16–80 ms, flip angle = 90°, FOV = 240 mm^2, ETL = 6, matrix = 256 × 256, 44 contiguous axial slices, 3 mm thick), 3D T1-weighted fast field echo (TR = 25, TE = 4.6 ms, flip angle = 30°, FOV = 230 mm^2, matrix = 256 × 256, 220 contiguous axial slices, 0.8 mm thick) and 200 sets of T2*-weighted echo planar

imaging sequence for RS fMRI (TR = 3000 ms, TE = 35 ms, flip angle = 90°, FOV = 240 mm^2, matrix = 128 × 128, 30 contiguous axial slices, 4 mm thick). By using the Ingenia scanner, we included the following acquisitions: 3D FLAIR (TR = 4800 ms, TE = 270 ms, TI = 1650 ms, ETL = 167, FOV = 256 × 256 mm^2, matrix = 256 × 256, 192 contiguous sagittal slices, 1 mm thick), 3D T2-weighted sequence (TR = 2500 ms, TE = 330 ms, ETL = 117, FOV = 256 × 256 mm^2, matrix = 256 × 256, 192 contiguous sagittal slices, 1 mm thick), 3D T1-weighted turbo field echo (TR = 7 ms, TE = 3.2 ms, TI = 1000 ms, flip angle = 8°, FOV = 256 × 256 mm^2, matrix = 256 × 256, 204 contiguous sagittal slices, 1 mm thick) and for adult subjects (i.e. age ≥ 18 years) 320 sets of T2*-weighted echo planar imaging sequence for RS fMRI (TR = 1567 ms, TE = 35 ms, flip angle = 70°, multi-band factor = 2, FOV = 240 × 240 mm^2, matrix = 96 × 96, 48 contiguous axial slices, 3 mm thick), while for children and adolescents 200 sets of T2*-weighted echo planar imaging sequence for RS fMRI (TR = 3000 ms, TE = 35 ms, flip angle = 90°, FOV = 240 mm^2, matrix = 128 × 128, 30 contiguous axial slices, 4 mm thick).

MRI analysis.

Structural MRI analysis. In MS patients scanned with the Achieva scanner, an experienced observer identified T2-hyperintense WM lesions on dual-echo scans, and LV was obtained using a semi-automated local thresholding segmentation technique (Jim 7.0, Xinapse Systems Ltd, Colchester, UK). In MS patients scanned with the Ingenia scanner, LV was calculated from T2-hyperintense WM lesions identified using a fully automated approach. This method was based on a cascade of two 3D patch-wise convolutional neural networks, using 3D fluid-attenuated inversion recovery and 3D T1-weighted MRI sequences as input images (Valverde *et al*, 2017). WM lesions were visually inspected by an experienced observer and corrected if necessary. NBV, NWMV and NCGMV were obtained for each subject using FSL SIENAx2 (Battaglini *et al*, 2018) software on lesion-filled 3D T1-weighted images. Using the FIRST tool (Patenaude *et al.*, 2011a), we calculated the normalized volumes of bilateral hippocampus and thalamus, which are the two primary subcortical substrates of cognitive dysfunction in both pediatric (Fuentes *et al.*, 2012; Rocca *et al.*, 2015d; Till *et al.*, 2011b; Till *et al.*, 2012a) and adult MS patients (Preziosa *et al.*, 2016). For all study participants who underwent MRI, normalized brain volumes were adjusted for

demeaned age, sex and scanner with linear models built on data from HCs, in order to remove the potential confounding effects of these variables (see Supplementary Table 4.1.1). For MS patients only, z-scores of log-transformed T2 LV were calculated separately for each scanner (Achieva and Ingenia) and for each age group (under and over 18 years).

In patients who underwent longitudinal assessment the percentage brain volume change (PBVC) at follow-up was obtained using FSL SIENA software (Jenkinson *et al*, 2012).

RS FC analysis. The CONN toolbox (https://web.conn-toolbox.org/) was utilized to process RS fMRI data. Images were first realigned to the mean of each session using a rigid-body transformation to correct for head movements. Subsequently, RS fMRI images were registered to the lesion-filled 3D T1-weighted scan, normalized to the MNI space using a non-linear transformation, and smoothed with a 6-mm3 Gaussian filter. By using WM principal components as nuisance covariates through the anatomical component-based noise correction method (aCompCor) and the first five cerebro-spinal fluid, denoising was performed. The six rigid motion parameters and their first temporal derivatives as well as any outliers detected by the ART toolbox and spurious effects from the first two time-points, were regressed out from the data. Finally, RS fMRI time series were linearly detrended and band-pass filtered between 0.01-0.1 Hz.

Independent component analysis (ICA) was conducted using the GIFT software (http://mialab.mrn.org/software/gift/) following these steps: (i) concatenation of scans from all participants, (ii) reduction of individual data to lower dimensionality, (iii) group spatial ICA, and (iv) back reconstruction (Calhoun *et al*, 2001). According to the minimum description length criterion (Calhoun *et al.*, 2001) we set the number of group independent components to 40. The statistical reliability of the resulting independent components was assessed with the ICASSO toolbox (Himberg *et al*, 2004). Visual inspection of spatial patterns, frequency analysis of the estimated independent components spectra and a template-matching procedure allowed the removal of components undoubtedly associated with artifacts and the selection of components related to cognitive functions. The remaining components after artifacts removal, identified as functionally relevant cognitive networks, were DMN (I and II), left and right working memory network (Damoiseaux *et al*, 2006), basal ganglia network

(Patenaude *et al.*, 2011a), FPN, salience network (Seeley *et al*, 2007), executive control network and temporoparietal network (Smith *et al*, 2009).

Using the REX toolbox, we obtained a global network mean RS FC z-score for each cognitive network by calculating the mean z-score of voxels within the familywise error-corrected mask of the RS network derived from an age- and scanner-adjusted SPM12 full factorial model.

Temporal association between time courses of each RS network, defined as pairwise Pearson's correlations, was assessed using the MANCOVAN toolbox included in the GIFT software. This allowed to derive an inter-network connectivity matrix for each subject at each time point. For each cognitive network, we calculated the degree, i.e., the weighted sum of inter-network correlation coefficients, representing strength of connection of the given network with all remaining ones.

Clinical and cognitive assessment. All MS patients underwent neurological examination including EDSS score (Kurtzke, 1983) assessment and record of ongoing disease-modifying treatments. Patients followed longitudinally underwent neurological examination at both baseline and follow-up, with changes in disease-modifying treatments and new relapses documented.

All MS patients underwent neuropsychological assessment. Verbal learning and memory were evaluated using the SRT (Portaccio *et al.*, 2009a; Tedone *et al*, 2024), visuospatial learning and memory were assessed with the SPART (Portaccio *et al.*, 2009a; Tedone *et al.*, 2024), attention and IPS were evaluated using the SDMT (Portaccio *et al.*, 2009a; Tedone *et al.*, 2024). In adult patients (age ≥18 years), verbal fluency was examined using the WLG (Tedone *et al.*, 2024), while pediatric patients were assessed with the Semantic Fluency Test (Portaccio *et al.*, 2009a). For each test, z-scores were calculated according to age-appropriate normative data (Portaccio *et al.*, 2009a; Tedone *et al.*, 2024). Patients who obtained a z-score below -1.5 in at least two tests assessing different cognitive domains were classified as cognitively impaired (Amato *et al*, 2018b). Participants who were followed longitudinally were assessed at both baseline and follow-up. Premorbid IQ was estimated using the WISC-IV (Orsini *et al.*, 2012) for patients aged 8 to 15 years, the WAIS-IV (Orsini & Pezzuti, 2013) for patients aged 16 to 17 years, and the Italian version of the National Adult Reading Test (Test di Intelligenza Breve) (Colombo, 2002) for patients aged 18 years and older.

Cognitive factor scores. Using the z-scores of MS patients without cognitive impairment (Amato *et al.*, 2018b), we conducted a factor analysis to identify the latent cognitive abilities assessed by the neuropsychological battery. To determine the appropriate number of factors, a parallel analysis (Horn, 1965) (paran package version 1.5.3 in R-4.4.1) was carried out. Exploratory factor analysis was then performed to determine the latent factor structure using the principal component analysis method followed by "varimax" rotation (psych package version 2.4.3). Further confirmatory factor analysis was completed using the lavaan package version 0.6-18. To determine the validity of the model, the cut-offs values for the fit indices were: Comparative Fit Index (CFI) > 0.95, Tucker-Lewis Index (TLI) > 0.95, Root Mean Square Error of Approximation (RMSEA) < 0.06 and Standardized Root Mean Square Residual (SRMR) < 0.08 (Dobyns *et al.*, 2023; Hu & Bentler, 1999). For each MS patient, the z-scores of the cognitive tests were multiplied by the corresponding standardized factor loading. These weighted z-scores were then summed to create a cognitive factor score for each latent variable (Dobyns *et al.*, 2023).

Residuals-based measures of resilience. We calculated measures of cognitive resilience by regressing cognitive factor scores against age, sex and structural MRI variables including T2 LV, NBV, NCGMV, NWMV, hippocampal and thalamic volumes. The resulting residual were considered measures of cognitive resilience, with higher values indicating better cognitive performance than expected based on demographics and the extent of brain structural damage. These residual terms were then entered into the Partial Least Squares (PLS) path model employed to compute consolidated resilience scores.

Consolidated resilience scores. By employing a multivariate PLS path model (plspm package version 0.5.1 in R-4.4.1) (Sanchez, 2013)we calculated a consolidated resilience score for each MS patient in the imaging cohort. The residual-based measures of resilience and the proxy-based measures of cognitive reserve (years of education and premorbid IQ) were used to build the model. The PLS path model consisted of an inner model and an outer model. The outer model specified the associations between the first-order latent variables and their manifest variables. In this case, it defined the relationship between the residual-based factor of cognitive resilience and the residual terms described above, as well as between the proxy-based factor of cognitive reserve

and years of education and the IQ. The inner model described the relationships between the latent variables (Dobyns *et al.*, 2023). We applied a two-step approach (Sanchez, 2013) to derive the second-order latent variables (consolidated resilience) from the two first-order latent variables. The goodness of fit of the PLS path model was assessed by calculating the average R^2, a value > 0.7 indicated an acceptable fit level (Dobyns *et al.*, 2023; Sanchez, 2013).

Longitudinal evaluation. We evaluated the association between consolidated resilience scores with RS FC networks' degree, reflecting connectivity strength, and longitudinal variations of cognitive factor scores in the longitudinal cohort using linear mixed models. In detail, we first checked for collinearity by performing a correlation analysis between consolidated resilience scores and RS FC measures. Then we employed a series of linear mixed models (package lmerTest version 3.1-3 in R-4.4.1) to evaluate the influence of consolidated resilience on changes in cognitive factor scores. Finally, to evaluate the effect of consolidated resilience and RS FC networks degrees (i.e., the strength of a network's connectivity to all other networks) on changes of cognitive performance over time, we modelled random effects for participant intercepts to account for individual variability, with age and sex as fixed effect covariates. Independent variables were mean-centered. The primary outcome was a significant three-way interaction between consolidated resilience, RS FC degree and time.

Statistical analysis. Demographic and clinical features were compared between groups using the Chi-square test for categorical variables and Two-sample t-test or Mann–Whitney U test for continuous variables. Linear models were used to compare MRI measures between groups. Normality of the distribution was tested with Kolmogorov–Smirnov and Shapiro–Wilk tests. For comparisons of demographic, clinical, cognitive and MRI variables between pediatric and adult MS patients in the longitudinal cohort as well as between these patients' groups and HCs, Benjamini-Hochberg FDR correction was employed.

Results

Participants. A total of 440 MS patients including the MS imaging cohort and the factor analysis cohort (58% female, mean [SD] age=37.2 [14.7] years, median [IQR]

disease duration=7.7 [2.0 – 16.0] years, median [IQR] EDSS=1.5 [1.0 – 3.5], 349 RRMS and 91 progressive MS) had complete neuropsychological test data.

Table 4.1.1 reports the main demographic, clinical, cognitive, and structural MRI characteristics of the HCs and the 268 out of 440 MS patients who underwent MRI assessment at baseline. Compared to HCs, MS patients had lower volumes in all brain regions analyzed (all $p < 0.001$). The division of participants into imaging, factor analysis and longitudinal cohorts is depicted in Figure 4.1.1.

Table 4.1.1. Demographic, clinical, neuropsychological and structural MRI data of the study participants who underwent MRI.

	MS (n=268)	**HC (n=123)**	**p**
Females, No (%)	150 (56)	67 (54)	0.87[a]
Age, median (range), y	36.9 (8.7 to 66.0)	29.5 (8.3 to 65.1)	0.14[b]
Education, median (IQR), y	13 (11 to 15)	13 (11 to 17)	0.54[b]
Disease duration, median (IQR), y	5.0 (1.2 to 14.0)	NA	NA
EDSS, median (IQR)	1.5 (1.0 to 3.0)	NA	NA
MS phenotype: RRMS/ PMS, No (%)	216 (81)/ 52 (19)	NA	NA
Subjects scanned with: S1/ S2, No (%)	127 (47)/ 141 (53)	47 (38)/ 76 (62)	0.11[a]
Verbal memory factor score, mean (SD)	-1.0 (3.0)	NA	NA
Visuospatial memory factor score, mean (SD)	0.0 (2.1)	NA	NA
Attention/executive functions factor score, mean (SD)	-0.4 (1.3)	NA	NA
Consolidated resilience, mean (SD)	0.0 (1.0)	NA	NA
T2-hyperintense Lesion Volume, median (IQR), mL	3.3 (1.0 to 7.9)	NA	NA
Normalized Brain Volume, mean (SD), mL	1568 (60)	1612 (38)	**<0.001[c]**
Normalized WM Volume, mean (SD), mL	725 (43)	748 (25)	**<0.001[c]**
Normalized Cortical GM Volume, mean (SD), mL	634 (42)	652 (30)	**<0.001[c]**
Normalized Hippocampal Volume, mean (SD), mL	10.1 (1.1)	10.6 (0.9)	**<0.001[c]**

Normalized Thalamic Volume, mean (SD), mL	20.9 (2.1)	22.6 (1.2)	**<0.001**[c]

[a]Chi-squared test; [b]Mann-Withney U test; [c]Linear model.

Abbreviations: EDSS=Expanded Disability Status Scale; GM=gray matter; IQR=interquartile range; HC=healthy controls; MS=multiple sclerosis; PMS=progressive multiple sclerosis; RRMS=relapsing-remitting multiple sclerosis; S=scanner; SD=standard deviation; WM=white matter.

Figure 4.1.1. Venn diagrams of the number of participants in each cohort. All 440 MS patients underwent cognitive assessment (MS cohort), 336 of them (MS factor analysis cohort) resulted cognitively preserved and factor analysis was run on their cognitive tests scores to derive factor loadings, which were then used to calculate cognitive factor scores for the entire MS cohort. Consolidated resilience scores were calculated for a subsample of 268 MS patients who underwent MRI (MS imaging cohort). Twenty-four pediatric and 25 sex- and disease duration-matched adult RRMS patients of the MS imaging cohort underwent longitudinal assessment (MS longitudinal cohort) to assess the effect of consolidated resilience on the trajectories of cognitive factor scores and its interaction with RS FC measures. A total of 123 HCs underwent MRI (HC imaging cohort), and normalized brain volumes of the MS imaging cohort were adjusted to control for demographic effects identified in the HC imaging cohort. Finally, 24 pediatric healthy subjects and 28 adult healthy individuals were selected as baseline control group for the MS longitudinal cohort.

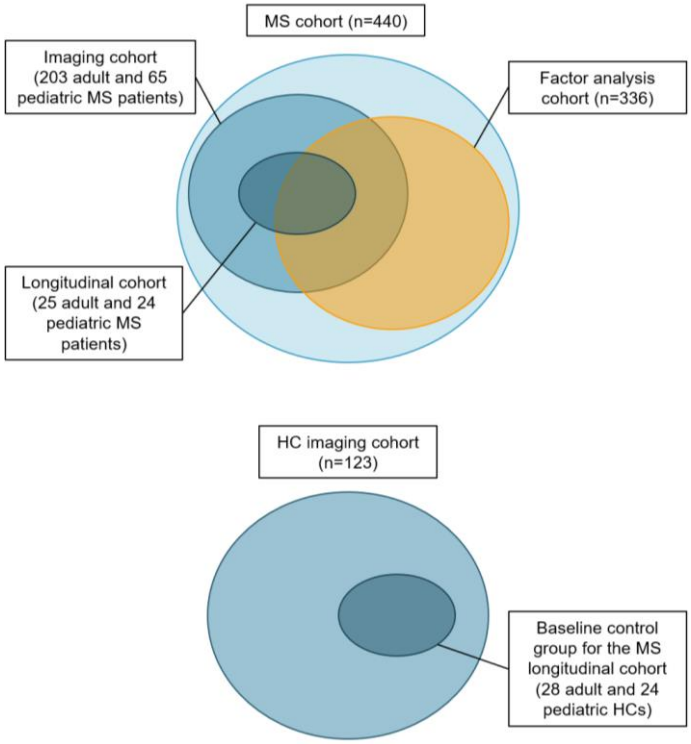


Table 4.1.2 summarizes the demographic, clinical, cognitive, and structural MRI characteristics of adult and pediatric RRMS patients included in the longitudinal cohort, along with their respective control groups.

At baseline, adult and pediatric RRMS patients were matched in term of sex, disease duration and EDSS (FDR-p ≥ 0.66). No significant differences were found in consolidated resilience, verbal memory scores, visuospatial memory scores and attention-executive scores (FDR-p ≥ 0.36). When compared with their respective control groups and with each other, adult and pediatric RRMS patients had no significant differences in structural MRI features and RS fMRI variables (FDR-p ≥ 0.18). The median follow-up duration was 2.0 years for adult RRMS patients and 2.4 years for pediatric patients (FDR-p = 0.84).

At follow-up, EDSS, changes in disease modifying treatment (DMT), cognitive scores, PBVC and T2 LV were not significantly different between adult and pediatric RRMS patients (FDR-p ≥ 0.25) (Table 4.1.2). No patients converted to a SP form of MS.

Table 4.1.2. Main demographic, clinical, neuropsychological, and structural MRI features of MS patients in the longitudinal cohort and HCs.

	Adult RRMS patients (n=25)	**Adult healthy controls (n=28)**	**Pediatric RRMS patients (n=24)**	**Pediatric healthy controls (n=24)**	**FDR-p**
Females, No (%)	15 (60)	14 (50)	15 (62)	15 (46)	≥ 0.80
Baseline Age, median (IQR), y	29.5 (26.6 to 38.2)	30.1 (23.6 to 35.1)	14.8 (11.9 to 16.6)	14.6 (11.9 to 16.6)	**0.004[a]**
Baseline Education, median (IQR), y	14.0 (13.0 to 17.0)	13.0 (13.0 to 16.0)	8.0 (7.0 to 9.0)	8.5 (7.0 to 11.0)	**0.004[a]**
Follow-up duration, median (IQR), y	2.0 (1.6 to 3.1)	NA	2.4 (1.6 to 4.1)	NA	0.84
Baseline Disease duration, median (IQR), y	1.6 (0.4 to 4.4)	NA	0.9 (0.2 to 1.8)	NA	0.66
Baseline EDSS, median (IQR)	1.5 (1.0 to 1.5)	NA	1.0 (1.0 to 1.5)	NA	0.66
FU EDSS, median (IQR)	1.5 (1.0 to 1.5)	NA	1.0 (1.0 to 1.5)	NA	0.25
Baseline DMTs: No treatment, 1st line, 2nd line, No. (%)†	2 (8)/ 18 (72)/ 5 (20)	NA	7 (29)/ 14 (58)/ 3 (13)	NA	0.66
DMT change: yes (%)/no (%)	7 (28)/ 18 (72)	NA	12 (50)/ 12 (50)	NA	0.76
Subjects scanned with: Scanner 1/ Scanner 2, No (%)	14 (56)/ 11 (44)	15 (54)/ 13 (46)	20 (83)/ 4 (17)	18 (75)/ 6 (25)	≥ 0.39
Baseline verbal memory factor score, mean (SD)	-0.4 (1.9)	NA	0.2 (3.2)	NA	0.87
Baseline Visuospatial memory factor score, mean (SD)	-0.2 (1.5)	NA	-0.2 (2.3)	NA	0.96
Baseline Attention-executive factor score, mean (SD)	-0.6 (1.0)	NA	0.1 (1.0)	NA	0.17
Consolidated resilience, mean (SD)	0.0 (0.5)	NA	-0.5 (1.0)	NA	0.36

Baseline T2 Lesion Volume, mean (SD), mL	0.5 (1.3)	NA	0.9 (1.5)	NA	0.68
FU T2 Lesion Volume, mean (SD), mL	0.7 (1.3)	NA	1.4 (1.2)	NA	0.76
Baseline Normalized Brain Volume, mean (SD), mL	1611 (48)	1627 (42)	1616 (57)	1638 (52)	≥ 0.62
PBVC, median (IQR); %	-0.7 (-1.9 to -0.1)	NA	-1.7 (-2.9 to -1.3)	NA	0.24

Comparisons were performed using the Chi-squared test (sex, baseline treatment, scanner and treatment change), Mann-Whitney U test (age, education, disease duration, EDSS, follow-up duration, PBVC), two-sample t test (cognitive scores and consolidated resilience), linear models (NBV), and scanner-adjusted linear models (T2 lesion volume, transformed on a logarithmic scale).

Letters indicate significant differences as follows: [a]adult MS patients *vs* pediatric MS patients.

†Classification of DMTs: first line=interferon beta, glatiramer acetate, teriflunomide, dimethyl fumarate; second line=natalizumab, rituximab, fingolimod, cyclophosphamide, mitoxantrone and ocrelizumab.

Abbreviations: DMT=disease modifying treatment; EDSS=Expanded Disability Status Scale; FU=follow-up; GM=gray matter; IQ=intelligence quotient; IQR=interquartile range; PBVC=percentage brain volume change; RRMS=relapsing-remitting multiple sclerosis; SD=standard deviation; WM=white matter; z=z-score.

Cognitive factor scores. Of the 440 MS patients with cognitive assessment, 366 (60.1% female, mean [SD] age=36.3 [14.7] years, age range 8.7 – 74.0 years, median [IQR] education=13 [10 – 16] years, median [IQR] disease duration=7.0 [1.4 – 15.0] years, median [IQR] EDSS=1.5 [1.0 – 3.0], 305 RRMS and 61 progressive MS) were cognitively preserved and were included in the factor analysis. Exploratory factor analysis was carried out to determine the number of dimensions and the factor structure of the neuropsychological battery. As depicted in Supplementary Figure 4.1.1, the

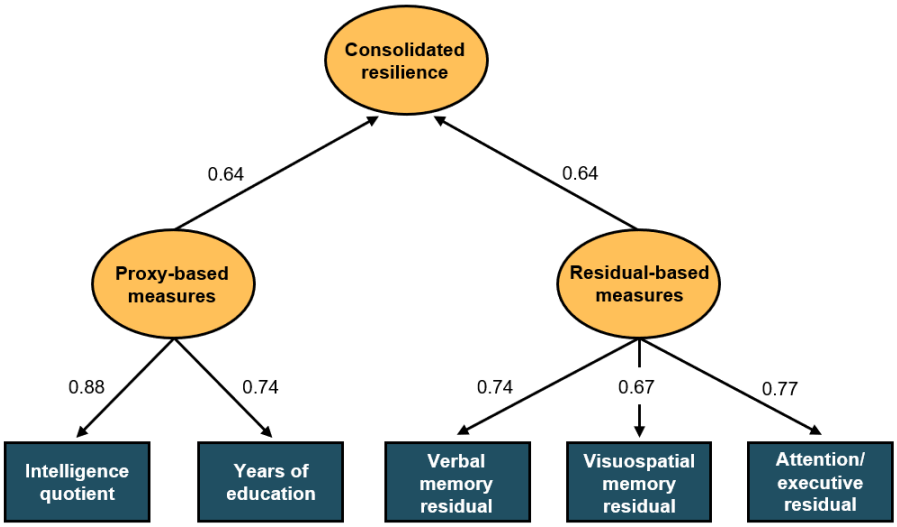
Horn's parallel analysis (Horn, 1965) suggested a three-factor solution. All cognitive measures loaded highly on one of the three factors: (1) Verbal memory, (2) Visuospatial memory and (3) Attention/executive function (see Supplementary Table 4.1.2) which explained 78% of the variance. Confirmatory factor analysis revealed that the three-factor model was a good fit for the data (CFI = 0.99, TLI = 0.99, RMSEA = 0.03, SRMR = 0.04). Finally, the standardized factor loadings were used as weights to calculate verbal memory, visuospatial memory and attention/executive function scores for each MS patient.

Residuals-based measures of resilience. Supplementary Table 4.1.3 reports the results of the regression models assessing the influence of structural MRI measures on cognition. In the group of 268 MS patients who underwent structural MRI, higher scores on tests assessing verbal memory were associated with female sex ($\beta = 0.75$, $p = 0.040$), lower T2 LV ($\beta = 0.49$, $p = 0.030$) and higher thalamic volume ($\beta = 0.22$, $p = 0.037$). Better visuospatial memory was predicted by lower T2 LV ($\beta = 0.31$, $p = 0.026$) and higher NBV ($\beta = 0.01$, $p < 0.001$). Higher attention/executive function scores were associated with female sex ($\beta = 0.39$, $p = 0.007$), lower T2 LV ($\beta = 0.35$, $p < 0.001$) and higher NWMV ($\beta = 0.01$, $p < 0.001$). The residuals of these models were entered in the PLS path model as measures of cognitive resilience.

PLS path model. To generate a consolidated resilience score for each MS patient in the imaging cohort we built a PLS path model using residual-based measures of cognitive resilience and proxy-based measures of cognitive reserve (years of education and IQ). Figure 4.1.2 shows a graphical representation of the PLS path model with loadings. The factor scores for the second-order latent variable were extracted and used as a measure of consolidated resilience. The goodness of fit index was 0.76, indicating a good model fit.

Figure 4.1.2. Results of PLS path modelling in MS patients. The manifest variables are displayed in boxes. The first-order latent variables (proxy-based measures of cognitive reserve and residuals-based measures of cognitive resilience) and the second-order latent variable (consolidated resilience) are depicted as ellipses. Arrows pointing to indicators represent constructs measured in a reflective way. Arrows

pointing to the second-order latent variable indicate that the construct is measured in a formative way. Factor loadings are reported next to the arrows.



Longitudinal evaluation. At baseline, no significant correlations were found between consolidated resilience scores and RS FC measures in either adult or pediatric MS patients, indicating that these variables were not collinear.

At follow-up, in adult RRMS patients, the model predicting attention/executive function showed significant main effects of consolidated resilience ($\beta = 0.88$, $p = 0.030$) and time ($\beta = 0.38$, $p = 0.001$), suggesting that higher consolidated resilience is linked to improvements in attentional/ executive performance over time. The two-way interaction of salience network RS FC degree and time ($\beta = 0.42$, $p = 0.018$) was significant, indicating that the effect of salience network RS FC degree on attentional/ executive factor scores depends on time. The primary outcome, the three-way interaction between consolidated resilience, time and RS FC degree of the salience network ($\beta = 1.42$, $p = 0.010$) was also significant (Supplementary Table 4.1.4), suggesting that the relationship between consolidated resilience and attentional/ executive performance is moderated by the RS FC degree of the salience network. A

further linear mixed model predicting attention/executive function scores revealed a significant effect of consolidated resilience ($\beta = 1.03$, $p = 0.019$) and time ($\beta = 0.47$, $p < 0.001$), as well as a significant three-way interaction between consolidated resilience, time and RS FC degree of the executive control network ($\beta = -0.70$, $p = 0.008$) (Supplementary Table 4.1.4).

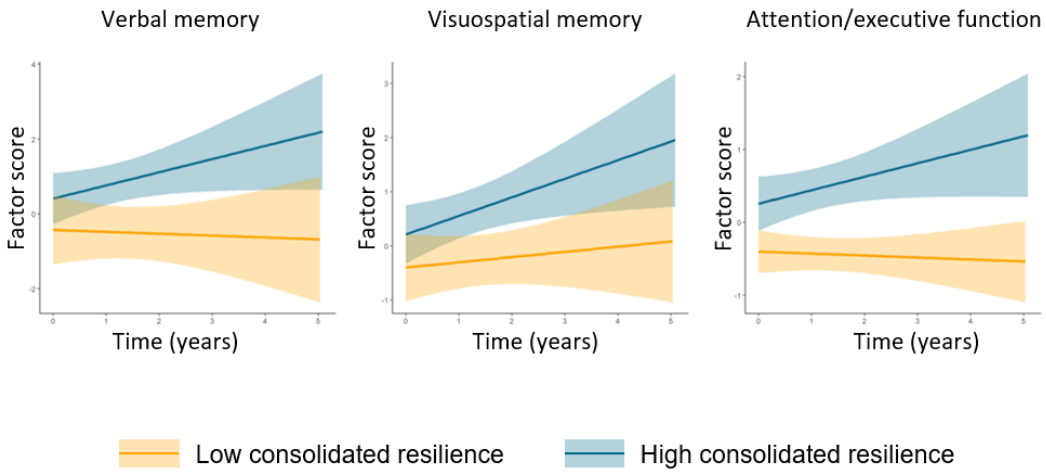
In pediatric RRMS patients, changes in verbal memory scores over time resulted associated with consolidated resilience ($\beta = 1.1$, $p = 0.043$), time ($\beta = 0.67$, $p = 0.045$) and age ($\beta = -0.50$, $p = 0.043$), indicating that higher consolidated resilience is linked to improvements in verbal memory over time. Moreover, the three-way interaction between consolidated resilience, time and RS FC degree of the FPN was significant ($\beta = 0.81$, $p = 0.032$), suggesting that the relationship between consolidated resilience and verbal memory performance is moderated by the FPN inter-network connectivity (Supplementary Table 4.1.5).

The three-way interaction between consolidated resilience, time, and within-network mean RS FC was not significant in any of the linear mixed models tested.

To better understand the relationship between consolidated resilience and changes of cognitive performance over time in the whole group of RRMS patients, we performed linear mixed models that included age, sex and time as covariates. In models predicting verbal memory, visuospatial memory and attention/executive function, consolidated resilience was significant ($p \leq 0.001$) (Figure 4.1.3). Similar results were obtained in linear mixed models performed on adult and pediatric RRMS patients separately. In adult patients, consolidated resilience was a significant predictor of changes in verbal memory ($\beta = 3.36$, $SE = 0.57$, $p < 0.001$) and attention/executive function scores ($\beta = 0.93$, $SE = 0.40$, $p = 0.030$). In pediatric patients, it resulted associated with verbal memory ($\beta = 1.30$, $SE = 0.48$, $p = 0.014$), visuospatial memory ($\beta = 0.79$, $SE = 0.31$, $p = 0.022$) and attention/executive function ($\beta = 0.63$, $SE = 0.15$, $p < 0.001$).

Figure 4.1.3. Consolidated resilience predicts cognitive performance in MS patients. Longitudinal changes in cognitive performance are plotted for lower and higher levels of consolidated resilience defined by values below and above the median. Shaded areas represent the 90% confidence interval. Higher consolidated resilience is

associated with better outcomes in verbal memory (β = 1.37, SE = 0.38, p < 0.001), visuospatial memory (β = 0.87, SE = 0.26, p = 0.002), and attention/executive function (β = 0.62, SE = 0.15, p < 0.001).



Discussion

In a cohort of MS patients aged 8 to 66 years we computed individual measures of consolidated resilience scores from proxies of cognitive reserve and residual-based measures of cognitive resilience using PLS path modelling (Dobyns *et al.*, 2023). In a subsample of 25 adult and 24 pediatric RRMS patients followed longitudinally, we found that higher consolidated resilience predicted better performance in all cognitive functions examined. In adult patients, consolidated resilience interacted with time and RS FC degrees of the salience and executive control network in linear mixed models predicting attention/executive function changes, suggesting that the connectivity strength of these networks with other cognitive networks moderates the relationship between consolidated resilience and decline in attention and executive function. In the pediatric RRMS group, linear mixed models revealed a significant interaction between consolidated resilience, time and RS FC degree of the FPN in predicting verbal memory scores, highlighting the role of the FPN connectivity with others cognitive network in maintaining auditory-verbal memory.

This was the first study in both pediatric and adult MS to apply a measure of consolidated resilience that combines the individual's total cognitive resources (cognitive reserve) with the capacity to adapt to structural brain alterations (cognitive resilience). While consolidated resilience has been previously validated in preclinical Alzheimer's disease, its interaction with RS FC of large-scale brain networks has not been explored before. Although our findings do not offer a complete understanding of the specific functional pathways of consolidated resilience, these results suggest that control processes may play a key role in mitigating the impact of MS-related pathological changes on cognition. In line with this notion, previous studies in healthy subjects indicated that higher cognitive reserve and resilience may imply the ability of the brain to use its resources efficiently through effective communication among cognitive networks (Conti *et al.*, 2021b; Gonzalez-Burgos *et al.*, 2020; Marques *et al.*, 2016; Stern *et al.*, 2007). Moreover, literature in adult MS supports a protective role of cognitive reserve on cognitive decline (Amato *et al.*, 2013; Artemiadis *et al.*, 2020; Sumowski *et al.*, 2013), suggesting that higher cognitive reserve may stem from more efficient network communication (Bizzo *et al.*, 2020; Sumowski *et al.*, 2009). To date, no fMRI investigations specifically focused on cognitive reserve or cognitive resilience have been performed in pediatric MS patients. However, three fMRI studies conducted on POMS patients with normal cognitive function demonstrated that their cognitive performance was sustained by compensatory recruitment of cognitive-related networks (Akbar *et al.*, 2015a; Akbar *et al.*, 2015b; Barlow-Krelina *et al.*, 2019).

To compute residual-based measures of cognitive resilience, we regressed cognitive scores against demographic and structural MRI variables. Consistent with previous works, female sex resulted associated with better performance on tests assessing verbal memory (Tedone *et al*, 2023), attention and executive function (Schoonheim *et al*, 2012). Among structural MRI measures, higher T2 LV correlated with lower scores across all cognitive domains, confirming the substantial contribution of focal WM lesions to cognitive impairment in MS (Rocca *et al*, 2015a). Reduced thalamic volume was linked to poorer verbal memory performance. The thalamus is indeed a relay and processing hub within thalamocortical circuits, playing a well-established role in several cognitive functions, including episodic memory (Amin & Ontaneda, 2021). Reduced NWMV was associated with poorer performance on tests evaluating attention and

executive function. These results are in line with a previous study (Preziosa *et al.,* 2016) demonstrating that WM atrophy is among the predictors of cognitive impairment in MS.

In 25 adult and 24 pediatric RRMS patients, longitudinal changes in verbal memory, visuospatial memory and attention/executive performance over a median follow-up of 2.4 years were found to be dependent on the baseline level of consolidated resilience. Results from linear mixed models showed that patients with higher consolidated resilience were less prone to cognitive decline across all functions examined, suggesting that the combination of cognitive reserve and cognitive resilience can mitigate the effect of MS pathology on cognition not only in adult but also in children and adolescents with MS. In adult RRMS patients, consolidated resilience interacted with the RS FC degree of the salience network and time in a linear mixed model accounting for attention and executive function scores. In this model, both consolidated resilience and time were significant predictors, indicating that higher consolidated resilience was associated with better attention/executive function scores, and that performance of adult RRMS patients generally improved independent of other factors. The RS FC degree of the salience network and its interaction with time were not predictive of cognitive changes. These findings indicate that these variables do not directly influence the performance on tests of attention and executive function. However, the three-way interaction between consolidated resilience, RS FC degree of the salience network and time was significant. This result suggests that increased connectivity between the salience network and other cognitive networks enhances the positive effect of consolidated resilience on mitigating declines of attention and executive function. The salience network is a large-scale brain network anchored by two main cortical hubs: the anterior cingulate and ventral anterior insular cortices. Other regions involved are the amygdala, hypothalamus, ventral striatum, thalamus, and specific brainstem nuclei (Seeley, 2019). The salience network plays a key role in cognitive control and attention by coordinating the switching between the DMN, involved in internally oriented cognition, and FPN, which is responsible for externally oriented attention, based on detection of salient events. This process is essential for efficiently managing cognitive resources and adapting behavioural responses to environmental changes (Menon & Uddin, 2010). Prior studies conducted on healthy subjects (La Cor*te et* al, 2016), patients with Alzheimer's disease (Ewers *et al,* 2021) and patients with cerebrovascular pathology (Bens*on et* al, 2018)

have demonstrated that cognitive resilience is associated with higher RS FC of the hubs in the salience network. Thus, our finding of an association between higher RS FC degree of the salience network and consolidated resilience with better attentional and executive performance over time may reflect compensatory mechanisms involving functional reorganization of cognitive control networks (Van Hoor*en et* al, 2018) to uphold the ability to switch between cognitive states, allowing for a more efficient allocation of brain resources.

In adult RRMS patients, we also found a significant interaction between consolidated resilience, RS FC degree of the executive control network and time in a linear mixed model predicting the performance on tests measuring attention and executive function. The significant effect of consolidated resilience and time were confirmed, while the RS FC degree of the executive control network and its interaction with time were not associated with cognitive changes. Unlike the previous model, the significant three-way interaction had a negative direction. These findings revealed that the protective effect of consolidated resilience on attention and executive function was higher in subjects with a lower RS FC between the executive control network and other cognitive networks. The executive control network includes hubs in the anterior cingulate, dorsolateral frontal and parietal cortices (Seeley *et al.*, 2007). It has been related to integration of sensory and mnemonic information, working memory and executive function that depend on top-down attention processes such as cognitive control and inhibition of automatic responses (Liu *et al*, 2021; Xu *et al*, 2020). In the early stages of the disease, adult RRMS patients who exhibit increased information flow from the executive control network to the salience network are characterized by poorer attentional performance (Wu *et al*, 2020). This finding is in line with another study (Sumowski *et al.*, 2009) suggesting that patients with MS require greater prefrontal recruitment than healthy subjects to maintain cognitive performance. Therefore, increased engagement of the prefrontal cortex may represent a marker of cognitive decline (Sumowski *et al.*, 2009). Our results indicate that higher connectivity between the executive control network and other cognitive networks diminishes the protective role of consolidated resilience, as hyperconnectivity of the executive network may correspond to increased cognitive effort due to reduced cognitive efficiency (Cader *et al*, 2006; Sumowski *et al.*, 2009).

In pediatric RRMS patients, consolidated resilience interacted with the RS FC degree of the FPN and time in a linear mixed model accounting for verbal memory scores. In this model, consolidated resilience, time and age were significant predictors, indicating that higher consolidated resilience was associated with better verbal memory scores, and that performance of pediatric RRMS patients generally improved over time. The RS FC degree of the FPN and its interaction with time were not predictive of cognitive changes, suggesting that these variables do not directly influence the performance on tests of verbal memory. However, the three-way interaction between consolidated resilience, RS FC degree of the FPN and time was significant. It is tempting to speculate that a stronger connectivity of the FPN with other cognitive networks increases the protective effect of consolidated resilience on verbal memory outcomes in pediatric RRMS. The FPN is highly connected with numerous cortical areas and modulates the activity of other cognitive systems depending on task requirements. It plays a key role in top-down attentional control, working memory and episodic verbal memory. In adolescents, the development of cognitive control abilities towards adult-level stability parallels the maturation of the FPN (Luna *et al*, 2015; Solis *et al*, 2021) and functional alterations of this network are major contributors of cognitive dysfunction in pediatric MS patients (Mistri *et al*, 2024). Prior studies (Chen *et al*, 2022; Ersoezlue *et al*, 2023) have indicated that the connectivity of frontal regions within the FPN supports flexible regulation of brain functions underlying the effect of cognitive reserve on cognition. However, these investigations were conducted on adult subjects with WM hyperintensities (Chen *et al.*, 2022) and patients with amyloid pathology (Ersoezlue *et al.*, 2023). Furthermore, the functional mechanisms that support cognitive reserve are known to vary with age (Stern *et al.*, 2007). Therefore, caution is warranted when interpreting our results in RRMS pediatric patients.

Of note, values of RS FC within each cognitive network did not moderate the association between consolidated resilience and cognition in either adult or pediatric RRMS patients, suggesting that the contribution of inter-network connectivity to consolidated resilience is more relevant in these patients.

Although our study has multiple methodological strengths, including the use of a consolidated resilience score that encompasses all dimensions of cognitive reserve and cognitive resilience, the inclusion of RRMS patients across different age groups, and the

analysis of RS FC both within and between cognitive networks, it presents some limitations. First, the relative limited sample size of the longitudinal cohort may have reduced the statistical power. Second, the lack of longitudinal assessment in HCs does not allow to fully characterize changes in RS FC and cognitive performance observed in patients over time. Third, we did not account for the potential confounding effects of depressive symptoms on cognition in MS patients.

In conclusion, our study demonstrated the protective effect of consolidated resilience in both pediatric and adult patients with RRMS. Therefore, a latent factor approach to compute consolidated resilience scores could be employed to identify MS patients at higher risk of progression to cognitive impairment. Our results also highlight the crucial role played by cognitive networks in moderating the relationship between consolidated resilience and cognitive outcomes. In adult patients, higher RS FC degree of the salience network and lower degree of the executive network enhanced the protective effect of consolidated resilience on attention and executive function. In pediatric patients, higher RS FC degree of the FPN moderated the relationship between consolidated resilience and verbal memory. These results suggest that the link between consolidated resilience and cognition is age-dependent and shaped by distinct patterns of connectivity between high-order cognitive networks.

Supplementary Material

Supplementary Table 4.1.1. Effect of scanner and demographic variables on normalized brain volumes.

Dependent variable	Independent variable	β	p	Adjusted R^2
NBV	Age	-3.26	**<0.001**	0.75
	Sex	25.58	**<0.001**	
	Scanner	-68.20	**<0.001**	
	Age*Scanner	2.27	**<0.001**	
NCGMV	Age	-3.43	**<0.001**	0.71
	Age^2	0.07	**<0.001**	
	Sex	18.68	**<0.001**	
	Scanner	19.01	**0.002**	
	Age*Scanner	1.10	**0.008**	0.84
NWMV	Age	0.15	0.32	
	Sex	1.13	0.80	
	Scanner	-119.31	**<0.001**	

Hippocampal volume	Age	0.01	0.50	0.18
	Sex	0.59	**<0.001**	
	Scanner	0.61	**<0.001**	
Thalamic volume	Age	-0.02	**0.003**	0.11
	Sex	0.29	0.21	
	Scanner	-0.49	**0.043**	

Supplementary Table 4.1.2. Standardized factor loadings for the three-factor solution.

	Factor 1	**Factor 2**	**Factor 3**
SRT-lts	**0.92**	0.05	0.06
SRT-cltr	**0.92**	0.05	0.06
SRT-recall	**0.86**	0.06	0.16
SPART	0.06	**0.91**	0.05
SPART-recall	0.06	**0.91**	0.05
SDMT	0.03	0.27	**0.75**
WLG/ SFT	0.17	-0.14	**0.78**

Extraction method: principal component analysis; rotation method: varimax.

Abbreviations: cltr=consistent long-term retrieval; lts=long term storage; SDMT=symbol digit modalities test; SPART=10/36 spatial recall test; SFT=Semantic Fluency Test; SRT=selective reminding test; WLG=word list generation.

Supplementary Table 4.1.3. Multiple regression models assessing the association between cognitive scores and structural MRI variables.

Dependent variable	**Independent variable**	**B**	**p**	**Adjusted R^2**
Verbal memory	Sex	0.75	**0.040**	0.09
	z-T2 lesion volume	0.49	**0.030**	
	Thalamic volume	0.22	**0.037**	
Visuospatial memory	z-T2 lesion volume	0.31	**0.026**	0.11
	Normalized brain volume	0.01	**<0.001**	
Attention/executive function	Sex	0.39	**0.007**	0.17
	z-T2 lesion volume	0.35	**<0.001**	
	Normalized white matter volume	0.01	**<0.001**	

Supplementary Table 4.1.4. Longitudinal predictions of attention and executive function scores through consolidated resilience and resting state functional connectivity network degree in adult RRMS patients.

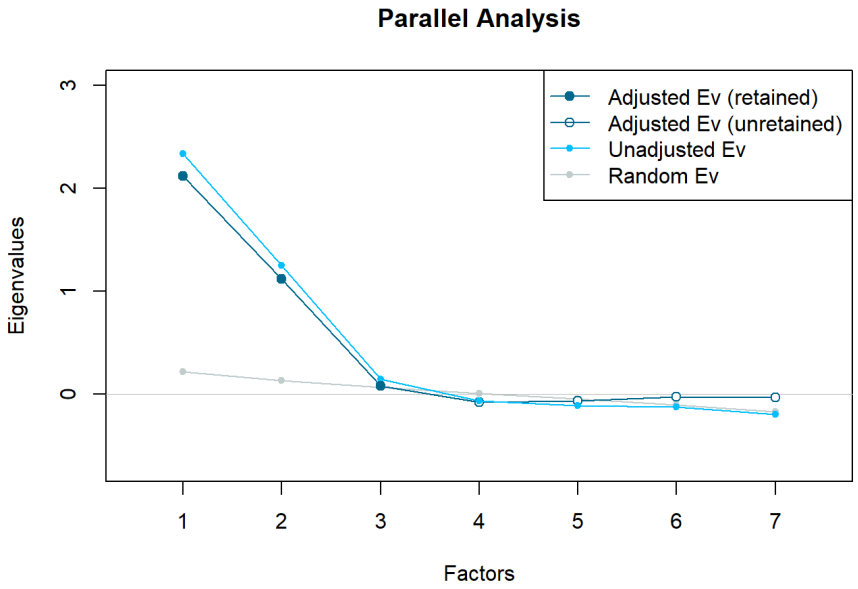
Dependent variable	B	Standard error	p
First model: salience network degree			
Intercept	0.14	0.21	0.51
Salience network degree	0.07	0.22	0.74
Consolidated resilience	0.88	0.38	**0.030**
Time	0.38	0.10	**0.001**
Sex	-0.39	0.33	0.26
Age	0.006	0.02	0.77
Salience network degree*Consolidated resilience	0.007	0.65	0.99
Salience network degree*Time	0.42	0.17	**0.018**
Consolidated resilience *Time	0.004	0.25	0.98
Salience network degree*Consolidated resilience*Time	1.42	0.53	**0.010**
Second model: executive network degree			
Intercept	0.15	0.22	0.52
Executive network degree	-0.31	0.17	0.078
Consolidated resilience	1.03	0.39	**0.019**
Time	0.47	0.10	<**0.001**
Sex	-0.49	0.36	0.19
Age	-0.002	0.02	0.92
Executive network degree*Consolidated resilience	-0.06	0.32	0.85
Executive network degree*Time	-0.13	0.12	0.30
Consolidated resilience *Time	0.33	0.21	0.14
Executive network degree*Consolidated resilience*Time	-0.70	0.24	**0.008**

Supplementary Table 4.1.5. Longitudinal predictions of verbal memory scores through consolidated resilience and resting state functional connectivity network degree in pediatric RRMS patients.

Dependent variable	B	Standard error	p
Intercept	1.28	0.57	**0.037**
Frontoparietal network degree	-0.44	0.51	0.39
Consolidated resilience	1.13	0.53	**0.043**
Time	0.67	0.32	**0.045**
Sex	-2.00	0.97	0.053
Age	-0.50	0.21	**0.029**
Frontoparietal network degree*Consolidated resilience	0.34	0.51	0.51
Frontoparietal network degree*Time	-0.002	0.41	0.99

Consolidated resilience *Time	-0.41	0.25	0.11
Frontoparietal network degree*Consolidated resilience*Time	0.81	0.36	**0.033**

Supplementary Figure 4.1.1. Parallel analysis. The graph was produced using the R package paran.



Abbreviations: EV=eigenvalues.

5. Superficial white matter and cortical gray matter abnormalities in pediatric-onset multiple sclerosis and association with cognitive performance

Abstract

Introduction. Superficial white matter (SWM) is a thin layer of WM underneath the cortex that undergoes myelination throughout childhood and adolescence and is particularly vulnerable to multiple sclerosis (MS) pathology. The aim of this study was to assess the impact of pediatric-onset MS (POMS) on maturational changes in SWM, cortical thickness and cortical volume across the four lobes.

Methods. One hundred and five POMS patients and 34 age- and sex-matched healthy controls (HCs) underwent clinical and MRI assessment. Structural MRI variables included T2 hyperintense lesion volume (LV), cortical volume and thickness. Fractional anisotropy (FA) values, indicative of SWM myelination and microstructural integrity, were obtained using diffusion tensor imaging. MRI scans from 174 HCs from the National Institutes of Health (NIH)-funded MRI Study of Normal Brain Development were included to estimate the influence of demographics on SWM FA, cortical thickness and cortical volume in healthy subjects. Deviation from age-expected values was calculated as z-scores for POMS patients. All patients underwent cognitive assessment, and the association between cognitive scores and MRI measures was estimated using correlation analysis.

Results. POMS patients showed lower than age-expected thickness and volume across the entire cortical mantle ($p \leq 0.002$), as well as reduced FA values in SWM beneath the frontal ($p < 0.001$), temporal ($p < 0.001$) and parietal lobe ($p = 0.017$), but not in the occipital lobe ($p = 0.088$). In POMS patients, higher T2 LV was associated with lower FA of the SWM beneath the temporal ($p < 0.001$) and parietal ($p = 0.009$) lobe ($p = 0.024$). In POMS patients, reduced parietal lobe thickness was linked to verbal memory impairment ($r = 0.25$, $p = 0.019$), while higher FA in SWM beneath the occipital lobe was associated with better information processing speed (IPS) ($r = 0.30$, $p = 0.017$) and visuospatial abilities ($r = 0.30$, $p = 0.014$). In POMS patients no significant

correlation was found between cortical thickness, cortical volume and SWM FA measures and disease duration and Expanded Disability Status Scale (EDSS).

Conclusions. POMS is associated with impaired microstructural integrity of the SWM and reduced cortical thickness and volume. The SWM beneath the occipital lobe appeared preserved, supporting visuospatial processing in POMS.

Introduction

MS is a chronic, inflammatory disease of the CNS that leads to both demyelination and neurodegeneration (Filippi *et al.*, 2018). Although the hallmark of MS pathology is the presence of widespread focal lesions in the WM, structural damage to the GM also plays a significant role in the accumulation of physical disability and cognitive deficits since the early stages of the disease (Filippi *et al*, 2013; Rocca *et al.*, 2015a). Most of the research has focused on patients with AOMS, since POMS (i.e., MS onset before the age of 18) is rare, accounting for 2 - 10% of individuals with MS (Boiko *et al.*, 2002; Charvet *et al.*, 2014; Margoni *et al.*, 2022a; Renoux *et al.*, 2007). However, POMS patients represent a unique group for studying MS, as the disease onset occurs during a critical period of life for maturation processes, affecting the physiological trajectory of development of WM pathways (Longoni *et al.*, 2017) and GM structures (De Meo *et al.*, 2019b). A previous DTI study (Longoni *et al.*, 2017) demonstrated that pediatric MS patients and children with a single demyelinating attack show impaired maturation of WM tracts and progressive loss of WM integrity. Failure of age-expected GM development was observed in a longitudinal study of POMS patients (De Meo *et al.*, 2019b) which detected impaired cortical, cerebellar, thalamic and caudate growth. The Authors found an association between WM lesions and the volume of closely integrated and late-developing GM regions, supporting the hypothesis of an age-dependent regional vulnerability to MS (De Meo *et al.*, 2019b), as observed in WM (Rocca *et al.*, 2015f). However, the absence of this association in several cortical areas confirmed the existence of an early primary involvement of GM (De Meo *et al.*, 2019b).

Superficial WM (SWM) is a thin layer of WM underneath the cortex composed of cortico-cortical U-shaped fibers that connect adjacent gyri (Schilling *et al,* 2023; Wu *et al*, 2013). SWM contains most of the brain connections, constitutes the majority of the total WM volume, and its myelination occurs later than most brain regions (Schilling *et al.*, 2023). Due to high inter-subject variability and absence of major tracts in SWM, applying diffusion MRI fiber tractography can be challenging (Schilling *et al.*, 2023), consequently, most previous studies of SWM have focused on regional analyses using automated parcellation techniques (Wu *et al.*, 2013). During development, increased myelination, reflected by higher FA, is observed in SWM beneath the frontal, temporal, parietal and occipital cortices (Ben Bashat *et al*, 2005; Wu *et al.*, 2013). Moreover,

increases in FA over time in children and adolescents correlates with cortical thinning (Tamnes *et al*, 2010), an age-related phenomena possibly linked to selective synaptic pruning (Shaw *et al*, 2008). SWM plays a key role in information processing and integration (Colombo, 2018), and SWM abnormalities have been identified as a relevant substrate of cognitive impairment in several neurologic and psychiatric conditions including Alzheimer's disease (Bigham *et al*, 2022), small vessel disease (Wang *et al*, 2022), autism (Zikopoulos & Barbas, 2010) and schizophrenia (Nazeri *et al*, 2013). Multiple studies in AOMS (Buyukturkoglu *et al*, 2022; Komnenić *et al*, 2024; Luo *et al*, 2024) have reported patterns of reduced microstructural integrity of the SWM that are associated with poor cognitive performance. However, the potential impact of POMS on SWM and cortical development remains unexplored.

This cross-sectional study aimed to investigate the impact of POMS on maturational changes in SWM FA, cortical thickness and cortical volume by comparing them with age-specific reference values and assessing their correlation with T2-hyperintense WM lesions and brain atrophy. In addition, to gain insight into the clinical relevance of impaired maturation of SWM, cortical thickness and cortical volume in POMS, we evaluated their correlation with clinical and cognitive outcomes.

Methods

Participants. A total of 105 patients with pediatric onset (i.e., disease onset before the age of 18) RRMS (Thompson *et al.*, 2018b) underwent clinical, cognitive and MRI evaluation at San Raffaele Hospital (SRH) in Milan, Italy. Inclusion criteria were: (1) absence of primary systemic, neurologic (other than MS) or mental disorders; (2) no use of antidepressant or psychoactive drugs; (3) being relapse- and steroid-free for at least one month prior the clinical, cognitive and MRI examinations. Genetic testing was performed when necessary to exclude leukodystrophies and myelin oligodendrocyte glycoprotein antibody-associated disorders.

Given the difficulty of enrolling large cohorts of pediatric HCs, we included data from two groups of healthy subjects. The first group consisted of 34 sex- and age-matched HCs recruited at SRH, who underwent MRI examination using the same scanners as the POMS patients. The second group included 174 healthy children,

adolescents and young adults from the NIH-funded MRI Study of Normal Brain Development (NIH HC) with MRI (Evans, 2006; Walker *et al*, 2016) and neuropsychological assessments (Waber *et al*, 2007). The NIH HC data were downloaded in October 2023.

Clinical evaluation. Within 48 hours of the MRI, all POMS patients underwent neurological examination with record of ongoing disease-modifying treatments. Physical disability was assessed using the EDSS (Kurtzke, 1983). To estimate IQ, an experienced neuropsychologist administered the WISC (Orsini *et al.*, 2012) to POMS patients aged 6 to 15 years, and the WAIS (Orsini & Pezzuti, 2013) to patients aged ≥ 16 years. Verbal memory was assessed using the SRT, and z-scores were calculated according to age-appropriate normative data (Portaccio *et al.*, 2009a; Tedone *et al.*, 2024). A short-term verbal memory z-score was obtained by averaging the two immediate recall subscores of the SRT (z-SRT-lts and z-SRT-cltr), while the long-term verbal memory z-score corresponded to the SRT delayed recall z-score. Information processing speed was assessed with Digit Symbol Coding (DSC) subtest of the WAIS/WISC, visuospatial abilities were evaluated using the Block design (BD) subtest of the WAIS/WISC, verbal reasoning and concept formation were examined with the Similarities subtest of the WAIS/WISC and, word knowledge and concept formation were assessed with the Vocabulary subtest of the WAIS/WISC. DSC, BD, Similarities and Vocabulary raw scores were age-corrected using Italian normative data and converted into scaled scores with a mean of 10 and a SD of 3 (Orsini & Pezzuti, 2013; Orsini *et al.*, 2012).

In the NIH HC group, IQ was assessed using the WASI (Wechsler, 1999). Verbal memory was evaluated with the California Verbal Learning Test for Children (CVLTC) (Delis *et al.*, 1994) or CVLT-II (Delis *et al.*, 2000), and z-scores were calculated for both the immediate recall (short-term verbal memory) and delayed recall (long-term verbal memory) scores. As was done for the POMS patients, the DSC, BD, Similarities and Vocabulary subtests of the WASI were administered to all subjects and age-corrected scaled scores (mean = 10, SD =3) were calculated based on normative data (Wechsler, 1999). Cognitive data were available in the NIH HC data repository (Waber *et al.*, 2007).

MRI acquisition. All 105 POMS patients and 34 age- and sex-matched SRH HCs underwent brain MRI using two 3.0 T scanners (Achieva [72 POMS patients and 22 HCs] and Ingenia [33 MS patients and 12 HCs], Philips Medical Systems, Eindhoven, The Netherlands). The following acquisitions were obtained using the Achieva scanner: dual-echo turbo spin echo (TR = 2599 ms, TE = 16–80 ms, flip angle = 90°, FOV = 240 mm^2, ETL = 6, matrix = 256 × 256, 44 contiguous axial slices, 3 mm thick), 3D T1-weighted fast field echo (TR = 25, TE = 4.6 ms, flip angle = 30°, FOV = 230 mm^2, matrix = 256 × 256, 220 contiguous axial slices, 0.8 mm thick) and, pulsed-gradient spin echo planar imaging (TR/TE = 8938/58 ms, FOV = 240 × 231 mm^2, acquisition matrix = 112 × 88, slice thickness = 2.3 mm, 55 contiguous axial slices) with SENSE (acceleration factor = 2) and diffusion gradients applied in 35 non-collinear directions. In order to acquire diffusion weighted images two optimized b factors were used (b1= 0, b2 = 900 s/mm^2).

By using the Ingenia scanner, we included the following acquisitions: 3D FLAIR (TR = 4800 ms, TE = 270 ms, TI = 1650 ms, ETL = 167, FOV = 256 × 256 mm^2, matrix = 256 × 256, 192 contiguous sagittal slices, 1 mm thick), 3D T2-weighted sequence (TR = 2500 ms, TE = 330 ms, ETL = 117, FOV = 256 × 256 mm^2, matrix = 256 × 256, 192 contiguous sagittal slices, 1 mm thick), 3D T1-weighted turbo field echo (TR = 7 ms, TE = 3.2 ms, TI = 1000 ms, flip angle = 8°, FOV = 256 × 256 mm^2, matrix = 256 × 256, 204 contiguous sagittal slices, 1 mm thick) and, axial pulsed-gradient spin echo single shot diffusion-weighted echo planar imaging (TE = 78 ms, TR = 5900 ms, FOV = 240 × 233 mm^2, matrix = 112 × 85, pixel size = 2.14 × 2.69 mm, 56 slices, 2.3 mm-thick). Three shells (b-value = 700/1000/2855 s/mm^2) were acquired with diffusion weight along 6/30/60 non-collinear directions and 10 b = 0 volumes were acquired. In addition, three b = 0 volumes with reversed polarity of gradients were acquired for distortion correction.

Brain images of NIH HC were acquired at five centers using 1.5 T scanners (GE Medical Systems, Cleveland, OH [62 subjects] and Siemens, Munich, Germany [112 subjects]) (Walker *et al*, 2016). The following acquisitions were obtained: dual-echo turbo spin echo (TR/TE = 3500/5–119 ms, FOV = 256 × 224 mm^2, matrix size = 256 × 256, flip angle = 90°, ETL = 8, 2-mm-thick axial slices), whole brain 3D T1-weighted radiofrequency spoiled gradient echo sequence (TR = 22–25 ms, TE = 10 – 11 ms, FOV

= 256 × [160 – 180] mm^2, matrix = 256 × 256, flip angle = 30°, slice thickness = 1.0 mm) (Evans, 2006), axial diffusion weighted spin echo echo planar imaging (minimum TR = 3000 ms, minimum TE achievable with full echo acquisition, excitation pulse angle = 90°, FOV, matrix, and slice thickness adjusted to give 3 × 3 × 3 mm voxels, with FOV and matrix adjusted according to head size, 48 – 60 contiguous slices with no slice gaps, b values = 0/1000 along 6 non collinear directions), images were reconstructed at their original resolution, without zero filling or interpolation (Walker *et al.*, 2016).

Conventional MRI analysis. In POMS patients scanned with the Achieva scanner, an experienced observer identified T2-hyperintense WM lesions on dual-echo scans, and LV was obtained using a semi-automated local thresholding segmentation technique (Jim 7.0, Xinapse Systems Ltd, Colchester, UK). In POMS patients scanned with the Ingenia scanner, LV was calculated from T2-hyperintense WM lesions identified using a fully automated approach. This method was based on a cascade of two 3D patch-wise convolutional neural networks, using 3D fluid-attenuated inversion recovery and 3D T1-weighted MRI sequences as input images (Valverde *et al.*, 2017). WM lesions were visually inspected by an experienced observer and corrected if necessary. NBV was obtained for each subject using FSL SIENAx2 software (Battaglini *et al.*, 2018) on lesion-filled 3D T1-weighted images.

Cortical thickness and volume analysis. Cortical surface reconstruction and the calculation of cortical thickness and volume on T1w images were performed using FreeSurfer v7 (version 7.4.1 https://surfer.nmr.mgh.harvard.edu/). Automated linear registration of T1w images to the Talairach space was applied (Talairach & Tournoux, 1988). Then, bias correction was performed on the T1w images based on bias field derived from B1 inhomogeneity, computed using the changes in WM intensity. After skull stripping (Ségonne *et al*, 2007), WM segmentation was performed based on neighboring constraints and signal intensity. The resulting brain mask was examined, and control points were included to correct erroneous WM segmentation. The GM-WM boundary was derived by tessellation of the WM outer surface and adjusted according to the intensity gradient between WM and GM. The pial surface was generated by expanding the WM surface to model the GM-pial boundary. Cortical areas were labelled according to the FreeSurfer's Desikan-Killiany default atlas (Klein & Tourville, 2012).

Cortical thickness of each region in the Desikan-Killiany atlas was estimated by computing the distance between the vertices of the WM and pial surfaces. Then, the values of regions within same lobe were averaged to calculate the mean cortical thickness of each lobe. Finally, we calculated cortical lobe volumes by summing the GM volumes of each region in the Desikan-Killiany atlas based on their corresponding lobe.

Superficial white matter analysis. For all subjects acquired at SRH, the diffusion-weighted images were corrected for off-resonance and eddy current induced distortions, as well as for subject movements, using the Eddy tool within the FSL library (FSL version 6.0.1, www.fmrib.ox.ac.uk). A b0 image with reversed polarity of gradients was not available for DW acquired with the Achieva scanner; therefore, an undistorted b0 image was simulated (Schilling *et al*, 2019) from the 3D T1-weighted sequence to be used for distortion correction.

The present study used the DTI data available in the NIH HC data repository (Walker *et al.*, 2016). For all NIH HC (Walker *et al.*, 2016), diffusion-weighted images were corrected for motion and eddy current distortions (reorientation of the b-matrix for the rotation of the coordinate system) (Rohde *et al*, 2004).

Using the FMRIB's Diffusion Toolbox (FDT tool), the diffusion tensor was estimated in each voxel by linear regression (Basser *et al*, 1994) using the shells at $b \leq 1000$ s/mm^2 and FA maps were derived. Using a six-parameter rigid body transformation, the non-diffusion-weighted volume in the DTI data was registered to the T1w image. The FA values of the SWM were averaged along the direction perpendicular to the WM surface at a depth of 3 mm after applying the rigid transformation. Finally, according to the Desikan-Killiany atlas the FA values of regions within the same lobe were averaged to calculate the mean FA of each lobe.

Statistical analysis. Demographic and clinical features were compared between groups using the Chi-square test for categorical variables and linear models or Mann–Whitney U test for continuous variables. Normality of the distribution was tested with Kolmogorov–Smirnov and Shapiro–Wilk tests.

Age-expected values of cortical thickness, cortical volume and SWM FA were estimated using linear models accounting for sex and acquisition site on HCs. For each subject, these age-expected values were used to calculate z-scores, reflecting the

deviation of the observed measure from the reference population. The z-scores were computed by dividing the difference between the observed measure and the age-, sex- and site-specific expected value by the σ of the linear model. One-sample t-tests were conducted to compare cortical thickness, cortical volume and SWM FA z-scores of POMS patients with the reference value. The association between cortical thickness, cortical volume and SWM FA z-scores and T2 LV and NBV was evaluated with linear models in HCs and POMS patients separately, while accounting for the effect of different scanners. Correlation analysis was performed to assess the association between cortical thickness, cortical volume and SWM FA z-scores and disease duration, EDSS and cognitive variables.

Results

Clinical, cognitive and conventional MRI data. Table 5.1.1 presents the main clinical, cognitive and conventional MRI data from the three study groups. No significant differences were found in age and sex when comparing POMS patients and SRH HCs. Compared with NIH HC, POMS patients had lower IQ ($p < 0.001$) and exhibited poorer performance on all cognitive tests (all $p < 0.038$). Compared with SRH HCs, POMS patients had lower brain volume ($p < 0.001$).

Table 5.1.1. Main demographic, clinical, neuropsychological, and structural MRI features of POMS patients and HCs.

	POMS patients (n = 105)	**SRH HC (n = 34)**	**NIH HC (n = 174)**	**p**
Females, No (%)	71 (68)	17 (50)	89 (51)	0.10[a]
Age, median (IQR), y	15.6 (14.2 to 17.0)	15.3 (12.3 to 18.1)	11.7 (9.4 to 15.4)	0.82[a]
Disease duration, median (IQR), y	1.0 (0.4 to 2.2)	NA	NA	NA
EDSS, median (IQR)	1.0 (1.0 to 1.5)	NA	NA	NA
DMTs: No treatment, 1st line, 2nd line, No. (%)†	36 (34)/ 42 (40)/ 27 (26)	NA	NA	NA
Subjects scanned with: Achieva Scanner/ Ingenia Scanner, No (%)	72 (69)/ 33 (31)	22 (65)/ 12 (35)	NA	NA
IQ, mean (SD)	100.6 (16.4)	NA	111.4 (11.6)	**<0.001[b]**

Short-term verbal memory z-score, mean (SD)	-0.2 (1.1)	NA	0.3 (0.9)	**<0.001[b]**
Long-term verbal memory z-score, mean (SD)	-0.2 (1.1)	NA	0.3 (0.9)	**<0.001[b]**
DSC scaled score, mean (SD)	9.6 (3.6)	NA	10.6 (3.3)	**0.038[b]**
BD scaled score, mean (SD)	10.6 (2.8)	NA	12.0 (2.9)	**0.002[b]**
Vocabulary scaled score, mean (SD)	9.6 (3.5)	NA	11.8 (2.4)	**<0.001[b]**
Similarities scaled score, mean (SD)	9.9 (3.3)	NA	11.9 (2.2)	**<0.001[b]**
T2 LV, mean (SD), mL	5.4 (7.3)	0.0 (0.0)	NA	**<0.001[a]**
NBV, mean (SD), mL	1654 (66)	1690 (69)	NA	**<0.001[a]**

Comparisons were performed using the Chi-squared test (sex), Mann-Whitney U test (age), linear models (cognitive scores) and scanner-adjusted linear models (T2 LV transformed on a logarithmic scale and normalized brain volume).

Letters indicate significant differences as follows: [a]POMS patients *vs* HCs; [b]POMS patients *vs* NIH HCs.

†Classification of DMTs: first line = interferon beta and glatiramer acetate; second line = natalizumab, rituximab, fingolimod, cyclophosphamide, and mitoxantrone.

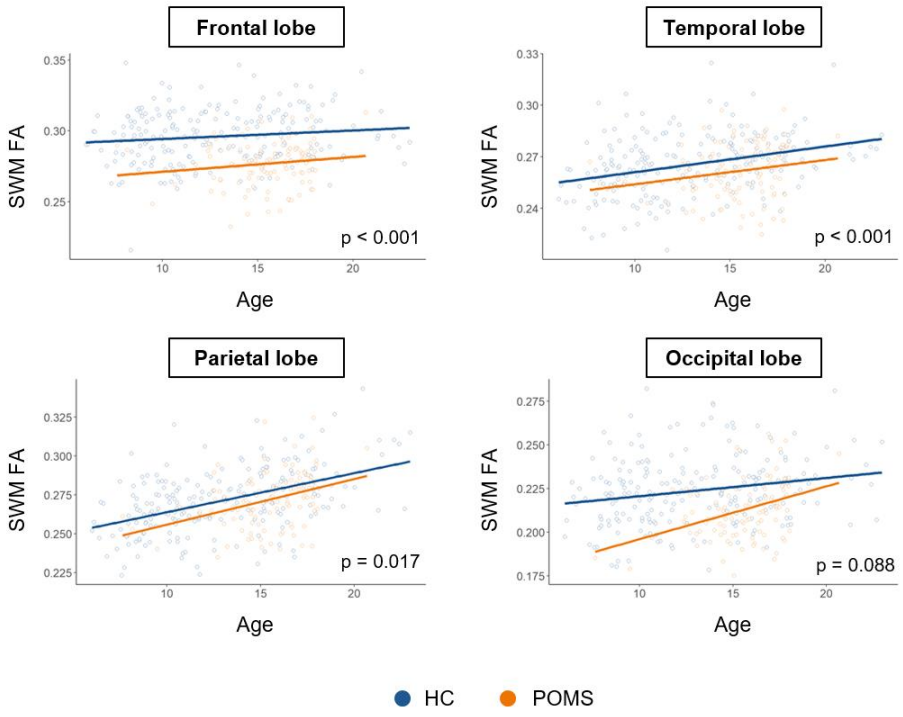
Abbreviations: BD=Block design test; DSC= Digit Symbol Coding test; EDSS=Expanded Disability Status Scale; HC=healthy controls; IQ=intelligence quotient; IQR=interquartile range; NBV=normalized brain volume; POMS=pediatric-onset multiple sclerosis; SD=standard deviation.

Cortical thickness, cortical volume and SWM FA in HC. In the whole HCs group (including both the SRH HCs and NIH HCs), we evaluated the influence of age, sex and site of acquisition on cortical thickness, cortical volume and SWM FA in the different lobes. Cortical thickness and cortical volume were negatively associated with age in all four lobes (β range = -0.0015 to -2.7, all $p < 0.001$) (Supplementary Table 5.1.1 and Supplementary Table 5.1.2). A significant positive effect of male sex was observed in the volumes of all four lobes (β range = 6.4 to 20.5, all $p < 0.001$) (Supplementary Table 5.1.2). FA values were positively correlated with age in all four lobes (β range = 0.0012 to 0.0023, all $p < 0.001$), while a significant positive effect of male sex was detected only in FA values in the occipital lobe (β = 0.006, all $p = 0.012$) (Supplementary Table

5.1.2).

Cortical thickness, cortical volume and SWM FA in POMS. After calculating the z-scores of cortical thickness, cortical volume and SWM FA (representing the standardized difference from the age- and sex-expected value) in all study participants, we ran one-sample t-tests to evaluate the difference between the POMS patients' z-scores and the reference value. The results showed that POMS patients exhibited decreased cortical thickness and cortical volume in all cortical lobes ($p \leq 0.002$). Reduced z-SWM FA values were detected in the frontal ($p < 0.001$), temporal ($p < 0.001$) and parietal lobe ($p = 0.017$), but not in the occipital lobe ($p = 0.088$) (Figure 5.1.1).

Figure 5.1.1 Scatterplots with linear trend of FA of the SWM in POMS and HCs. POMS patients exhibited lower SWM FA than HCs in the frontal, temporal and parietal lobe. P values of the one-sample t-test on age-, sex- and scanner-adjusted SWM FA z-scores are reported.



Correlation analysis. z-SWM FA were significantly correlated with the z-cortical thickness of the corresponding lobe in both HCs (r range = 0.17 to 0.41, $p < 0.001$) and POMS patients (r range = 0.24 to 0.46, $p \leq 0.014$). In HC, significant associations were found between z-SWM FA and z-cortical volume in the temporal ($r = 0.18$, $p = 0.009$), parietal ($r = 0.15$, $p = 0.032$) and occipital lobe ($r = 0.36$, $p < 0.001$) (Table 5.1.2), whereas in POMS a significant correlation was observed only in the occipital lobe ($r = 0.25$, $p = 0.016$) (Table 5.1.3).

In SRH HC no significant association was detected between z-cortical thickness, z-cortical volume and z-SWM FA and NBV (Table 5.1.2). On the other hand, in POMS patients NBV was associated with z-cortical thickness of the frontal ($\beta = 0.007$, $p < 0.001$) and parietal lobe ($\beta = 0.007$, $p < 0.001$). In addition, significant associations between T2 LV and z-cortical volume of the parietal lobe ($\beta = -0.34$, $p = 0.044$) and z-SWM FA of the temporal ($\beta = -0.66$, $p < 0.001$), parietal ($\beta = -0.42$, $p = 0.009$) and occipital lobe ($\beta = -0.32$, $p = 0.024$) were found (Table 5.1.3).

Table 5.1.2. Correlations between FA of the SWM and cortical thickness and volume in HCs.

	z-SWM FA			
	Frontal	**Temporal**	**Parietal**	**Occipital**
NBV	NS	NS	NS	NS
z-cortical thickness				
Frontal	r=0.25, p<0.001	NS	r=0.23, p<0.001	r=0.15, p=0.031
Temporal	NS	r=0.17, p=0.016	r=0.15, p=0.032	NS
Parietal	r=0.26, p<0.001	r=0.24, p<0.001	r=0.38, p<0.001	r=0.28, p<0.001
Occipital	r=0.23, p<0.001	r=0.26, p<0.001	r=0.21, p=0.002	r=0.41, p<0.001
z-cortical volume				
Frontal	NS	r=0.17, p=0.012	NS	NS
Temporal	NS	r=0.18, p=0.009	NS	NS
Parietal	NS	r=0.24, p<0.001	r=0.15, p=0.032	r=0.20, p=0.003
Occipital	r=0.20, p=0.004	r=0.30, p<0.001	r=0.19, p=0.007	r=0.36, p<0.001

Association between z-SWM FA and NBV was evaluated using scanner-adjusted linear models.

Abbreviations: HC=healthy controls; NBV=normalized brain volume; NS=not

significant; SWM=superficial white matter.

Table 5.1.3. Correlations between FA of the SWM and NBV, T2 LV, cortical thickness and volume in POMS.

	z-SWM FA			
	Frontal	**Temporal**	**Parietal**	**Occipital**
NBV	NS	β=0.004, p=0.019	NS	NS
T2 LV	NS	β=-0.66, p<0.001	β =-0.42, p=0.009	β=-0.32, p=0.024
z-cortical thickness				
Frontal	r=0.45, p<0.001	r=0.24, p=0.015	r=0.31, p=0.002	NS
Temporal	r=0.26, p=0.008	r=0.24, p=0.014	r=0.26, p=0.008	NS
Parietal	r=0.36, p<0.001	r=0.31, p=0.001	r=0.46, p<0.001	r=0.26, p=0.007
Occipital	NS	NS	NS	r=0.41 p<0.001
z-cortical volume				
Frontal	NS	NS	NS	NS
Temporal	NS	NS	NS	NS
Parietal	NS	NS	NS	NS
Occipital	NS	NS	NS	r=0.25, p=0.011

Association between z-SWM FA and NBV and logaritmic transformation of T2 LV were evaluated using scanner-adjusted linear models.

Abbreviations: LV= lesion volume; NBV=normalized brain volume; NS=not significant; POMS=pediatric-onset multiple sclerosis; SWM=superficial white matter.

In POMS patients no significant correlation was found between cortical thickness, cortical volume and SWM FA measures and disease duration and EDSS.

In NIH HC, IQ was correlated with z-cortical volume of the frontal ($r = 0.25$, $p < 0.001$), temporal ($r = 0.24$, $p = 0.001$) and parietal lobes ($r = 0.25$, $p < 0.001$). DSC scaled scores were negatively correlated with z-cortical thickness ($r = -0.17$, $p = 0.040$) and z-SWM FA ($r = -0.18$, $p = 0.025$) of the parietal lobe. BD scaled scores were associated with z-cortical volume of the frontal ($r = 0.17$, $p = 0.026$), parietal ($r = 0.19$, $p = 0.009$) and occipital lobes ($r = 0.16$, $p = 0.036$). Similarities scaled scores resulted associated with z-cortical volume of the frontal ($r = 0.20$, $p = 0.009$), temporal ($r = 0.19$,

p = 0.011) and parietal lobes (r = 0.20, p = 0.007). Vocabulary scaled scores were positively associated with z-cortical volume of the frontal (r = 0.22, p = 0.004) and temporal lobes (r = 0.18, p = 0.016). No significant correlations were observed for z-short-term or z-long-term verbal memory.

In POMS patients, similar to NIH HC, IQ was positively correlated with z-cortical volume of the frontal (r = 0.26, p = 0.020) and parietal lobes (r = 0.30, p = 0.006). POMS patients showed unique associations, including a positive correlation between z-long-term verbal memory and z-cortical volume of the parietal lobe (r = 0.25, p = 0.019), as well as between DSC scaled scores and z-SWM FA of the occipital lobe (r = 0.30, p = 0.017), and between BD scaled scores and z-SWM FA of the occipital lobe (r = 0.30, p = 0.014).

Discussion

In this cross-sectional investigation we explored the impact of POMS on developmental changes of SWM FA, cortical thickness and cortical volume. In line with previous studies in healthy children and adolescents (Schilling *et al.*, 2023; Wu *et al.*, 2013) we found a positive association between SWM FA and age and a negative correlation between cortical thickness, cortical volume and age in HC. Higher FA may reflect increasing myelination (Beaulieu, 2002), while reductions of cortical thickness and volumes are possibly related to use-dependent synaptic reorganization, which involves synaptic pruning (Shaw *et al.*, 2008). Male healthy subjects showed higher occipital lobe volumes and SWM FA beneath this region than females, indicating faster maturation of this region (Mutlu *et al*, 2013). Lower than age-expected FA values were observed in SWM beneath the frontal, temporal and parietal lobe in POMS patients. In addition, increased cortical thinning and decreased cortical volume were found across the entire cortical mantle. In healthy subjects, SWM FA was positively correlated with cortical volume in most regions, while in POMS patients this association was only observed in the occipital lobe. In POMS patients higher T2 LV was associated with lower FA of the SWM beneath the temporal, parietal and occipital lobe. When exploring the associations with cognitive outcomes, reduced parietal lobe thickness was linked to verbal memory impairment, while higher FA in SWM beneath the occipital lobe was

associated better IPS and visuospatial abilities.

To the best of our knowledge, this is the first study that investigated the age-related patterns of microstructural abnormalities of SWM in POMS and showed that SWM microstructural damage can be detected since the early stages of POMS as previously reported in AOMS (Komnenić *et al.*, 2024).

In POMS patients, higher T2 LV was negatively associated with FA of the SWM beneath the temporal, parietal, and occipital lobes supporting the notion that SWM may be particularly vulnerable to inflammatory activity (Werring *et al*, 2000). While no significant correlations were observed between T2 LV and cortical thickness or volume in most lobes, an exception was the parietal lobe volume, possibly indicating its vulnerability to downstream effects of WM lesions (Riccitelli *et al*, 2012).

Of note, a linear relationship between SWM FA and cortical volume was observed in HC in most regions. However, in POMS patients, it was only found in the occipital lobe, suggesting a selective susceptibility of maturational processes to POMS pathology (De Meo *et al.*, 2019b). In line with this, no significant difference was found in SWM beneath the occipital lobe between POMS and HCs. These findings suggest that while the occipital lobe follows the physiological trajectory of maturation due to preserved SWM microstructural integrity, it may still be subject to MS-related structural alterations.

No significant correlation was found between disease duration and SWM FA, cortical thickness and cortical volume in POMS. This result contrasts with previous findings in AOMS (Rocca *et al*, 2021a) and POMS (Bonacchi *et al.*, 2021), however, the limited variability of disease duration within our sample may have reduced statistical power, hindering our ability to detect such associations. Indeed, previous investigations (Bonacchi *et al.*, 2021; Rocca *et al.*, 2021a) included patient groups with wider ranges of values that contributed to observing stronger correlations. Similarly, no significant correlation was found between the level of physical disability and SWM FA, cortical thickness and cortical volume in POMS.

The most relevant findings of this study are the correlations between regional SWM FA, cortical thickness and cortical volume and cognitive variables. IQ scores were positively associated with frontal and parietal lobes volumes in both HC and POMS patients. This result is in line with previous studies highlighting the prominent role of

the communication between multimodal areas within frontal and parietal cortices in sustaining reasoning and rational thinking (Jung & Haier, 2007). Consistent with the well-established link between volumetric measures of GM and cognitive abilities in children and adolescents (Torre & Eden, 2019; Wilke *et al*, 2003), we observed a pattern of associations between cognitive outcomes and cortical volumes in healthy subjects, with the only exception being verbal memory scores. We found a positive correlation between long-term verbal memory and z-cortical volume of the parietal lobe in POMS. This result aligns with the association between verbal memory impairments and reduced cortical volume and altered RS FC within the FPN observed in pediatric MS patients (see Chapter 2) (Mistri *et al.*, 2024), suggesting that the parietal cortex may play a crucial role in memory retrieval (Cabeza *et al.*, 2008).

In POMS patients, performance on BD and DSC was positively correlated with the FA of the SWM beneath the occipital lobe, which was the only region where we did not detect significant reductions of FA in the SWM. The occipital lobe is highly involved in in visuospatial abilities (Grill-Spector *et al*, 1998; Polat *et al*, 2012), and in this case its association with a measure of pattern construction and spatial reasoning (BD) indicates that the integrity of the SWM underlying the occipital lobe may play a critical role in supporting processing of visuospatial information. The DSC measures the ability to rapidly match symbols with numbers, which involves IPS, visuospatial abilities, motor speed, memory and executive function (Crowe *et al*, 1999). In this case, the preserved connections in the SWM beneath the occipital lobe likely facilitate fast visual processing supporting the performance on tests that require visual symbol-digit matching.

The present study has some limitations. The cross-sectional design limits the ability to fully explore the age-related changes of SWM FA, cortical volume and cortical thickness in HCs and POMS patients. NIH HC and the POMS sample were not matched in terms of demographic variables, potentially limiting the validity of the comparisons of MRI and cognitive measures. However, MRI variables were adjusted for age and sex effects, while raw cognitive scores were transformed into age-corrected scaled scores based on normative data. MRI scans were obtained with different scanners. Despite the statistical adjustments were made, this variability could still have influenced the results. Finally, in POMS patients the potential confounding effect of fatigue and depression on

cognitive performance were not assessed.

Our results suggest that POMS is associated with impaired microstructural integrity of the SWM and reduced cortical thickness and volume. The SWM beneath the occipital lobe appeared preserved and was found to support visuospatial processing in POMS. Future longitudinal studies are needed to assess SWM changes over time and their association with GM structural measures as well as clinical and cognitive variables.

Supplementary Material

Supplementary Table 5.1.1. Effect of acquisition site and demographic variables on cortical thickness measures in healthy controls.

Lobe	Independent variable	β	p	Adjusted R^2
Frontal	Age	-0.016	<0.001	0.54
	Sex	-0.015	0.28	
	Site 1-7	0.17 to 0.25	< 0.001	
Temporal	Age	-0.015	<0.001	0.49
	Sex	-0.023	0.14	
	Site 1-7	0.27 to 0.41	< 0.001	
Parietal	Age	-0.018	<0.001	0.55
	Sex	-0.006	0.67	
	Site 1-7	0.13 to 0.30	< 0.001	
Occipital	Age	-0.019	<0.001	0.63
	Sex	0.020	0.17	
	Site 1-7	0.30 to 0.43	< 0.001	

The site variable was binarized with Site 1 and 2 representing SRH HC (1 = Achieva scanner, 2 = Ingenia scanner), and Site 3 to 7 representing the acquisition sites of NIH HC.

Supplementary Table 5.1.2. Effect of acquisition site and demographic variables on cortical volume measures in healthy controls.

Lobe	Independent variable	β	p	Adjusted R^2
Frontal	Age	-2.74	<0.001	0.37
	Sex	20.51	<0.001	
	Site 4	11.54	0.08	
	Site 1,2,3,5,6,7	22.43 to 24.76	< 0.025	
Temporal	Age	-1.20	<0.001	0.31
	Sex	10.23	<0.001	
	Site 1-7	16.79 to 21.47	< 0.001	
Parietal	Age	-2.48	<0.001	0.42
	Sex	13.20	<0.001	
	Site 4	6.38	0.14	
	Site 1,2,3,5,6,7	12.84 to 18.59	≤ 0.001	
Occipital	Age	-0.72	<0.001	0.41
	Sex	6.41	<0.001	
	Site 1-7	9.38 to 14.34	<0.001	

The site variable was binarized with Site 1 and 2 representing SRH HC (1 = Achieva scanner, 2 = Ingenia scanner), and Site 3 to 7 representing the acquisition sites of NIH HC.

Supplementary Table 5.1.3. Effect of acquisition site and demographic variables on fractional anisotropy of the superficial white matter in healthy controls.

Lobe	Independent variable	β	p	Adjusted R^2
Frontal	Age	0.0012	<0.001	0.30
	Sex	0.0019	0.39	
	Site 2	-0.0028	0.62	
	Site 4	0.0005	0.91	
	Site 6	0.007	0.09	
	Site 1,3,5,7	0.020 to 0.028	< 0.001	
Temporal	Age	0.0015	< 0.001	0.20
	Sex	0.0032	0.15	
	Site 3	-0.002	0.63	
	Site 5	-0.005	0.15	
	Site 6	-0.003	0.43	
	Site 1,2,4,7	-0.019 to -0.013	≤ 0.019	
Parietal	Age	0.0024	<0.001	0.27
	Sex	0.0039	0.11	
	Site 3	0.0093	0.057	
	Site 4	0.0013	0.80	
	Site 5	0.0037	0.41	

	Site 1,2,6,7	0.012 to 0.19	≤ 0.027	
Occipital	Age	0.0012	<0.001	0.18
	Sex	0.006	0.012	
	Site 1-7	0.017 to 0.029	≤ 0.001	

The site variable was binarized with Site 1 and 2 representing SRH HC (1 = Achieva scanner, 2 = Ingenia scanner), and Site 3 to 7 representing the acquisition sites of NIH HC.

6. Validation and standardization of the Consecutive-Letter Substitution Test in an Italian population sample

Abstract

Introduction. The aim of the present work was to present preliminary results of the validation process and the calculation of normative data for the Italian population for the Consecutive-Letter Substitution Test (CST), a new tool for assessing cognitive processing speed.

Methods. The CST consists of a sheet of paper displaying letters of the alphabet, and the examinee's task is to orally report the consecutive letter of the alphabet as quickly as possible. The CST was administered to a cohort of 298 healthy subjects, along with the Brief Repeatable Battery of Neuropsychological Tests and the Digit Span Backward Task. Normative data for the Italian population were calculated using a regression model on scaled scores. Construct validity and factor structure was explored with correlation analysis and factor analysis. Healthy volunteers were administered the CST at baseline and again after a fifteen-day follow-up to assess test-retest reliability using intra-class correlation coefficients.

Results. A total of 298 healthy volunteers completed the baseline evaluation, and 276 were assessed at follow-up. Regression analysis revealed a negative association between CST scores and age ($\beta = -0.025$, $p = 0.034$), a positive correlation with education ($\beta = 0.24$, $p < 0.001$), and no significant relationship with sex ($\beta = -0.47$, $p = 0.14$). Correlation analysis revealed a positive correlation between CST and all other neuropsychological tests (r range = 0.20 to 0.52, $p \leq 0.0007$), except those assessing visuospatial memory. Exploratory factor analysis identified a two-factor structure. Factor 1 was related to measures of working memory, attention and information processing speed, while Factor 2 reflected memory and verbal fluency. The CST heavily loaded on the first factor ($\lambda = 0.77$), while the Symbol Digit Modalities Test was almost equally associated with both factors ($\lambda = 0.42$ and $\lambda = 0.40$ respectively). Test-retest reliability was excellent (intra-class correlation = 0.928, $p < 0.001$).

Conclusions. Preliminary results suggest that the CST is a valid and reliable tool for assessing cognitive processing speed.

Introduction

Information processing speed (IPS) is defined as the ability to perform simple, repetitive cognitive tasks quickly and fluently (McGrew, 2005). It is considered a complex process that results from the interplay of multiple factors (Costa *et al*, 2017). Slowed IPS is frequently reported in patients suffering from MS, chronic fatigue syndrome and traumatic brain injury (Chiaravalloti *et al*, 2003). Although various IPS tests have been developed, the neuropsychological construct underlying these measures is not well understood (Costa *et al.*, 2017). Over time, three distinct theoretical models have been proposed to describe the neuropsychological basis of IPS (Costa *et al.*, 2017; McGrew, 2005; Salthouse, 1996).

The Cattell-Horn-Carroll hierarchical theory of cognitive abilities (McGrew, 2005) is the most comprehensive and empirically supported model of the hierarchical structure of cognitive functions. It includes one overall g or general ability, 16 broad cognitive abilities and over 80 narrow abilities that are most consistently represented in tests of cognitive and academic abilities. IPS is considered a broad cognitive ability composed of five distinct narrow abilities: perceptual speed (i.e., the ability to quickly search for visual stimuli), rate-of-test-taking (i.e., the ability to quickly execute simple tests), number facility (i.e., the ability to rapidly perform numerical operations), reading speed and writing speed (McGrew, 2005).

Salthouse (Salthouse, 1996) proposed a theory to account for some of the age-related cognitive slowing phenomena. The central hypothesis suggests that ageing is associated with a decline in the speed at which many processing operations can be executed, and this slowdown leads to decline in cognitive functioning. According to this theory, reduced sensorimotor speed (i.e., the ability to rapidly write or draw) influences processing time but does not affect response accuracy. Conversely, perceptual speed is associated with decision accuracy but not decision time. Thus, impaired perceptual speed may impact the quality of cognitive processing and not simply the speed with which the processing outcomes are communicated (Salthouse, 1996).

The most recent theory of IPS was proposed by Costa et al. (Costa *et al.*, 2017) who conducted a review of IPS research in MS. After comparing several definitions and models of IPS, the Authors presented a tri-factor model. In this model, IPS consists of three steps: sensorial, cognitive, and motor processing, each of which can be selectively

impaired by MS-related pathology. Therefore, four profiles of IPS impairment can be identified: sensorial speed impairment (a delay in sensorial speed reduces the time available to perform the subsequent steps), cognitive speed impairment (a delay in cognitive processing speed), motor speed impairment (associated with impaired motor function) and impairments involving more than one function (Costa *et al.*, 2017).

IPS is the most frequently affected cognitive function in MS (Benedict *et al.*, 2020), and neuropsychologists are often asked to assess IPS in these patients. However, the SDMT (Smith, 1982), the most widely used cognitive test to evaluate IPS deficits in these patients (Benedict *et al*, 2017), has been criticized for its lack of validity (Leavitt, 2021). The SDMT is paper-and-pencil test that consists of a page headed by a key that pairs single digits with symbols. The rows below the key contain only symbols and the subject's task is to match the symbol with the corresponding number by quickly scanning back and forth between the key and the rows below. The score is the number of correct responses in 90 seconds (Benedict *et al.*, 2017). Therefore, as highlighted by multiple studies, performance on the test is heavily influenced by visual scanning, verbal fluency and visual memory (Chen *et al*, 2020; Leavitt, 2021).

In a previous study, Pavisian and colleagues (Pavisian *et al*, 2019) administered the oral version of the SDMT to 33 MS patients, while tracking eye movements. The results showed that the number of visits to the key area and the number of times the subjects maintained visual gaze on a symbol in the test area, were the strongest predictors of SDMT scores. This finding supports the notion that visual scanning abilities are heavily involved in the performance on the SDMT. Moreover, the Authors found a strong association between higher scores on the test and fewer visits to the key at the top of the page, suggesting that incidental visual memory also plays a key role in performance on the SDMT (Pavisian *et al.*, 2019). Consistently, a recent investigation employing principal component analysis on cognitive tests scores of two independent cohorts of MS patients, demonstrated that SDMT scores load on multiple factors including attention/IPS, verbal fluency and visual memory (Leavitt *et al*, 2020). Taken together, these findings suggest that the SDMT is a nonspecific cognitive marker (Leavitt, 2021). According to the IPS tri-factor model (Costa *et al.*, 2017), the performance on the SDMT requires all three components of IPS (sensory, cognitive and motor speed), preventing the identification of deficits in specific aspects of IPS (Costa *et al.*, 2017). To

our knowledge, an instrument capable of specifically investigating cognitive processing speed is not available. Therefore, we aim to validate a new clinical tool developed based on the IPS tri-factor model (Costa *et al.*, 2017) that, compared to SDMT, should be more sensitive to cognitive processing speed and less influenced by sensorial and motor speed. The Consecutive-Letter Substitution Test (CST) is a new clinical tool designed to quantify cognitive processing speed. It consists of a sheet of paper displaying letters of the alphabet arranged in a table. The subject's task is to orally report the consecutive letter of the alphabet as quickly as possible. The examinee is not required to quickly scan back and forth as for the SDMT, thus limiting the influence of sensory and motor speed (Chen *et al.*, 2020). The task required by the CST is simple cognitive processing, as reciting the alphabet is an easy and mostly automatic operation (Wheatley & Wegner, 2001) that even patients with major neurocognitive disorders can complete in less than 13 seconds (Mahurin & Cooke, 1996). The simplicity of the task is important because, when more complex measures are used (such as lexical decision speed), it becomes difficult to determine how much of the variability in the scores is due to differences in the speed of performing simple cognitive computations versus differences in semantic knowledge. Moreover, the cognitive speed variable should not be related to sensory and motor speed, otherwise it may not represent the duration of execution of cognitive computations (Salthouse, 1996). Given these premises, the present study aims to: (i) assess the psychometric properties of the CST and (ii) derive its normative values in an Italian population sample.

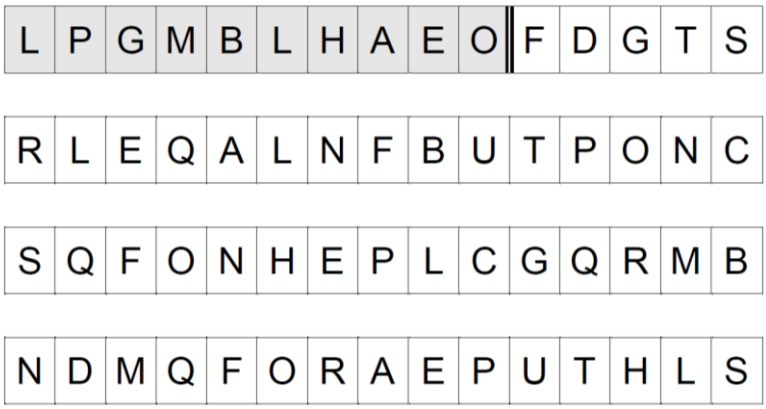
Methods

Participants. The present study aimed to recruit 480 healthy participants, in order to have a sample wide enough to represent the Italian population. Inclusion criteria were: (1) age ≥18 and ≤ 75 years, (2) Italian native speaker and, (3) no history of neurological and/or psychiatric diseases. Exclusion criteria were: (1) history of drug, alcohol or substance abuse and, (2) a score > 9 on the MADRS (Zimmerman *et al*, 2004), which is the lower limit for mild depressive symptoms. Some healthy subjects were asked their willingness to participate to the study by general practitioners that are accredited with "San Raffaele Scientific Institute", while other subjects were recruited through "Vita-

Salute San Raffaele University" staff and students. The sample size was determined based on recommendations from the literature indicating that at least 300 observations are needed for performing factor analysis with low communalities and a small number of factors (MacCallum *et al*, 2001). However, a robust reliability analysis requires a minimum of 400 subjects (Charter, 1999). Moreover, to account for a potential screen failure rate of approximately 6% to 7% (Gigantesco *et al*, 2015) and a potential drop-out rate estimated about 10% (Sakpal, 2010), 80 additional participants were included, resulting in a total sample of 480 subjects.

The Consecutive-Letter Substitution Test. The CST consists of a sheet of paper on which 135 letters of the alphabet are arranged in random order in a table with nine rows and fifteen columns. The CST includes the letters "A", "B", "C", "D", "E", "F", "G", "H", "L", "M", "N", "O", "P", "Q", "R", "S", "T" and "U", while the letters "I", "J", "K", "V", "W", "X", "Y" and "Z" were excluded to avoid ambiguous items. The subject's task is to orally report the consecutive letter of the alphabet as quickly as possible and move to the next one. The examinee is timed to determine how many correct responses can be made in 90 seconds. The first ten items are administered without a time limit to familiarize the examinee with the task before proceeding to the actual test (Figure 6.1.1).

Figure 6.1.1. Faux stimuli for the Consecutive-Letter Substitution Test (CST)



Neuropsychological evaluation. At baseline the cognitive assessment included the CST, the Digit Span Backward Task (Monaco *et al*, 2013), and the BRB-N (Tedone *et al.*, 2024). Moreover, the MADRS (Zimmerman *et al.*, 2004) was administered to detect the presence of depressive symptoms that could affect cognitive performance. Fifteen days after the baseline evaluation, the CST was re-administered.

Statistical analysis. All analyses were performed using R-4.0.3. The condition of a normal distribution was verified using the Kolmogorov-Smirnov and Shapiro-Wilk. BRB-Ns' z-scores and Digit Span Backward Task age- sex, and education-adjusted scores were calculated based on normative data (Monaco *et al.*, 2013; Tedone *et al.*, 2024).

To calculate regression-based normative values, we transformed all CST raw scores into scaled scores with mean of 10 and a SD of 3, based on their fractional ranks (Templeton, 2011). Linear regression models were then performed to assess the influence of demeaned age, age², years of education, and sex on the CST scaled scores. Age was demeaned to avoid multicollinearity, while the term age^2 was included to adjust for non-linear age effects on CST scores (Berrigan *et al*, 2014). The normal distribution of the residuals was evaluated using Kolmogorov-Smirnov and Shapiro-Wilk tests. Predicted CST scaled scores were calculated for each participant by multiplying the β obtained with the regression model for the corresponding demographic feature. Finally, to obtain the CST z-scores, we subtracted the predicted scaled scores from the actual scaled scores and divided the results by the root mean square error (Tedone *et al.*, 2024).

Test-retest reliability was estimated using intra-class correlation coefficients calculated from CST z-scores at baseline and CST z-scores at fifteen-day follow-up.

Convergent validity with centred Digit Span Backward Task scores and BRB-N z-scores was tested with Pearson's correlation. Prior to performing the exploratory factor analysis, to avoid multicollinearity issues and to reduce the number of variables, SRT-lts, SRT-clts and SRT-delayed recall z-scores were averaged, as were the SPART and SPART-delayed recall z-scores, and the PASAT 3" and PASAT 2" z-scores. Kaiser–Meyer–Olkin (KMO) test was performed to evaluate sampling adequacy, and the Bartlett' sphericity tests was conducted to assess whether the correlation matrix differed from an identity matrix. The optimal number of factors was determined with the

parallell analysis (paran package version 1.5.3). To explore the latent factor structure, we performed exploratory factor analysis using the principal component analysis method followed by "promax" rotation (psych package version 2.4.3). Further confirmatory factor analysis was completed using the lavaan package version 0.6-18. To assess the validity of the model, the cut-offs values for the fit indices were CFI > 0.95, TLI > 0.95, RMSEA < 0.06 and SRMR < 0.08 (Hu & Bentler, 1999).

Results

Participants. Figure 6.1.1 shows a flow diagram of the recruitment process of participants. Our goal to recruit 480 healthy subjects has not yet been reached and the study is still ongoing. In total, 323 subjects (67%) were screened for inclusion/exclusion criteria, 298 participants were included, and 276 participants completed the follow-up evaluation. Baseline demographic and cognitive features of the study's participants are reported in Table 6.1.1.

Figure 6.1.2. Recruitment flow chart.

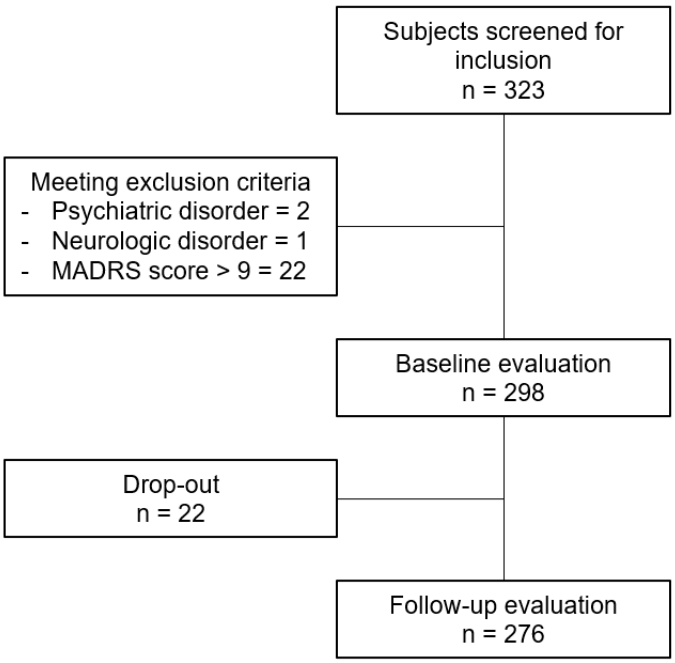


Table 6.1.1. Main demographic and cognitive features of the normative sample.

N.	298
N. of females (%)	168 (56)
Median Age (IQR) [years]	48.1 (30.3 – 59.5)
Median Education (IQR) [years]	13.0 (13.0 – 18.0)
Mean MADRS (SD)	1.6 (2.5)
Mean SRT-lts raw score (SD)	45.6 (13.6)
Mean SRT-cltr raw score (SD)	38.6 (15.6)
Mean SRT-recall raw score (SD)	8.7 (2.3)
Mean SPART raw score (SD)	21.2 (5.0)
Mean SPART-recall raw score (SD)	7.4 (2.2)
Mean SDMT raw score (SD)	54.8 (12.3)
Mean PASAT 3" raw score (SD)[a]	44.1 (11.2)
Mean PASAT 2" raw score (SD)[b]	34.7 (10.0)
Mean WLG raw score (SD)	27.6 (6.2)
Mean Digit Span Backward raw score (SD)	4.9 (1.3)
Mean CST raw score at baseline (SD)	45.5 (15.7)
Mean CST raw score at FU (SD)[c]	53.4 (17.9)

[a]Data available for 292 subjects

[b]Data available for 284 subjects

[c]Data available for 276 subjects

Abbreviations: cltr=consistent long-term retrieval; CST=Consecutive-Letter Substitution Test; FU=follow-up; IQR=interquartile range; lts=long term storage; MADRS=Montgomery-Åsberg Depression Rating Scale; PASAT = paced auditory serial addition test; SD=standard deviation; SDMT=symbol digit modalities test; SPART=10/36 spatial recall test; SRT=selective reminding test; WLG=word list generation.

Normative data and validation analysis. Table 6.1.2 presents the conversion grid of the CST raw scores to scaled scores. By using a regression model, we obtained CST demographically adjusted z-scores by applying the following formula:

CST predicted scaled score = $7.39 – 0.024 \times (Age – 46.2) - 0.0004 \times (Age – 46.2)^2 – 0.47 \times (1 = male; 2 = female) + 0.24 \times (years\ of\ education)$

CST z-score = (CST scaled score – CST predicted scaled score) / RMSE

CST scaled scores were negatively associated with age ($\beta = -0.025$, $p = 0.034$) and positively associated with education ($\beta = 0.24$, $p < 0.001$). Age^2 and sex did not predict CST scaled scores. The residuals of the regression model were normally distributed, and the RMSE was 2.74.

Table 6.1.2. Scaled scores conversion grid.

Scaled score	Raw score
18	> 99
17	79 - 99
16	76 - 78
15	71 - 75

14	63 - 70
13	59 - 62
12	54 - 58
11	49 - 53
10	43 - 48
9	38 - 42
8	34 - 37
7	28 - 33
6	22 - 27
5	19 - 21
4	14 - 18
3	12 - 13
2	< 12

Test-retest reliability analysis revealed excellent reliability, with an intra-class correlation coefficient between the z-scores of the CST at baseline and those at follow-up of 0.928 (95% CI = 0.908 – 0.944, $p < 0.001$).

To assess the convergent validity of CST-z with other cognitive measures, we ran a correlation analysis that showed a significant associations with SRT-z ($r = 0.20$, $p = 0.0007$), SDMT-z ($r = 0.34$, $p < 0.0001$), PASAT-z ($r = 0.52$, $p < 0.0001$), WLG-z ($r = 0.27$, $p < 0.0001$) and Digit Span Backward ($r = 0.40$, $p < 0.0001$).

KMO test's results indicated that SPART-z (0.56) was not suitable for factor analysis, while all other measures had a KMO value > 0.70 (Kaiser, 1974). Bartlett' sphericity tests was significant ($p < 0.0001$). Exploratory factor analysis was conducted to determine the number of dimensions and the factor structure of the neuropsychological battery. As depicted in Figure 6.1.2, the Horn's parallel analysis (Horn, 1965) suggested a two-factor solution. All cognitive measures loaded strongly on one of the two factors (Factor 1: working memory, attention and IPS; Factor 2: verbal memory and verbal fluency), except for the SDMT-z that loaded on both factors (Table 6.1.3). The cumulative proportion of variance explained by the two factors was 60% (Factor 1 = 36%; Factor 2 = 24%). The two factors were positively correlated ($r = 0.48$). Confirmatory factor analysis generated the following fit indices CFI = 0.97, TLI = 0.94, RMSEA = 0.06 and SRMR = 0.03 (Hu & Bentler, 1999).

Figure 6.1.3. Parallel analysis. The graph was produced using the R package paran.

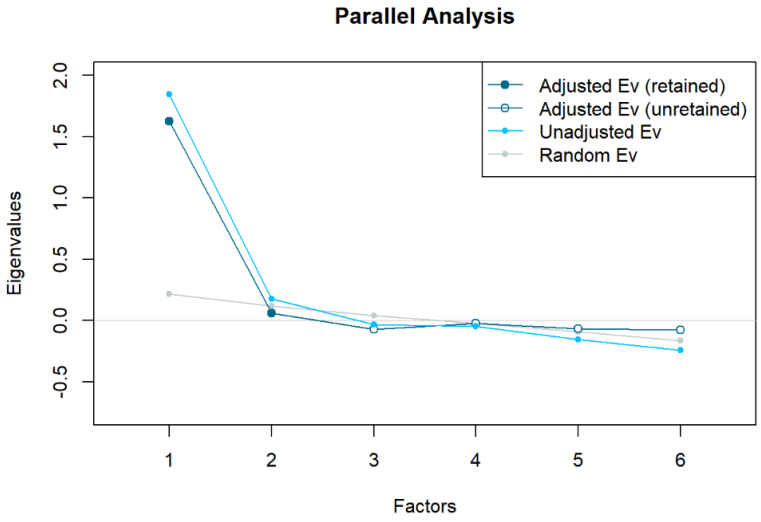


Table 6.1.3. Standardized factor loadings for the two-factor solution.

	Factor 1	**Factor 2**
SRT	-0.26	**0.95**
SDMT	**0.42**	**0.40**
PASAT	**0.74**	0.14
WLG	0.18	**0.61**
Digit Span Backward	**0.89**	-0.30
CST	**0.77**	-0.03

Extraction method: principal component analysis; rotation method: promax.

Abbreviations: CST=Consecutive-Letter Substitution Test; PASAT = paced auditory serial addition test; SDMT=symbol digit modalities test; SPART=10/36 spatial recall test; SRT=selective reminding test; WLG=word list generation.

Discussion

The present work provides preliminary results from the validation study of the CST, a paper-pencil cognitive test designed to assess IPS. The CST differs from the SDMT, the most used test in clinical practice, because it relies less on visual scanning abilities as much, thereby reducing the influence of perceptual and motor speed on results.

CST normative data were computed using a regression-based method with scaled scores. Our sample included 298 healthy subjects aged 18 to 75 years, selected using stringent inclusion and exclusion criteria that minimized the potential influence of even mild depressive symptoms. CST' scaled scores were negatively associated with age, suggesting that the cognitive functions underlying CST performance tend to decline over time. In addition, we found a positive correlation with years of education, indicating that individuals with higher educational attainment generally perform better than those with lower educational levels. No significant association was found with sex, as females and males of comparable age and education had similar scores.

We assessed test-retest reliability of the CST, between baseline and 15 days post-baseline. The intraclass correlation coefficient for the z-scores was 0.928, exceeding the 0.60 threshold for acceptable test-retest reliability (Baumgartner & Chung, 2001). This suggests that despite the practice effect found at follow-up, CST results are consistent across assessments.

The evaluation of the association between the CST z-scores and the other cognitive variables was conducted using correlation analysis and factor analysis. Correlation analysis revealed positive associations between CST scores and all other neuropsychological measures except SPART scores which represent visuospatial memory. Exploratory factor analysis was then performed to determine the factor structure of the neuropsychological battery. Before performing exploratory factor analysis, the conditions for its application were tested using the KMO test of sampling adequacy and the Bartlett's test of sphericity. The SPART scores were excluded due to insufficient sampling adequacy. Then the optimal number of factors was determined with a parallel analysis. The extraction method used in the exploratory factor analysis was the principal component analysis with promax rotation. The results showed that at least two factors are necessary to describe individual differences in performance on the neuropsychological battery. One factor was related to performance on working memory,

attention and IPS tests and accounted for 36% of the variance. The second factor accounted for 24% of the variance and loaded for verbal memory and verbal fluency. CST z-scores loaded heavily on the working memory, attention and IPS factor, while no significant association was found with the verbal memory and verbal fluency factor, suggesting that these latter cognitive functions are not involved in task performance. On the other hand, the SDMT loaded on both factors, possibly indicating that the SDMT involves multiple cognitive functions including memory and verbal fluency (Leavitt, 2021). Benedict et al. (Benedict *et al.*, 2017) hypothesized that IPS, assessed with SDMT, represents a basic cognitive domain that contributes to other cognitive processes including word retrieval and episodic memory. However, a recent investigation (Sandry *et al*, 2021) shifted this perspective by demonstrating a significant contribution of lexical access speed and memory to performance on the SDMT. In the present study, the positive correlation between the two factors was possibly related to the association of the SDMT with both factors. However, confirmatory factor analysis provided mixed results with some indices indicating that the two-factor structure was a good fit for the data (CFI > 0.95 and SRMR < 0.08), while others, despite being very close to the required threshold, did not (TLI = 0.94 and RMSEA = 0.06).

This work has some limitations. At present, we have enrolled 67% of the total sample of 480 healthy individuals. The limited sample size may have impacted the test-retest reliability analysis, which nonetheless showed very robust results, and more importantly the factor analysis. We excluded SPART scores from factor analysis due to sampling inadequacy, precluding the possibility of assessing the relationship between the CST and the SDMT with a measure of visuospatial memory. The limited sample size may also have affected the results of the parallel analysis and exploratory factor analysis when examining the factor structure of the neuropsychological test battery. Moreover, the confirmatory factor analysis showed that the two-factor solution was not a perfect fit for our dataset, confirming that robust factor solutions require a larger sample size.

In conclusion, the CST showed excellent reliability (Baumgartner & Chung, 2001) and a strong convergent validity (Chin & Yao, 2023) with measures of working memory, attention and IPS, demonstrating its robust psychometric proprieties. In line with expectations (Chen *et al.*, 2020; Leavitt, 2021), it provided a more focused

measure of cognitive speed compared to the SDMT, which also loaded on verbal memory and verbal fluency factor. Expanding the sample size will allow for a more reliable analysis of the CST's psychometric properties and its differences with SDMT (Charter, 1999; MacCallum *et al.*, 2001).

7. Discussion

During my PhD, I focused on the study of the MRI substrates of cognition in POMS and on the validation of the CST, a paper-pencil cognitive test to assess IPS.

I started from the identification of the different cognitive phenotypes in pediatric MS and their structural and functional MRI features (**Section 3 - *Structural and functional imaging features of cognitive phenotypes in pediatric multiple sclerosis***). Most previous investigations classified cognitive functioning according to a dichotomous view, namely, preserved *vs* impaired, overlooking the heterogeneity of the cognitive manifestations in pediatric MS (De *Meo et* al., 2021a). Recent studies in adult MS patients (De *Meo et* al., 2021a; P*odda et* al., 2021) deployed machine learning models to identify the recurring patterns of cognitive deficits and demonstrated the existence of four to five cognitive phenotypes ranging from intact cognition to severe and widespread impairment in cognitive functioning (De *Meo et* al., 2021a; P*odda et* al., 2021). However, work extending this cognitive phenotypes framework beyond AOMS was lacking. By employing the K-means clustering algorithm, we identified three distinct cognitive phenotypes corresponding to different degrees of impairment in patients with pediatric MS. We observed that two of the three neuropsychological profiles (PC and MI) align with the conventional notions of intact cognition and global cognitive impairment. Of note, a third cluster representing 38% of the sample, characterized by mild deficits in verbal memory and semantic fluency, introduced a novel cognitive phenotype that challenged the dichotomous view of cognitive function in pediatric MS. Both verbal fluency and verbal memory require ability to retrieve verbal information from long-term memory therefore its impairment may explain the co-occurence of these deficits (Kave & Sapir-Yogev, 2020). Verbal fluency develops during childhood and adolescence along with ongoing brain maturation (Gonz*alez et* al., 2021), and involves frontal, temporal and parietal cortical regions (Biesb*roek et* al., 2021). Verbal memory relies on the medial temporal lobe (memory formation and consolidation) and the frontal and parietal cortices (memory retrieval) retrieval (Cabeza *et al.*, 2008), therefore the neural region overlap may indicate that semantic fluency and verbal memory have a common cortical substrate. In line with this hypothesis, we found

that pediatric MS patients belonging to this cognitive phenotype showed reduced cortical GM volume compared to HC. In addition, the fMRI analysis revealed decreased RS FC within the FPN, which is consistent with previous studies showing reduced RS FC in the FPN in cognitively impaired adult (Cruz-G*omez et* al., 2014) and pediatric (R*occa et* al., 2014c) patients with MS.

Pediatric MS patients with intact cognition represented the 37% of the sample and had higher IQ and lower EDSS than pediatric patients with severe cognitive deficits. Our investigation revealed no significant differences in normalized brain volumes between HCs and the PC phenotype, suggesting that brain structural abnormalities in these patients are limited. On the other hand, PC patients had a significant reduction of RS FC within the DMN. Studies on adult MS patients (R*occa et* al., 2018b) have shown that WM damage may determine reduced RS FC within the DMN in the first phases of the disease, whereas, as disease progresses, RS FC may increase and reflect a maladaptive process potentially contributing to cognitive decline (Me*ijer et* al., 2017).

The prevalence of MI was 25%. Compared to patients with PC, patients with MI exhibited lower scores on all cognitive tests except the phonemic fluency test, lower intelligence quotient scores and more severe physical disability. The cognitive and clinical profile of these patients reflected the diffuse brain structural damage observed on MRI, affecting all brain regions examined except the hippocampus. Moreover, patients with MI exhibited significant alteration of structural connectivity in four of seven cortical networks (somatomotor network, dorsal attention network, limbic network and FPN) compared to patients with PC.

Our study provides insights into cognitive phenotypes in pediatric MS as well as associated clinical and MRI markers (Mar*goni et* al., 2022a).

In **Section 4** (***Inter-network connectivity moderates the protective effect of consolidated resilience in adult and pediatric multiple sclerosis patients***), we tested the association between consolidated resilience, inter-network functional connectivity changes and cognitive decline in different age groups of MS patients. Most previous MRI studies have found a correlation between brain T2-hyperintense focal lesions or other measures of brain structural damage and cognitive deficits in RRMS patients; however, the strength of these associations is heterogeneous. Over the years, two constructs have been proposed to explain this variability: cognitive reserve and

cognitive resilience. A recent study (Do*byns et* al., 2023) introduced and validated a theory-driven, multifactorial "consolidated resilience" measure combining common proxies of cognitive reserve and measures of cognitive resilience, which was shown to mitigate the detrimental effects of tau accumulation in older adults. In the context of adult MS, fMRI investigations showed that higher cognitive reserve in these patients was associated with increased FC of the DMN and decreased recruitment of prefrontal regions, suggesting that adult MS patients with lower lifetime intellectual enrichment may require greater cerebral resources to perform cognitive tasks (Sumo*wski et* al., 2009). Interestingly, the functional activation patterns associated with cognitive reserve in healthy subjects seem to change across the lifespan (Ha*beck et* al., 2022). In young adults, better neuropsychological task performance is linked to minimal, yet efficient use of brain resources, while in older adults it requires flexible and adaptive recruitment of cortical regions to compensate for age-related structural brain changes (Ha*beck et* al., 2022).

In a cohort of MS patients aged 8 to 66 years we computed individual measures of consolidated resilience scores from proxies of cognitive reserve and residual-based measures of cognitive resilience using PLS path modelling (Dobyns *et al.*, 2023). In a subsample of 25 adult and 24 pediatric RRMS patients followed longitudinally, we found that higher consolidated resilience predicted better performance in all cognitive functions examined. These findings suggested that cognitive reserve and cognitive resilience mitigate the effect of MS pathology on cognition not only in adults but also in children and adolescents with MS.

After identifying the main cognitive networks (DMN, left and right working memory network, basal ganglia network, FPN, salience network, executive control network and temporoparietal network) by independent component analysis, we calculated inter-network connectivity through pairwise correlations. At each time point, we calculated the degree of each network, i.e., the weighted sum of inter-network correlation coefficients, representing strength of connection of the given network with all remaining ones. Linear mixed models were used to evaluate the effect of consolidated resilience and network degree on cognitive decline.

In adult MS patients, consolidated resilience interacted with time and higher RS FC degree of the salience network and lower RS FC degree of the executive control

network in linear mixed models predicting higher attention/executive function scores. The salience network manages the allocation of cognitive resources by coordinating the switching between the DMN and the FPN, that are respectively involved in internally and externally oriented cognition (Menon & Uddin, 2010). Thus, our finding of an association between higher RS FC degree of the salience network and consolidated resilience with better attentional and executive performance over time may reflect compensatory mechanisms involving functional reorganization of cognitive control networks (Van Ho*oren et* al., 2018) to uphold the ability to switch between cognitive states, allowing for a more efficient allocation of brain resources. The protective effect of consolidated resilience on attention and executive function was higher in subjects with a lower RS FC between the executive control network and other cognitive networks. The executive control network has been related to integration of sensory and mnemonic information, working memory and executive function that depend on top-down attention processes such as cognitive control and inhibition of automatic responses *(Liu et* al., 2021*; Xu et* al., 2020). Our results indicate that higher connectivity between the executive control network and other cognitive networks diminishes the protective role of consolidated resilience, as hyperconnectivity of the executive network may correspond to increased cognitive effort due to reduced cognitive efficiency (C*ader et* al., 2006; Sumo*wski et* al., 2009).

In the pediatric RRMS group, linear mixed models revealed a significant interaction between consolidated resilience, time and RS FC degree of the FPN in predicting verbal memory scores. The FPN is highly connected with numerous cortical areas and modulates the activity of other cognitive systems depending on task requirements. It plays a key role in top-down attentional control, working memory and episodic verbal memory. In adolescents, the development of cognitive control abilities towards adult-level stability parallels the maturation of the FPN (*Luna et* al., 2015; S*olis et* al., 2021) and functional alterations of this network are major contributors of cognitive dysfunction in pediatric MS patients (Mi*stri et* al., 2024).

Our study demonstrated the protective effect of consolidated resilience in both pediatric and adult patients with RRMS. Therefore, a latent factor approach to compute consolidated resilience scores could be employed to identify MS patients at higher risk of progression to cognitive impairment. Finally our results suggested that he link

between consolidated resilience and cognition is age-dependent and shaped by distinct patterns of connectivity between high-order cognitive networks.

In **Section 5** (***Superficial white matter and cortical gray matter abnormalities in pediatric-onset multiple sclerosis and association with cognitive performance***), we explored the impact of POMS on maturational changes in SWM, cortical thickness and cortical volume across the four lobes. Multiple longitudinal studies in healthy children reported increasing FA in the SWM indicating increasing myelination (Ben Ba*shat et* al., 2005*; Wu et* al., 2013). These increases in FA correlated with cortical thinning (Ta*mnes et* al., 2010), an age-related phenomena linked to selective synaptic pruning (*Shaw et* al., 2008). SWM plays a key role in information processing and integration (Colombo, 2018), and SWM abnormalities have been identified as a relevant substrate of cognitive impairment in several neurologic and psychiatric conditions (Bi*gham et* al., 2022; Na*zeri et* al., 2013; *Wang et* al., 2014; Zikopoulos & Barbas, 2010). Multiple studies in AOMS (Buyukturk*oglu et* al., 2022; Komn*enić et* al., 2024; *Luo et* al., 2024) have reported an association between reduced SWM microstructural integrity and poor cognitive performance, but the presence of such association in POMS patients is still unknown.

In this cross-sectional study including data from 105 POMS patients and 208 HCs (34 HSR HCs and 174 NIH HCs), we detected lower than age-expected values of FA in SWM beneath the frontal, temporal and parietal lobe in POMS patients. In addition, increased cortical thinning and decreased cortical volume were found across the entire cortical mantle. Higher T2 LV was negatively associated with FA of the SWM beneath the temporal, parietal, and occipital lobes supporting the notion that SWM may be particularly vulnerable to inflammatory processes (Miki *et al*, 1998). While no significant correlations were observed between T2 LV and cortical thickness or volume in most lobes, an exception was the parietal lobe volume, possibly indicating its vulnerability to downstream effects of WM lesions (Riccit*elli et* al., 2012). Of note, a linear relationship between SWM FA and cortical volume was observed in HC in most regions. Conversely, in POMS patients, it was only found in the occipital lobe, suggesting a selective susceptibility of maturational processes of this lobe to POMS pathology (De *Meo et* al., 2019b). In line with this, no significant difference was found in SWM beneath the occipital lobe between POMS and HCs. These findings suggested

that while the occipital lobe follows the physiological trajectory of maturation due to preserved SWM microstructural integrity, it may still be subject to MS-related structural alterations.

The most relevant findings of this study are the correlations between regional SWM FA, cortical thickness, cortical volume and cognitive parameters. In POMS patients, performance on BD and DSC was positively correlated with FA of the SWM beneath the occipital lobe, the only region where no significant SWM FA reductions were detected. The occipital lobe is highly involved in in visuospatial abilities (Grill-Spector *et al.*, 1998; Polat *et al.*, 2012), and in this case its association with a measure of pattern construction and spatial reasoning (BD) indicates that the integrity of the SWM underlying the occipital lobe may play a critical role in supporting processing of visuospatial information. The DSC measures the ability to rapidly match symbols with numbers, which involves IPS, visuospatial abilities, motor speed, memory and executive function (Crowe *et al.*, 1999). In this case, the preserved connections in the SWM beneath the occipital lobe likely facilitated fast visual processing supporting the performance on tests that require visual symbol-digit matching.

Our results suggested that POMS is associated with impaired microstructural integrity of the SWM and reduced cortical thickness and volume. The SWM beneath the occipital lobe appeared preserved and was found to support visuospatial processing in POMS. Future longitudinal studies are needed to assess SWM changes over time and their association with GM structural measures, clinical and cognitive variables.

In **Section 6** (***Validation and standardization of the Consecutive-Letter Substitution Test in an Italian population sample***), we provided preliminary results of the validation study of the CST, a new tool for assessing cognitive processing speed. IPS is the ability to perform simple, repetitive cognitive tasks quickly and fluently (McGrew, 2005). IPS is the most frequently affected cognitive function in MS (Bene*dict et* al., 2020), and neuropsychologists are often asked to assess IPS in these patients. However, the SDMT (Smith, 1982), the most widely used cognitive test to evaluate IPS deficits in these patients (Bene*dict et* al., 2017), has been criticized for its lack of validity (Leavitt, 2021) because the performance on the SDMT is heavily influenced by visual scanning abilities, verbal fluency and visual memory (*Chen et* al., 2020; Leavitt, 2021). The CST consists of a sheet of paper displaying letters of the alphabet arranged in a table. The

subject's task is to orally report the consecutive letter of the alphabet as quickly as possible. The examinee is not required to quickly scan back and forth as for the SDMT, thus limiting the influence of visual scanning abilities and visual memory (*Chen et* al., 2020).

CST normative data were computed using a regression-based method with scaled scores. Our sample included 298 healthy subjects aged 18 to 75 years. CST' scaled scores were negatively associated with age, suggesting that the cognitive functions underlying CST performance tend to decline over time. In addition, we found a positive correlation with years of education, indicating that individuals with higher educational attainment generally perform better than those with lower educational levels. No significant association was found with sex, as females and males of comparable age and education had similar scores.

We assessed test-retest reliability of the CST, between baseline and 15 days post-baseline. The intraclass correlation coefficient was 0.928, suggesting that despite the practice effect found at follow-up, CST results were consistent across assessments.

Exploratory factor analysis conducted on neuropsychological tests battery including SRT, SPART, SDMT, WLG, digit span backward task and CST, revealed a two-factor structure. One factor was related to performance on working memory, attention and IPS tests and the second factor loaded for verbal memory and verbal fluency. CST z-scores loaded heavily on the working memory, attention and IPS factor, while no significant association was found with the verbal memory and verbal fluency factor, suggesting that these latter cognitive functions are not involved in task performance. On the other hand, the SDMT loaded on both factors, possibly indicating that the SDMT involves multiple cognitive functions including memory and verbal fluency (Leavitt, 2021). However, confirmatory factor analysis provided mixed results with some indices indicating that the two-factor structure was a good fit for the data (CFI > 0.95 and SRMR < 0.08), while others, despite being very close to the required threshold, did not (TLI = 0.94 and RMSEA = 0.06).

At present, we have enrolled 67% of the total sample of 480 healthy individuals planned. The relatively limited sample size may have impacted the test-retest reliability analysis and the factor analysis (Charter, 1999; MacCa*llum et* al., 2001).

The CST showed excellent reliability (Baumgartner & Chung, 2001) and a strong

convergent validity (Chin & Yao, 2023) with measures of working memory, attention and IPS, demonstrating its robust psychometric proprieties. In line with expectations (*Chen et* al., 2020; Leavitt, 2021), it provided a more focused measure of cognitive speed compared to the SDMT, which also loaded on verbal memory and verbal fluency factor. Expanding the sample size will allow for a more reliable analysis of the CST's psychometric properties and its differences with SDMT (Charter, 1999; MacCa*llum et* al., 2001).

In conclusion, in this PhD project we combined conventional and advanced neuroimaging techniques with cognitive data to elucidate the substrates of cognitive decline and consolidated resilience in POMS. In addition we introduced the CST, a new reliable test for assessing IPS, paving the way for more focused assessments in MS patients.

8. Conclusions

During the present PhD by applying advanced MRI techniques, we gained additional insight into the neuroanatomical basis of cognitive deficit in POMS. Specifically, fMRI provided evidence of the prominent role of the FPN in sustaining verbal memory and verbal fluency performance, as well as in mediating the protective effect of consolidated resilience on verbal memory in pediatric MS patients. The association between consolidated resilience and cognition was found to be age-dependent and shaped by distinct patterns of connectivity between high-order cognitive networks, as demonstrated by the differing results between pediatric and adult MS patients. Using DTI, we explored the maturation process in the SWM in POMS and identified widespread reductions in myelination levels compared to age-expected values, also demonstrating their association with IPS and visuospatial abilities. The application of conventional MRI allowed us to detect lower cortical thickness and volume in POMS compared to HC, as well as the association between reduced brain volumes and poorer cognitive performance in pediatric MS patients. In addition, we provided preliminary evidence of the validity and reliability of the CST, an IPS test that, compared to the SDMT, aims to be less influenced by visual scanning abilities and visuospatial memory.

Future longitudinal studies should evaluate the stability of the cognitive phenotypes and should assess maturational changes in SWM and their association with cognition in POMS. Completion of the CST validation study, through the enrolment of the final sample, will allow determination of its psychometric proprieties and potential advantages compared to the SDMT.

9. Additional publications of the PhD course

9.1 The association between cognition and motor performance is beyond structural damage in relapsing-remitting multiple sclerosis

Mistri et al., Journal of Neurology 2022

This chapter describes the work published in Journal of Neurology (PMID: 35278104, DOI: 10.1007/s00415-022-11044-8).

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https://doi.org/10.1007/s00415-022-11044-8

ORIGINAL COMMUNICATION

The association between cognition and motor performance is beyond structural damage in relapsing–remitting multiple sclerosis

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Abstract

Background Previous studies demonstrated an association between motor and cognitive performance in multiple sclerosis (MS). However, disease-related brain damage might represent a common substrate to both phenomena, which was not considered before.

Objective Aim of this study is to investigate whether the association between cognition and motor function is beyond structural damage in patients with MS.

Methods Eighty-one healthy controls and 106 relapsing–remitting (RR) MS patients underwent a 3.0 T MRI with quantification of T2-lesion volumes, T1-lesion volumes and normalized brain volumes. A functional examination [Nine-Hole Peg Test (9-HPT), Timed 25-Foot Walk test (T25FW) and Expanded Disability Status Scale] and a neuropsychological evaluation (Brief Repeatable Battery of Neuropsychological Tests) were also administered. Association between demographic, clinical, cognitive, MRI and functional measures were analysed with univariate analyses and hierarchical linear regression.

Results In RRMS patients, Spatial Recall Test and Symbol Digit Modalities Test were positively correlated with 9-HPT ($p < 0.001$) and T25FW ($p \leq 0.035$); Paced Auditory Serial Addition Test (PASAT) correlated with 9-HPT ($p \leq 0.009$). 9-HPT and T25FW were significantly associated with normalized brain volumes ($p \leq 0.016$), T2- and T1-lesion volumes ($p \leq 0.009$). Hierarchical regression models selected age and normalized deep gray matter volume as predictors of T25FW (adjusted-$R^2 = 0.109$). Younger age, female sex, higher normalized gray matter volume and higher PASAT 2″ scores predicted higher 9-HPT scores (adjusted-$R^2 = 0.337$).

Conclusions In RRMS patients, deficit in information processing speed and executive function may contribute to hand motor dysfunction beyond the effect of structural disease-related burden, supporting the integration of motor and cognitive assessment in clinical settings.

Keywords Multiple sclerosis · Movement · Cognition · Magnetic resonance imaging · Walking speed · Executive function

Introduction

MS is a chronic, inflammatory and neurodegenerative disease of the CNS characterized by inflammation, demyelination and neurodegeneration (Filippi *et al.*, 2018). Motor dysfunction, deficits in gait and balance, and cognitive impairment are clinical hallmarks of MS that impact daily life activities (Buzaid *et al*, 2013). Cognitive impairment affects 43-70% of MS patients. The most frequently involved cognitive domains are information processing speed, executive functions, attention, working memory and episodic memory (Rocca *et al.*, 2015a).

Cognitive and motor symptoms often occur simultaneously and their relationship is well-known in numerous neurological disorders (Allali *et al*, 2008; Bayot *et al*, 2018; Benedict *et al*, 2011; Kelly *et al*, 2012; Salvadori *et al*, 2020). In Alzheimer's disease impaired executive functions can lead to a significant increase of stride time and stride time variability during walking (Allali *et al.*, 2008). Gait deficits in patients with Parkinson's disease are exacerbated while performing simultaneous cognitive or motor tasks, with reduction in walking speed, left and right steps coordination and stride length (Kelly *et al.*, 2012). In elderly individuals the coexistence of cognitive deficits and mobility impairment (i.e., slow gait speed) is associated with elevated risk of progression to dementia (Grande *et al*, 2020; Peel *et al*, 2019). There is a growing recognition that mobility and cognitive impairments should be considered interrelated to develop new risk assessment procedures in clinical and research settings (Grande *et al*, 2019). Since the likelihood of cognitive impairment increases in subjects with slower walking speed, gait speed assessment was proposed to detect prodromal dementia (Grande *et al.*, 2019).

The simultaneous performance of cognitive tasks while walking, termed dual tasking, has been suggested as an useful tool to detect the impact of motor–cognitive impairment in patients suffering from MS (Argento *et al*, 2021; Fritz *et al*, 2019). Several existing studies demonstrated that dual-task generates cognitive–motor interference causing loss of efficacy in one or both tasks (Wajda & Sosnoff, 2015). In MS patients, cognitive-motor interference intensifies functional impairments by reducing gait speed (Motl *et al*, 2014) and increasing fall risk (Wajda *et al*, 2013). Alterations in motor performance under dual-task condition are often explained within the context of the “limited-capacity model of attention”, according to which both the

motor and cognitive tasks compete for finite attentional resources (Wajda *et al.*, 2013). More recently, Plummer and Eskes (Plummer & Eskes, 2015) proposed the "task prioritization model" suggesting that the magnitude and direction of dual-task interference is also influenced from the way in which individuals spontaneously prioritize their attention.

In MS, deficits in executive functions and IPS have been reported to be correlated with slower gait speed measured with Timed 25-Foot Walk test (T25FW) (Benedict *et al.*, 2011; Bollaert *et al*, 2019; D'Orio *et al*, 2012; Motl *et al*, 2013; Motl *et al*, 2021), poorer walking endurance assessed with the 6-minute walk test (Bollaert *et al.*, 2019; Motl *et al.*, 2013; Motl *et al.*, 2021) and lower functional mobility (Bollaert *et al.*, 2019). Moreover, cognitive dysfunction is associated with higher step length variability and increased fall frequency (Hsieh *et al*, 2017; Sosnoff *et al*, 2013).One possible explanation is that slower information processing speed prevents a prompt response to postural threats, resulting in increased step length, step time and risk of falling (Hsieh *et al.*, 2017; Sosnoff *et al.*, 2013)

In physiological normal conditions, walking is mainly automatic and rhythmic, however, moving across a complex environment requires higher-order cognitive functions to plan movements and avoid obstacles (Bayot *et al.*, 2018). Gait control involves both the direct and indirect motor pathways, the former including the primary motor cortex, cerebellum, and spinal cord, and the latter involving the basal ganglia, premotor area and prefrontal cortex (Bayot *et al.*, 2018). Existing studies of neurological patients and older adults (Allali *et al.*, 2008; Kelly *et al.*, 2012; Salvadori *et al.*, 2020) have mainly focused on associations between cognitive dysfunction and gait impairment, while the link between cognitive performance and hand motor function has received little attention. A few investigations demonstrated an association between hand dexterity and cognitive tests measuring information processing speed and executive functions in older adults (Kobayashi-Cuya *et al*, 2018) and MS patients (Benedict *et al.*, 2011). Yet, the pathological mechanisms underpinning motor and cognitive dysfunctions remain unclear.

In one study (Benedict *et al.*, 2011), 211 MS patients underwent extensive functional and neuropsychological evaluation to assess the influence of cognitive impairment on motor dysfunction. Information processing speed and executive function tests predicted

variability in gait speed and hand dexterity. However, the possible role of structural brain damage has not been explored. Gray matter atrophy and white matter abnormalities are known to correlate with both motor and cognitive dysfunction (Cordani *et al*, 2020b; Riccitelli *et al*, 2020), which can therefore develop in parallel, and not necessarily directly influencing each other. The detected association between motor and cognitive performance may be due to the tendency of MS patients with preserved mobility to perform better on neuropsychological tests than those with motor impairment.

Aim of this work is to investigate the association between cognitive function, motor performance and brain structural damage (i.e., WM lesions and atrophy) in patients with MS. To gain additional insights into the mechanisms underlying the different aspects of motor deficits in these patients and their interplay with cognitive dysfunction, we also assessed the effect of demographic, clinical, cognitive, and brain structural measures on gait speed measured with the T25FW and hand dexterity evaluated with the nine-hole peg test (9-HPT).

Methods

Ethics committee approval. The local ethical standards committee on human experimentation approved this study. Written informed consent was obtained from all participants.

Participants. We recruited 106 RRMS patients and 81 sex- and age-matched HCs. To mitigate a potential bias due to the exhaustion of functional compensatory mechanisms and the severity of structural damage, which could mask any effects of cognition on motor performance (Rocca *et al*, 2005), we excluded patients with the progressive phenotypes of the disease. Inclusion criteria were: age ≥18 years, right handedness (Edinburgh Handedness Inventory pre-MS condition ≥50) (Oldfield, 1971), Italian native language, no previous history of neurological disorders (other than MS for patients), and no psychiatric disorders. RRMS patients were steroid-free for at least one month, and treatment-stable for at least three months.

Clinical and functional assessment. Within 48 hours from the MRI acquisition, all participants underwent a neurological and functional assessment. The neurological

evaluation included the EDSS (Kurtzke, 1983), right- and left 9-HPT, and T25FW. All functional tests were repeated twice and results were averaged.

Neuropsychological assessment. Patients also underwent cognitive assessment. Experienced neuropsychologists conducted the neuropsychological assessment on the day of MRI, using the Brief Repeatable Battery of Neuropsychological Tests (BRB-N), which assesses verbal memory (SRT), visuospatial memory (SPART and delayed-recall), attention and information processing speed (SDMT, Paced Auditory Serial Addition Test [PASAT] 3" and 2") and verbal fluency (WLG) (Amato *et al*, 2006). Corrected scores for age, sex, and education according to normative values (Amato *et al.*, 2006) were derived based on normative data, obtaining z-scores for each cognitive test.

MRI acquisition. Using a Philips Intera 3.0 T scanner, the following sequences of the brain were acquired from all subjects: (i) dual-echo turbo spin echo (DE-TSE) (TR/TE = 2599/16-80 ms; flip angle 90°; FOV 240 mm^2; matrix 256 x 256; ETL 6; 44 contiguous, 3-mm-thick axial slices) and (ii) 3D T1-weighted fast field echo (TR/TE = 25/4.6 ms, flip angle 30°, FOV 230 mm^2, matrix 256 x 256, slice thickness 1 mm, 220 contiguous axial slices, in-plane resolution 0.89 x 0.89 mm^2).

MRI Analysis. T2-hyperintense and T1-hypointense LV were measured on DE-TSE and 3D T1-weighted images, using a local thresholding segmentation technique (Jim 7.0, Xinapse Systems Ltd, Colchester, UK). After T1-hypointense lesion refilling (Chard *et al*, 2010), normalized volumes of the whole brain (NBV), GM (NGMV) and WM (NWMV) were measured using the SIENAx software (Smith *et al*, 2002). Normalized deep GM volume (NDGMV), corresponding to the sum of the normalized volumes of bilateral thalamus, caudate, putamen, pallidum, amygdala and accumbens, measured with the FIRST tool (Patenaude *et al*, 2011b), was also calculated.

Statistical analysis. The statistical analysis was performed using R-4.0.3 and SPSS software, version 22.0. Between-group comparisons of demographic and brain MRI measures were assessed with the chi-square test, Mann–Whitney U test, or two-sample t test (according to normality assumption).

The inverse of 9-HPT and T25FW scores were converted to z-scores using HC's mean and standard deviation. The significance of z-scores deviation from the HC population was tested with a one-sample t test.

T2-LV and T1-LV underwent a logarithmic transformation before statistical analysis. Right and left 9-HPT z-scores were averaged to reduce the number of variables.

Univariate analyses (Pearson's correlations) were used to assess the association between MRI measures, cognitive scores and motor performance (z-scores of T25FW and 9-HPT). We investigated the differences of correlation between the two considered functional scores by Steiger's z-test (Steiger, 1980). Hierarchical regression analysis was performed to select the independent predictors of motor performance. We used a within-block stepwise approach: block 1 included demographic and clinical variables (age, sex, disease duration and education); block 2 comprised all MRI measures (NBV, NGMV, NWMV, NDGMV, T2-LV and T1-LV) and block 3 contained BRB-N tests z-scores (SRT, SPART, SDMT, PASAT 3", PASAT 2" and WLG). Since the EDSS score is not an independent variable explaining disability, but a measure of disability itself, it was not included among the demographic and clinical variables of the models. For all analyses, statistical significance was set at $p<0.05$.

Results

Demographic, clinical, functional, neuropsychological and MRI features. Table 9.1.1 summarizes the main demographic, clinical, functional, neuropsychological and MRI findings in MS patients and HC. Compared to HC, RRMS patients showed significant motor impairment measured with 9-HPT (mean z-score = -1.0 ± 0.8; $p < 0.001$) and T25FW (mean z-score = -0.5 ± 0.8; $p = 0.001$).

RRMS patients performed below the normative values in all neuropsychological tests (mean z-score ≤ -0.3) except for SPART (mean z-score = 0.0 ± 0.9) and SPART recall (mean z-score = 0.0 ± 0.9).

Compared to HC, RRMS patients had lower NBV ($p = 0.02$), NGMV ($p = 0.01$) and NDGMV ($p < 0.001$).

Table 9.1.1. Main demographic, clinical, functional, neuropsychological and structural MRI characteristics of the subjects included in the study. Bold text indicates a statistically significant result ($p < 0.05$).

	RRMS patients (n=106)	HC (n=81)	p
Demographic and clinical variables			
Number of women / men (%)	65 (61) / 41 (39)	44 (54) / 37 (46)	0.41[a]
Median Age (IQR) [years]	34.4 (26.1-44.1)	39.3 (24.7-53.9)	0.13[b]
Median Education (IQR) [years]	**13.0 (13.0-16.7)**	**16.0 (13.0-18.0)**	**0.07[b]**
Mean Disease Duration (SD) [years]	10.3 (6.4)	-	-
Median EDSS (IQR)	1.5 (1.0 - 2.0)	-	-
Functional measures			
Mean Right 9-HPT [seconds] (SD)	**22.9 (4.5)**	**18.8 (3.1)**	**<0.001[c]**
Mean Right z-9-HPT (SD)	**-1.1 (0.8)**	**0.0 (1.0)**	**<0.001[c]**
Mean Left 9-HPT [seconds] (SD)	**24.5 (4.4)**	**21.1 (4.7)**	**<0.001[c]**
Mean Left z-9-HPT (SD)	**-0.8 (0.8)**	**0.0 (1.0)**	**<0.001[c]**
Mean z-9-HPT (SD) [both hands]	**-1.0 (0.8)**	**0.0 (0.9)**	**<0.001[c]**
Mean T25FW [seconds] (SD)	**5.9 (2.8)**	**5.0 (2.1)**	**0.02[c]**
Mean z-T25FW (SD)	**-0.5 (0.8)**	**0.0 (1.0)**	**0.001[c]**
Neuropsychological tests [z-scores]			
Mean SRT lts	-0.5 (1.0)	-	-
Mean SRT cltr	-0.5 (1.0)	-	-
Mean SRT recall	-0.4 (1.0)	-	-
Mean SPART	0.0 (0.9)	-	-
Mean SPART recall	0.0 (0.9)	-	-
Mean SDMT	-0.3 (1.5)	-	-
Mean PASAT 3"	-0.5 (1.1)	-	-
Mean PASAT 2"	-0.8 (0.9)	-	-
Mean WLG	-0.5 (1.0)	-	-
MRI measures			
Median T2-LV (IQR) [mL]	**3.8 (2.4-8.5)**	**0.1 (0.0-0.2)**	**<0.001[d]**
Median T1-LV (IQR) [mL]	**2.4 (1.2-5.9)**	**0.0 (0.0-0.2)**	**<0.001[d]**
Mean NBV (SD) [mL]	**1532 (99)**	**1568 (94)**	**0.02[b]**
Mean NGMV (SD) [mL]	**710 (69)**	**737 (63)**	**0.01[b]**
Mean NWMV (SD) [mL]	822 (47)	831 (50)	0.15[b]
Mean NDGMV (SD) [mL]	**49 (4.5)**	**52 (3.4)**	**<0.001[b]**

[a]Chi-squared test; [b]Mann-Withney U test; [c]One-sample t test; [d] Mann-Withney U test performed on log-scale.

Abbreviations: 9-HPT= nine-hole peg test; cltr= consistent long-term retrieval; EDSS= Expanded Disability Status Scale; IQR= interquartile range; lts= long term storage; LV= lesion volume; NBV= normalized brain volume; NDGMV= normalized

deep grey matter volume; NGMV= normalized grey matter volume; NWMV= normalized white matter volume; PASAT= paced auditory serial addition test; RRMS= relapsing-remitting multiple sclerosis; SD= standard deviation; SDMT= symbol digit modalities test; SPART= 10/36 spatial recall test; SRT= selective reminding test; T25FW= 25-foot walk test; WLG= word list generation; z= z-scores.

Correlations between cognitive, MRI and motor performance. Table 9.1.2 shows the univariate associations between functional measures (z-scores) and neuropsychological tests (z-scores) as well as between functional measures (z-scores) and MRI measures in RRMS patients. Linear relationship between motor and cognitive performance is presented in Figure 9.1.1 and Figure 9.1.2.

Visuospatial memory assessed with SPART was associated with 9-HPT ($r = 0.359$; $p < 0.001$) and T25FW ($r = 0.236$; $p = 0.015$). Reduced information processing speed measured with SDMT, PASAT 3" and PASAT 2" was related to poorer performance on 9-HPT ($r \geq 0.254$; $p \leq 0.009$). SDMT z-scores resulted also significantly associated with T25FW ($r = 0.205$; $p = 0.035$). Verbal fluency significantly correlated with hand dexterity assessed with 9-HPT ($r = 0.226$; $p = 0.020$).

All functional measures were significantly associated with NBV ($r \geq 0.346$; $p < 0.001$), NGMV ($r \geq 0.340$; $p < 0.001$), NWMV ($r \geq 0.234$; $p \leq 0.016$) and NDGMV ($r \geq 0.317$; $p \leq 0.001$). Higher T2-LV and T1-LV were correlated with worse performance at 9-HPT ($r \leq -0.430$; $p < 0.001$) and slower walking speed ($r \leq -0.254$; $p \leq 0.009$).

Applying Steiger's z-test to assess differences between correlations, 9-HPT showed stronger association with PASAT 3" ($p = 0.018$), PASAT 2" ($p = 0.021$) and NGMV ($p = 0.035$) than T25FW.

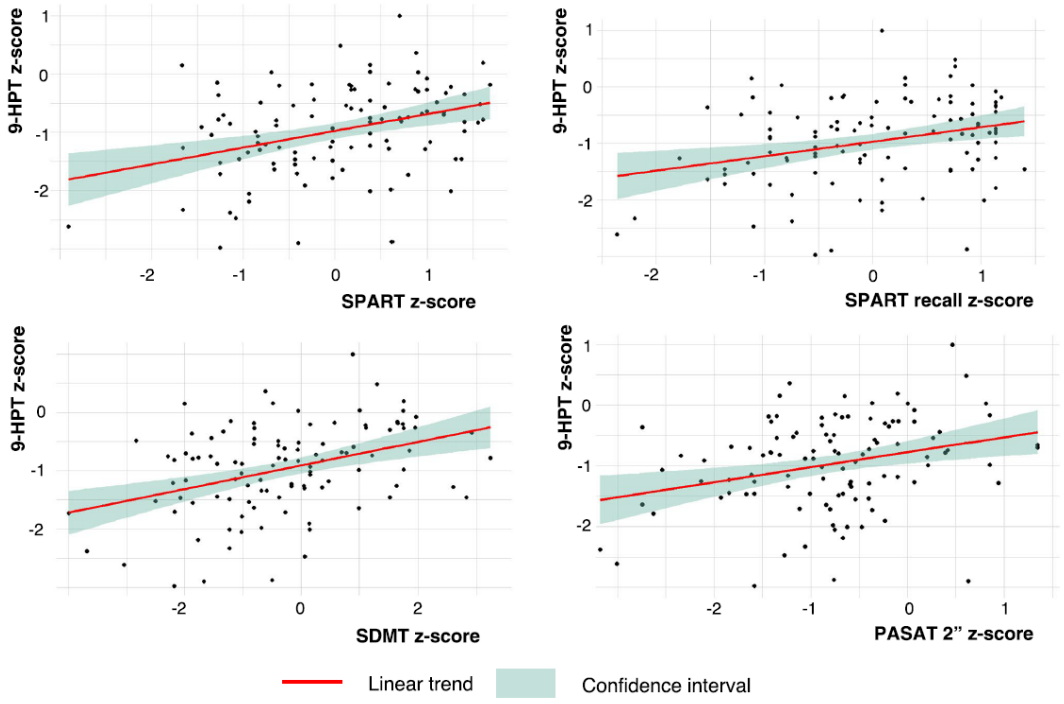
Table 9.1.2. Univariate association (Pearson's correlations) between neuropsychological tests z-scores, MRI measures and functional measures in RRMS patients. Bold text indicates a statistically significant correlation (p value < 0.05).

	9-HPT		**T25FW**		
	r	**p**	***r***	**P**	**p***
Neuropsychological tests [z-scores]					
SRT lts	0.184	0.058	0.120	0.221	0.566
SRT cltr	0.117	0.230	0.090	0.361	0.806
SRT recall	0.168	0.086	0.085	0.387	0.466
SPART	**0.359**	**<0.001**	**0.236**	**0.015**	0.253
SPART recall	**0.297**	**0.002**	0.178	0.067	0.279
SDMT	**0.388**	**<0.001**	**0.205**	**0.035**	0.088
PASAT 3"	**0.254**	**0.009**	-0.013	0.896	**0.018**
PASAT 2"	**0.294**	**0.002**	0.036	0.715	**0.021**
WLG	**0.226**	**0.020**	0.094	0.339	0.241
MRI measures					
T2-LV	**-0.430**	**<0.001**	**-0.254**	**0.009**	0.092
T1-LV	**-0.436**	**<0.001**	**-0.268**	**0.006**	0.106
NBV	**0.524**	**<0.001**	**0.346**	**<0.001**	0.069
NGMV	**0.545**	**<0.001**	**0.340**	**<0.001**	**0.035**
NWMV	**0.312**	**0.001**	**0.234**	**0.016**	0.474
NDGMV	**0.462**	**<0.001**	**0.317**	**0.001**	0.154

p* p value from Steiger's z-test performed to assess differences between correlations.

Abbreviations: 9-HPT= nine-hole peg test; cltr= consistent long-term retrieval; lts= long term storage; LV= lesion volume; NBV= normalized brain volume; NDGMV= normalized deep grey matter volume; NGMV= normalized grey matter volume; NWMV= normalized white matter volume; PASAT= paced auditory serial addition test; RRMS= relapsing-remitting multiple sclerosis; SDMT= symbol digit modalities test; SPART= 10/36 spatial recall test; SRT= selective reminding test; T25FW= 25-foot walk test; WLG= word list generation.

Figure 9.1.1. Scatterplots for significant positive correlations between 9-HPT and cognitive performance tests (Mistri et al., 2022). Linear relationship between 9-HPT and SPART (r = 0.359; p < 0.001), SPART recall (r = 0.297; p = 0.002), SDMT (r = 0.388; p < 0.001) and PASAT 2" (r = 0.294; p = 0.002).



Abbreviations: 9-HPT=nine-hole peg test; PASAT=paced auditory serial addition test; SDMT=symbol digit modalities test; SPART=10/36 spatial recall test

Figure 9.1.2. Scatterplots for significant positive correlations between T25FW and cognitive performance tests (Mistri et al., 2022). Linear relationship between T25FW and SPART (r = 0.236; p = 0.015) and SDMT (r = 0.205; p = 0.035).



Abbreviations: SDMT= symbol digit modalities test; SPART= 10/36 spatial recall test; T25FW= 25-foot walk test

Predictors of motor performance. Table 9.1.3 summarizes the results of the hierarchical regressions analysis.

Older age (p = 0.001; ΔR^2 = 0.103), male sex (p = 0.042; ΔR^2 = 0.035), lower NGMV (p < 0.001; ΔR^2 = 0.187) and lower scores at PASAT 2" (p = 0.017; ΔR^2 = 0.037) predicted worse performance at 9-HPT (adjusted R^2 = 0.337). Older age (p = 0.013; ΔR^2 = 0.058) and lower NDGMV (p = 0.005; ΔR^2 = 0.068) predicted slower gait speed measured with the T25FW (adjusted R^2 = 0.109).

Table 9.1.3. Hierarchical regression models assessing the predictors of motor performance for RRMS patients.

Dependent variable	Block	Significant IVs	Standardized β weights	R^2	R^2 change	p	Adjusted R^2
9-HPT	1	Age	-0.016	0.103	-	0.001	0.337
		Sex	-0.183	0.138	0.035	0.042	
	2	NGMV	0.472	0.325	0.187	<0.001	

	3	PASAT 2"	0.199	0.362	0.037	0.017	
T25FW	1	Age	-0.167	0.058	-	0.013	0.109
	2	NDGMV	0.272	0.126	0.068	0.005	
	3	-	-	-	-	-	

Abbreviations: 9-HPT=nine-hole peg test; EDSS=Expanded Disability Status Scale; IV=independent variable; NDGMV=normalized deep grey matter volume; NGMV=normalized grey matter volume; PASAT=paced auditory serial addition test; RRMS=relapsing-remitting multiple sclerosis; T25FW=25-foot walk test.

Discussion

In this study, we assessed the correlation between functional measures of upper and lower limbs, cognitive performance, and structural brain damage in RRMS patients. We subsequently identified the predictors of hand motor dexterity and walking speed among a set of demographic, clinical, MRI and cognitive variables using hierarchical regression analysis.

Better performance at tests measuring visuospatial memory and information processing speed correlated with higher degree of hand dexterity and faster walking speed. Motor performance resulted also associated with all MRI measures analysed (T2-LV, T1-LV, NBV, NGMV, NWMV and NDGMV).

As a second step, we searched for the predictors of motor impairment. Younger age, female sex, higher NGMV and higher PASAT 2" scores predicted better hand motor performance assessed with 9-HPT, while younger age and higher NDGMV were selected as significant predictors of faster walking speed.

Our results confirm the positive correlation between motor and cognitive performance in MS (Benedict *et al.*, 2011; D'Orio *et al.*, 2012; Drake *et al*, 2010; Hsieh *et al.*, 2017). However, our work differs from available literature as we assessed the influence of potential confounding variables such as brain atrophy and focal WM lesions on the association between motor and cognitive dysfunction. It could therefore be hypothesized that information processing speed and executive function deficit contribute to explain hand motor dysfunction. Complex movements require cognitive planning and monitoring, especially in subjects with motor impairment (Bayot *et al.*, 2018; D'Orio *et al.*, 2012; Kelly *et al.*, 2012) and a high order cognitive control

dysfunction can affect the performance at complex motor tasks independently from physical disabilty and structural brain damage.

Significantly higher correlation coefficients were found between 9-HPT and PASAT 3" and PASAT 2" than T25FW. Our results are in line with those of previous studies which observed stronger correlations between SDMT (Benedict *et al.*, 2011; Drake *et al.*, 2010), PASAT (Benedict *et al.*, 2011; Drake *et al.*, 2010), the Brief Visuospatial Memory Test-Revised (Benedict *et al.*, 2011) and hand dexterity compared to walking speed. We hypothesize that eye-hand coordination needed to execute timely and skilful movements requires more cognitive resources than movement coordination and integration of sensory feedback necessary for walking forward (Bayot *et al.*, 2018; Rizzo *et al*, 2020). The greater level of cognitive complexity assumed for hand dexterity tasks compared to gait speed measures can thus explain why correlations with cognitive performance were more often statistically significant and higher for 9-HPT than T25FW.

Hand motor functioning and gait speed resulted significantly correlated with all measures of structural brain damage, as consistently demonstrated by previous studies (Peterson & Fling, 2018). In particular, 9-HPT scores resulted more closely associated with NGMV than walking ability. Such aspect might be due to the higher functional demand required for upper limb function, which could make it more influenced by brain damage accumulation. On the other hand, it is well-known that involvement of CNS areas, such as the spinal cord, may be more clinically relevant to explain locomotor dysfunction (Rocca *et al*, 2019).

Univariate analysis results confirmed significant relationships between neuropsychological and motor outcomes (Benedict *et al.*, 2011; Bollaert *et al.*, 2019; D'Orio *et al.*, 2012; Motl *et al.*, 2013; Motl *et al.*, 2021). However, in our study lower correlation coefficients were found compared with previous works (Bollaert *et al.*, 2019; Motl *et al.*, 2021). This can be explained by the fact that our sample was younger and less heterogeneous in terms of clinical phenotypes than the samples included in earlier studies (Bollaert *et al.*, 2019; Motl *et al.*, 2021).

Applying hierarchical regression analysis, we identified the predictors of hand motor function and gait speed. Age was selected as significant predictor of motor dysfunction, confirming that slower ambulation and poorer hand dexterity are associated with

advancing age in MS (Kalinowski *et al*, 2021; Oxford Grice *et al*, 2003). The regression models also indicated that NDGMV is associated with T25FW. Our results align with previous works demonstrating the involvement of thalamic and basal ganglia structures in motor control (Motl *et al*, 2015; Motl *et al*, 2016).

For the 9-HPT, predictors of performance were age, sex, NGMV and PASAT performance. The effect of sex on hand motor function is in agreement with previous studies showing that men need more time to complete the task than women (Solaro *et al*, 2019). A previous study in a large cohort of RRMS patients showed that NGMV was one of the main predictors of upper limb motor performance (Cordani *et al*, 2020a). As reported by the literature, atrophy of cerebellum, basal ganglia and fronto-parietal regions may play a critical role in eye-hand coordination (Cordani *et al.*, 2020b; Rizzo *et al.*, 2020).

Nine-HPT was confirmed significantly associated with PASAT 2" even after controlling for demographic variables, clinical features and structural brain damage. PASAT has been developed to measure the rate of information processing (Tombaugh, 2006b), however numerous studies pointed out that PASAT is also related to other cognitive functions such as working memory and sustained attention (Tombaugh, 2006b). Sherman and colleagues (Sherman *et al*, 1997) tested PASAT construct validity and observed that, despite mathematical ability and attention accounted for a substantial amount of variance, verbal ability and even complex motor skills were also moderately correlated with its performance. The amount of variance shared between PASAT and complex motor skills such as manual dexterity is thought to be due to attentional/executive functioning deficits.

Evidence supports high-order cognitive functions involvement in complex movements. Our study confirmed the 9-HPT as a complex task requiring the interaction between motor- and cognitive abilities beyond the effect of disease progression and structural brain damage.

This study has a few limitations. First, our protocol did not include a spinal cord MRI acquisition that could have improved the ability of the final models to predict motor impairment. Second, we did not consider fatigue, depression and anxiety symptoms, which may influence cognitive performance in MS patients (Rocca *et al.*, 2015a). Third, it is cross-sectional, thus not allowing evaluating whether the observed predictors are

related to motor worsening. Finally, the selected demographical, clinical, neuropsychological and MRI variables were able to explain only 34% and 11% of variance in the 9-HPT and T25FW respectively. The inclusion of other MRI measures, such as those of structural and functional connectivity, could have increased the proportion of variance accounted.

Overall, this study provides evidence for an additional contribution of high order cognitive control dysfunction to motor hand dexterity impairment in MS, beyond the effect of structural brain damage.

Future studies should investigate the longitudinal evolution of cognitive and motor dysfunction in MS and clarify eventual common pathological mechanisms.

Our results support the idea that a multidimensional approach should be implemented in the clinical setting to improve the assessment of functional and cognitive deficits and enhance rehabilitation programs (Grande *et al.*, 2019). Also, this study provides further evidence for using composite scores for MS evaluation, including both motor and cognitive outcomes (Drake *et al.*, 2010).

9.2 Cognitive function in primary and secondary progressive multiple sclerosis: a multiparametric MRI study

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ORIGINAL ARTICLE

Cognitive function in primary and secondary progressive multiple sclerosis: A multiparametric magnetic resonance imaging study

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Abstract

Background and purpose: The differences in cognitive function between primary progressive and secondary progressive multiple sclerosis (MS) remain unclear. We compared cognitive performance between primary progressive multiple sclerosis (PPMS) and secondary progressive multiple sclerosis (SPMS), and explored the structural and functional magnetic resonance imaging (MRI) correlates of their cognitive functions.

Methods: Seventy-five healthy controls and 183 MS patients (60 PPMS and 123 SPMS) underwent 3.0-T MRI. MS patients were administered the Brief Repeatable Battery of Neuropsychological Tests; cognitive domain *z*-scores were calculated and then averaged to obtain a measure of global cognition. Using hierarchical linear regression analysis, the contribution of lesion volumes, normalized brain volumes, white matter (WM) fractional anisotropy (FA) and mean diffusivity abnormalities, and resting state (RS) functional connectivity (FC) alterations to global cognition in PPMS and SPMS was investigated.

Results: PPMS and SPMS had similar *z*-scores in all investigated cognitive domains. Poor global cognitive function was associated with decreased FA of the medial lemniscus ($\Delta R^2 = 0.11$, $p = 0.011$) and lower normalized gray matter volume ($\Delta R^2 = 0.29$, $p < 0.001$) in PPMS, and with decreased FA of the fornix ($\Delta R^2 = 0.35$, $p < 0.001$) and lower normalized WM volume ($\Delta R^2 = 0.05$; $p = 0.034$) in SPMS.

Conclusions: PPMS and SPMS had similar neuropsychological performance. Cognitive dysfunction in PPMS and SPMS was related to distinct patterns of structural MRI abnormalities and involvement of different WM tracts, whereas RS FC alterations did not contribute to explaining their global cognitive functioning.

KEYWORDS
executive function, memory, MRI, multiple sclerosis

Introduction

Cognitive deficits are reported to be more frequent and severe in patients with progressive MS compared to those with a relapsing-remitting disease course (Huijbregts *et al*, 2004). However, it remains still controversial whether patients with primary progressive (PP) and SP MS have similar frequency and patterns of cognitive dysfunction or not (Foong *et al*, 2000; Huijbregts *et al.*, 2004; Renner *et al*, 2020; Wachowius *et al*, 2005).

A number of cross-sectional investigations found higher prevalence of cognitive impairment in patients with SPMS (55-86%) than those with PPMS (Foong *et al.*, 2000; Huijbregts *et al.*, 2004) (53-73%) and PPMS patients were reported to perform significantly better on tasks involving visuospatial abilities (Huijbregts *et al.*, 2004), working memory (Foong *et al.*, 2000) and verbal fluency (Huijbregts *et al.*, 2004).

In the last few years, a body of evidence has suggested that progressive MS phenotypes have similar degree of cognitive dysfunction (Ruano *et al*, 2017; Wachowius *et al.*, 2005). A large nationwide study detected comparable measures of cognitive function between PPMS and SPMS (45% and 46% were classified as cognitively impaired, respectively) (Renner *et al.*, 2020).

Several factors can contribute to explain discrepancies among studies, including the use of different neuropsychological assessments and matching biases, since SPMS and PPMS often differ in terms of age, disease duration and physical disability (Huijbregts *et al.*, 2004; Renner *et al.*, 2020).

The application of advanced MRI techniques is contributing to identify the substrates associated with cognitive dysfunction in progressive MS. T2-hyperintense lesions(Rocca *et al*, 2015b) and GM atrophy (Riccitelli *et al*, 2011) have been correlated with the presence and severity of cognitive deficits in both PPMS and SPMS. T1-hypointense lesions (Camp *et al*, 1999), GM integrity (Tur *et al*, 2011) and microstructural alterations to the interhemispheric callosal pathways (Bodini *et al*, 2013) have been related to cognitive dysfunction in PPMS; while microstructural damage of specific WM tracts such as the corpus callosum, fornix, superior longitudinal fasciculus and forceps major have been shown to play a crucial role to explain cognitive deficits in SPMS (Manca *et al*, 2019; Meijer *et al*, 2016).

RS fMRI studies have demonstrated different patterns of recruitment of cognitively-related networks according to the stage of the disease (Rocca *et al*, 2018b). At the beginning of MS, increased RS FC within these networks has been interpreted as a compensatory mechanism limiting the consequences of disease-related structural damage, while at later stages a reduced RS FC has been associated with a worse cognitive performance (Conti *et al*, 2021a; Rocca *et al.*, 2018b).

Only a limited number of studies explored the MRI correlates of cognitive impairment in both PPMS and SPMS patients (Foong *et al.*, 2000; Rocca *et al*, 2010c). In most of available studies, a single or a few MRI techniques have been applied. It is arguable that a multiparametric approach, combining structural and functional MRI techniques, might contribute to improve the understanding of the mechanisms associated with cognitive deficits in PPMS and SPMS, allowing to identify shared and distinctive features of these disease clinical phenotypes.

In this study, we administered the BRB-N (Amato *et al.*, 2006) to a large sample of progressive MS patients to compare the cognitive performance between PPMS and SPMS. We then combined different MRI modalities to explore the structural and functional substrates of cognitive dysfunction in the two progressive phenotypes of MS.

Methods

Ethics committee approval. Approval was received from the local ethical standards committee on human experimentation and written informed consent was obtained from all subjects prior to study participation.

Participants. We recruited 183 MS patients (60 PPMS and 123 SPMS) and 75 age- and sex-matched HCs. Inclusion criteria were age ≥18 years, Italian native language, right handedness, and no previous history of neurological (other than MS for patients) or psychiatric disorders. MS patients were enrolled from the Neuroimaging Research Unit of San Raffaele Institute, Milan, Italy. HC were mainly recruited among the spouses of patients and by word of mouth. To be included, patients with MS had to have a diagnosis of MS according to the 2017 revised McDonald criteria.

Clinical and neuropsychological assessment. Within 48 hours from the MRI acquisition, all MS patients underwent a complete neurological examination, with rating of the Expanded Disability Status Scale (EDSS) (Kurtzke, 1983).

Experienced neuropsychologists administered the BRB-N, which assesses verbal memory (SRT long-term storage, SRT consistent long term retrieval and SRT delayed-recall), visuospatial memory (SPART and delayed-recall), attention and processing speed (SDMT, PASAT 3" and 2"), and semantic verbal fluency (WLG) (Amato *et al.*, 2006). Fatigue was also assessed through the Modified Fatigue Impact Scale (Fisk *et al*, 1994).

Age-, sex-, and education-adjusted scores were calculated based on normative data, and z-scores were obtained for each cognitive test (Amato *et al.*, 2006). A z-score for each cognitive domain was subsequently calculated by averaging the z-scores of corresponding tests: z-verbal memory, z-visuospatial memory, z-attention/processing speed, and z-verbal fluency. Then, a global z-cognitive function score (z-BRB-N), corresponding to the mean z-score obtained from the abovementioned cognitive domains, was calculated (Sepulcre *et al.*, 2006). Patients who scored below the 5th percentile of the normative sample on tests that explore at least two different cognitive domains were classified as cognitively impaired (Ruano *et al.*, 2017).

MRI acquisition. Each subject underwent a brain MRI scan performed using two 3.0 T scanners (Scanner 1: Achieva; Scanner 2: Ingenia; Philips Medical Systems, Eindhoven, The Netherlands). Images acquired using the Achieva scanner included the following scans: 1) 200 sets of T2*-weighted echo planar imaging sequence for RS fMRI; 2) dual-echo turbo spin echo; 3) 3D T1-weighted fast field echo; and 4) pulsed-gradient spin echo echo planar imaging with SENSE (acceleration factor=2) and diffusion gradients applied in 35 non-collinear directions.

Images acquired using the Ingenia scanner included: 1) 320 sets of T2*-weighted echo planar imaging sequence for RS fMRI; 2) 3D FLAIR; 3) 3D T2-weighted sequence; 4) 3D T1-weighted turbo field echo; and 5) axial pulsed-gradient spin echo single shot diffusion-weighted echo planar imaging (see Supplementary Methods).

Conventional MRI analysis. T2-hyperintense lesion masks and their volume (LV), NBV, NGMV, NWMV, and NDGMV (i.e., the sum of the normalized volumes of bilateral thalamus, caudate, putamen, pallidum, amygdala and accumbens) were calculated (see Supplementary Methods).

Tract based spatial statistic (TBSS) analysis. Pre-processing of diffusion-weighted images included correction for off-resonance (Scanner 2) - eddy current distortions and

movements (http://white.stanford.edu/mrdiff [Scanner 1]; Eddy tool FSL library (Andersson & Sotiropoulos, 2016) [scanner 2]). The DT was estimated by linear regression on diffusion-weighted imaging data at b=900 s/mm^2 (Scanner-1) or b=700/1000 s/mm^2 (Scanner-2) (Basser *et al.*, 1994). Then, maps of FA and MD were derived.

TBSS analysis was used for voxelwise analysis of whole brain WM DT MRI measures (http://www.fmrib.ox.ac.uk/fsl/tbss/index.html). In detail, individual FA images were non-linearly registered to the FMRIB58_FA atlas (Rueckert *et al*, 1999) provided within FSL and averaged. The resulting mean FA image was thinned to create a WM tract "skeleton", which was thresholded at a FA>0.2 to include only WM voxels.

Individual subjects' FA values were projected onto this group skeleton by searching perpendicular from the skeleton for maximum FA values. Maximum FA values were chosen to restrict the analysis to the center of WM tracts (where maximum FA values are found), rather than considering voxels at the edge of tracts, that may suffer from partial volume effects. The individual registration and projection vectors obtained during the above-described process were also applied to MD data and lesion masks coregistered to b0 image. Nineteen regions part of a combined atlas (JHU WM Labels-2 mm and JHU ICBM-Tracts-maxprob-thr25-2 mm) and listed in Supplementary Table 9.2.1 were overlaid to skeletonised FA maps from patients and HC to calculate average values in each region. Given the similar behaviour of right and left tract values, left and right WM tracts were averaged.

RS fMRI analysis. After pre-processing, RS FC was assessed using independent component analysis (see Supplementary Methods).

The visual inspection of the spatial patterns, a frequency analysis of the spectra of the estimated independent components, and a template-matching procedure allowed removing components clearly related to motion-related artifacts and physiological noise, and to select nine components of interest: one sensorimotor network (Smith *et al.*, 2009) one basal ganglia network (Patenaude *et al.*, 2011a), two DMNs (DMN I and II) (Damoiseaux *et al.*, 2006), one executive control network (Seeley *et al.*, 2007), one salience network (Seeley *et al.*, 2007), one left and one right working memory networks (Damoiseaux *et al.*, 2006). For each network, global network mean RS FC z-score

(mean z-score of voxels within the family-wise error corrected mask of the network) was computed and extracted using the Marsbar toolbox.

Statistical analysis. Statistical analysis was performed using R-4.0.3 and SPSS software (version 22.0). Between-group comparisons of demographic characteristics and neuropsychological z-scores were assessed with the chi-square test (qualitative variables) or the Mann–Whitney U test/two-sample t test/linear models, according to normality assumption (quantitative variables).

Between groups voxelwise differences in FA, MD and lesions were tested using a permutation-based inference for nonparametric statistical thresholding (the "randomize" program within FSL) and two-sample t tests with age, sex and disease duration as covariates. T2-hyperintense WM lesion masks was included as voxel-wise covariate in TBSS analyses to focus only on normal appearing white matter. A correlation analysis between lesions and z-BRB-N was also performed in PPMS and SPMS. The number of permutations was set to 5000. A p value less than 0.05 (family-wise error corrected for multiple comparisons) using the threshold-free cluster enhancement option in the "randomize" tool was set.

WM tract DT-derived measures and RS FC z-scores were compared between HC and MS patients as well as between PPMS and SPMS patients using age-, sex- and scanner number-adjusted linear models. Disease duration was included as covariate in linear models assessing the differences between PPMS and SPMS.

Hierarchical regression analysis was performed to select the independent predictors of cognitive status (z-BRB-N) in PPMS and SPMS, separately. We used a within-block stepwise approach: block 1 included demographic and clinical variables (age, sex, disease duration and education); block 2 comprised all MRI measures which significantly differed between HC and MS patients. For all analyses, statistical significance was set at $p<0.05$.

All analyses were performed on the whole sample and separately on subjects acquired on Scanner 1 to assess the influence of the scanner hardware on the obtained results (i.e., validation analysis).

Results

Demographic, clinical, and neuropsychological features. PPMS and SPMS had similar sex distribution, age, years of education, EDSS, and level of fatigue. Compared to PPMS, SPMS patients had longer disease duration ($p = 0.001$) (Table 9.2.1).

Nineteen PPMS (31.6%) and 53 SPMS (43.1%) were classified as cognitively impaired ($p = 0.19$). No significant differences in z-BRB-N and cognitive domains z-scores were found between PPMS and SPMS (Table 9.2.1).

Table 9.2.1. Main demographic, clinical and neuropsychological characteristics of the subjects included in the study. Bold text indicates a statistically significant result ($p<0.05$).

	HC (n=75)	**MS (n=183)**	**p: HC vs MS**	**PPMS (n=60)**	**SPMS (n=123)**	**p: PPMS vs SPMS**
Demographic and clinical variables						
Number of women / men (%)	42 (56) / 33 (44)	102 (56) / 81 (44)	1.00[a]	29 (48) / 31 (52)	73 (60) / 50 (40)	0.21[a]
Mean Age (SD) [years]	48.2 (10.8)	50.6 (9.8)	0.10[b]	52.6 (10.6)	49.6 (9.2)	0.06[b]
Median Education (IQR) [years]	-	13.0 (8.0 – 13.0)	-	12.5 (8.0 – 13.0)	13.0 (8.0 – 13.0)	0.18[c]
Median Disease Duration (IQR) [years]	-	17.4 (11.8 – 23.0)	-	**12.7 (6.7 – 18.6)**	**17.0 (13.0 – 25.0)**	**0.001[c]**
Median EDSS (IQR)	-	6.0 (5.5 – 6.5)	-	6.0 (5.0 – 6.5)	6.0 (5.5 – 6.5)	0.80[c]
Mean MFIS (SD)	-	41.1 (16.3)	-	39.5 (19.3)	42.0 (14.3)	0.44[b]
No. of subjects scanned with S 1/ S 2 (%)	62 (83) / 13 (17)	155 (85) / 28 (15)	0.82[a]	51 (85) / 9 (15)	104 (84) / 19 (16)	1.00[a]
Cognitive performance						
Number of cognitively impaired subjects (%)	-	72 (39.3)	-	19 (31.6)	53 (43.1)	0.19[a]
Mean z-BRB-N (SD)	-	-1.0 (1.1)	-	-1.0 (1.2)	-1.1 (1.0)	0.77[d]
Mean z-Verbal memory (SD)	-	-1.3 (1.1)	-	-1.3 (1.1)	-1.3 (1.1)	0.83[d]
Mean z-Visuospatial memory (SD)	-	-1.0 (1.0)	-	-1.0 (1.0)	-1.0 (1.0)	0.51[d]
Mean z-Attention/processing speed (SD)	-	-1.2 (1.3)	-	-1.0 (1.3)	-1.3 (1.3)	0.34[d]
Mean z-Verbal fluency (SD)	-	-0.8 (1.1)	-	-0.7 (1.2)	-0.8 (1.1)	0.46[d]

[a]Chi-squared test; [b]Two-sample t test; [c]Mann-Withney U test; [d]Disease duration-adjusted linear models

Abbreviations: BRB-N=Brief Repeatable Battery of Neuropsychological Tests; EDSS=Expanded Disability Status Scale; IQR=interquartile range; HC=healthy controls; MFIS=modified fatigue impact scale; MS=multiple sclerosis; PPMS=primary progressive multiple sclerosis; S=scanner; SD=standard deviation; SPMS=secondary progressive multiple sclerosis; z=z-score.

MRI findings. Compared with HC, MS patients had lower NBV, NGMV, NWMV and NDGMV (Table 9.2.2).

TBSS revealed decreased FA and increased MD in the entire WM skeleton in MS patients compared to HC (Figure 9.2.1). Similarly, at the WM tract analysis, MS patients showed decreased FA and increased MD in all WM tract analysed (Supplementary Table 9.2.2).

Compared to PPMS, SPMS had higher frequency of T2-hyperintense WM lesions in the corpus callosum, left superior corona radiata, left anterior corona radiata, bilateral posterior corona radiata, right superior longitudinal fasciculus and bilateral inferior longitudinal fasciculus (Supplementary Figure 9.2.1).

At RS FC analysis, compared to HC, MS patients showed decreased RS FC within the sensorimotor network, DMN I and salience network (Table 9.2.3).

PPMS and SPMS had similar T2-LV, T1-LV, NBV, NGMV, NWMV and NDGMV (Table 9.2.2).

TBSS analysis showed no FA and MD differences between PPMS and SPMS patients, whereas at WM tract analysis, compared to PPMS, SPMS patients had decreased FA and increased MD values in the fornix, and higher MD values in the optic radiation and posterior corona radiata (Figure 9.2.2, Supplementary Table 9.2.2).

Finally, compared to PPMS, SPMS patients showed decreased RS FC within the basal ganglia network (Table 9.2.3).

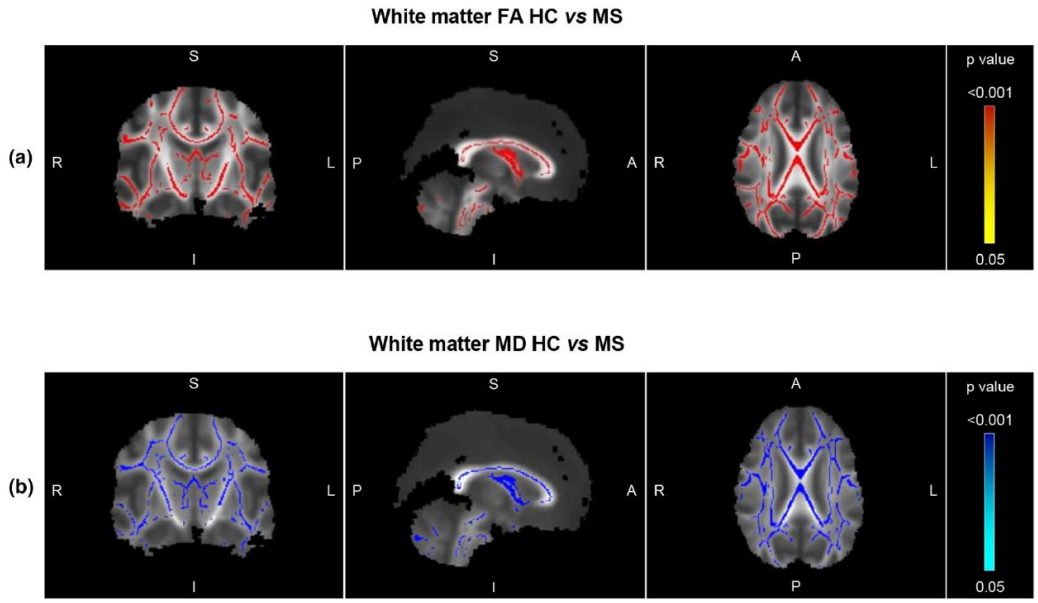
Table 9.2.2. Main MRI characteristics of healthy subjects and patients with MS. Unless otherwise specified, data are presented as mean (95% confidence interval). Bold text indicates a statistically significant result ($p<0.05$).

	HC (n=75)	**MS (n=183)**	**p Value: HC vs MS**	**Statistically significant covariates**	**PPMS (n=60)**	**SPMS (n=123)**	**p: PPMS vs SPMS**	**Statistically significant covariates**
Median T2-LV [mL]	**0.3 (0.0 – 2.8)**	**13.2 (11.6 – 14.8)**	**<0.001[a]**	Scanner number	11.7 (8.3 – 15.0)	14.0 (11.8 – 16.3)	0.13[b]	Disease duration; Scanner number
Median T1-LV [mL]	**0.2 (0.0 – 2.3)**	**9.5 (8.1 – 10.8)**	**<0.001[a]**	-	8.2 (5.3 – 11.0)	10.2 (8.3 – 12.2)	0.33[b]	Disease duration
Mean NBV [mL]	**1532 (1512 - 1552)**	**1443 (1430 - 1455)**	**<0.001[c]**	Age	1451 (1428 – 1475)	1432 (1417 – 1448)	0.19[d]	Age; Disease duration
Mean NGMV [mL]	**694 (681 - 708)**	**632 (624 - 640)**	**<0.001[c]**	Age; Scanner number	640 (624 – 657)	625 (614 - 636)	0.15[d]	Age; Disease duration; Scanner number
Mean NWMV [mL]	**801 (790 - 812)**	**775 (769 - 782)**	**<0.001[c]**	Age; Scanner number	778 (764 – 791)	774 (765 – 783)	0.64[d]	Scanner number
Mean NDGMV [mL]	**52 (51 - 53)**	**45 (44 - 46)**	**<0.001[c]**	Sex; Scanner number	46 (45 – 48)	45 (44 – 46)	0.09[d]	Disease duration; Scanner number

[a]Age-, sex- and scanner-adjusted linear models performed on log-scale; [b]Age-, sex-, disease duration- and scanner-adjusted linear models performed on log-scale; [c]Age-, sex- and scanner-adjusted linear models; [d]Age-, sex-, disease duration- and scanner-adjusted linear models.

Abbreviations: HC=healthy controls; LV=lesion volume; MS=multiple sclerosis; NBV=normalized brain volume; NDGMV=normalized deep gray matter volume; NGMV=normalized gray matter volume; NWMV=normalized white matter volume; PPMS=primary progressive multiple sclerosis; SPMS=secondary progressive multiple sclerosis.

Figure 9.2.1. Voxelwise differences in FA and MD values between HC and MS patients. A) Image shows clusters of voxels with significant (red for p value, FWE-corrected) decreased FA in MS patients compared to HC. Background image is the mean FA map derived from all subjects. B) Image shows clusters of voxels with significant (blue for p value, FWE-corrected) increased MD in MS patients compared to HC. Background image is the mean FA map derived from all subjects.



Abbreviations: FA= fractional anisotropy; FWE= family-wise error; HC= healthy controls; MD= mean diffusivity; MS= multiple sclerosis.

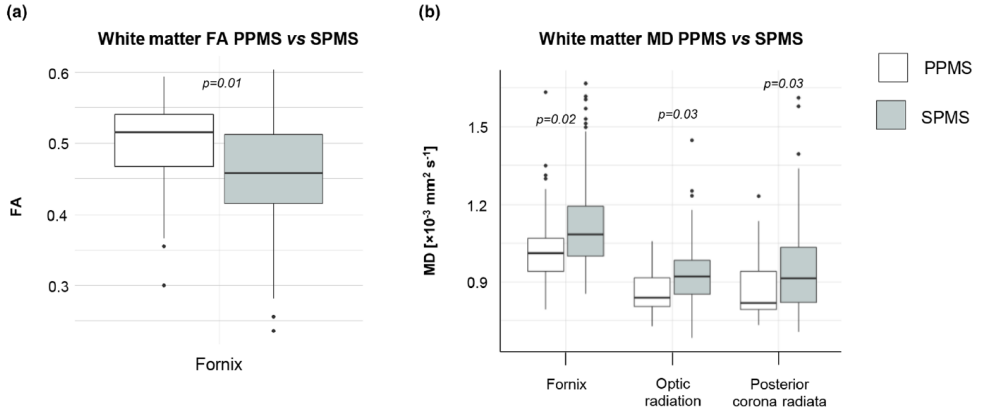
Table 9.2.3. Average z-scores of resting state functional connectivity for each group in all selected resting state networks. Unless otherwise specified, data are presented as mean (standard deviation). Bold text indicates a statistically significant result ($p<0.05$).

	HC (n=75)	**MS (n=183)**	**p: HC vs MS**	**PPMS (n=60)**	**SPMS (n=123)**	**p: PPMS vs SPMS**
Sensorimotor network	**1.67 (0.40)**	**1.49 (0.44)**	**0.005[a]**	1.42 (0.39)	1.53 (0.46)	0.07[b]
DMN I	**1.61 (0.26)**	**1.51 (0.28)**	**0.01[a]**	1.46 (0.30)	1.53 (0.27)	0.19[b]
R-working memory network	1.17 (0.14)	1.12 (0.18)	0.06[a]	1.12 (0.20)	1.12 (0.17)	0.76[b]
Executive network	0.98 (0.17)	0.98 (0.18)	0.70[a]	1.00 (0.20)	0.98 (0.17)	0.45[b]
DMN II	1.07 (0.19)	1.02 (0.23)	0.14[a]	1.00 (0.27)	1.03 (0.20)	0.56[b]
Salience network	**1.01 (0.17)**	**0.93 (0.19)**	**0.003[a]**	0.91 (0.21)	0.93 (0.18)	0.74[b]
Working memory network	0.96 (0.16)	0.95 (0.19)	0.69[a]	0.90 (0.19)	0.97 (0.19)	0.84[b]
Basal ganglia network	0.85 (0.16)	0.82 (0.17)	0.20[a]	**0.86 (0.17)**	**0.79 (0.17)**	**0.005[b]**
L-working memory network	1.16 (0.20)	1.12 (0.20)	0.36[a]	1.11 (0.23)	1.13 (0.20)	0.85[b]

[a]Age-, sex- and scanner-adjusted linear models; [b]Age-, sex-, disease duration- and scanner-adjusted linear models.

Abbreviations: DMN=default-mode network; HC=healthy controls; L=left; MS=multiple sclerosis; PPMS=primary progressive multiple sclerosis; R=right; SPMS=secondary progressive multiple sclerosis.

Figure 9.2.2. Comparisons of diffusion tensor metrics between PPMS and SPMS patients. Boxplot of FA of the fornix (A) and MD of the fornix, optic radiation and posterior corona radiata (B) in PPMS and SPMS patients. Boxes show the first and third quartiles with the line denoting the median. The upper whisker extends from the hinge to the largest value no further than 1.5 × IQR from the hinge. The lower whisker extends from the hinge to the smallest value at most 1.5 × IQR of the hinge. Data beyond the end of the whiskers are plotted individually.



Abbreviations: FA=fractional anisotropy; IQR=interquartile range; MD=mean diffusivity; PPMS=primary progressive multiple sclerosis; SPMS=secondary progressive multiple sclerosis.

Associations between MRI features and cognitive status. Table 9.2.4 summarizes the results of the hierarchical regressions analysis.

No significant correlations between z-BRB-N and lesion distribution were found in either PPMS or SPMS.

In PPMS, lower NGMV ($p < 0.001$; $\Delta R^2 = 0.29$) and decreased FA of the medial lemniscus ($p = 0.01$; $\Delta R^2 = 0.11$) were selected as significant predictors of lower z-BRB-N (adjusted $R^2 = 0.36$).

In SPMS, decreased FA of the fornix ($p < 0.001$; $\Delta R^2 = 0.35$ and lower NWMV ($p = 0.03$; $\Delta R^2 = 0.05$) and were associated with worse cognitive status (adjusted $R^2 = 0.37$).

Table 9.2.4. Hierarchical regression models assessing the predictors of global cognitive function score for PPMS and SPMS patients.

Dependent variable	Block	Significant IVs	Standardized β weights	R^2	R^2 change	P	Adjusted R^2
PPMS							
z-BRB-N	1	-	-	-	-	-	0.36
	2	NGMV	0.45	0.29	-	<0.001	
		Medial lemniscus FA	0.34	0.36	0.11	0.01	
SPMS							
z-BRB-N	1	-	-	-	-	-	0.37
	2	Fornix FA	0.53	0.35	-	<0.001	
		NWMV	0.22	0.39	0.05	0.03	

Abbreviations: BRB-N=Brief Repeatable Battery of Neuropsychological Tests; FA=fractional anisotropy; IV=independent variable; NGMV=normalized gray matter volume; NWMV=normalized white matter volume PPMS=primary progressive multiple sclerosis; SPMS=secondary progressive multiple sclerosis; z=z-score.

Validation analysis. The subgroup of participants acquired on Scanner 1 consisted of 150 MS (51 PPMS and 99 SPMS) and 62 age- and sex-matched HC. PPMS and SPMS were similar for demographic, clinical, neuropsychological and MRI variables, except for a longer disease duration in SPMS patients (Supplementary Table 9.2.3).

TBSS analysis showed no FA and MD differences between PPMS and SPMS patients. Reduced FA in the optic radiation and increased MD in the superior cerebellar peduncle, corticospinal tract and superior longitudinal fasciculus in SPMS resulted significant also at the WM tract analysis (Supplementary Table 9.2.4).

PPMS and SPMS had similar RS FC within the investigated networks.

In PPMS, both lower NGMV ($p < 0.001$; $\Delta R^2 = 0.29$) and decreased FA of the medial lemniscus ($p = 0.01$; $\Delta R^2 = 0.11$) were confirmed significantly associated with lower z-BRB-N.

Hierarchical regression confirmed the prominent association of reduced FA in the fornix and worse cognitive status in SPMS ($p < 0.001$; $\Delta R^2 = 0.23$), with the potential additional contribution of decreased FA in the medial lemniscus ($p = 0.004$; $\Delta R^2 = 0.08$).

Discussion

To determine whether there are differences in frequency and pattern of cognitive dysfunction between PPMS and SPMS patients, we administered a comprehensive battery of neuropsychological tests (Amato *et al.*, 2006). We then combined different MRI modalities, specific towards different disease-related pathological substrates, to identify the MRI variables associated with global cognitive functioning in PPMS and SPMS.

Patients with PPMS and SPMS had similar cognitive performance. Global cognitive functioning was negatively associated with lower NGMV and decreased FA of the medial lemniscus in PPMS, and with lower NWMV and decreased FA of the fornix in SPMS, the latter confirmed also in the validation analysis.

In our study, the overall prevalence of cognitive impairment in progressive MS patients investigated by the BRB-N was 39.3%, which is at the lower limit of the rates reported in previous studies, ranging from 37% to 86% (Foong *et al.*, 2000; Renner *et al.*, 2020; Ruano *et al.*, 2017; Wachowius *et al.*, 2005). The high degree of variability can be attributed to the differences in the sample characteristics, the cognitive assessment procedures and the chosen cut-off scores (Renner *et al.*, 2020). In previous studies, cognitive impairment was most commonly defined as performing 2.0 standard deviations below the normative mean on two tests (Foong *et al.*, 2000; Wachowius *et al.*, 2005), while one study applied a more liberal criterion based on an impaired performance (a score below the 5th percentile) on a single test (Renner *et al.*, 2020). To reduce the risk of false positive, we adopted a strict definition of cognitive impairment based on consensus recommendations: an age-, sex-, and education-adjusted score

below the 5th percentile of the normative sample on tests that explore at least two cognitive domains (Ruano *et al.*, 2017).

In comparison with PPMS, the prevalence of severe cognitive impairment was higher in SPMS (31.7% vs 41.3%), but the difference was not statistically significant. Our results are in line with a recent study (Renner *et al.*, 2020) which found a similar frequency of cognitive dysfunction in the two progressive phenotypes of MS.

Domain-wise comparison revealed that the composite scores for verbal memory, visuospatial memory, attention/processing speed and verbal fluency were similar in PPMS and SPMS after controlling for disease duration. Previous works reported differences between PPMS and SPMS in the performance on tests measuring information processing speed (Achiron *et al*, 2013), attention (Achiron *et al.*, 2013), memory (Achiron *et al.*, 2013; Huijbregts *et al.*, 2004; Wachowius *et al.*, 2005) and verbal fluency (Huijbregts *et al.*, 2004; Wachowius *et al.*, 2005). However, in one study patients' groups were not matched for age, sex and level of physical disability (Huijbregts *et al.*, 2004), while in others disease duration was considerably longer for patients with SPMS than for those with PPMS (Achiron *et al.*, 2013; Wachowius *et al.*, 2005).

Taken together these results suggest that PPMS and SPMS share a similar pattern of cognitive impairment in terms prevalence, severity and affected domains. Our results do appear to be robust, considering that, despite SPMS had longer disease duration than PPMS, the two patients' groups were matched for age, sex, education, physical disability, level of fatigue and no significant differences were found in terms of T2-LV, T1-LV, NBV, NGMV, NWMV and NDGMV (including the thalamus).

Compared to HC, both PPMS and SPMS showed widespread brain damage in terms of LV, NBV, NGMV, NWMV, NDGMV, WM microstructural abnormalities, and RS FC alterations in the sensorimotor network, DMN and salience network. In line with the results of previous DT MRI studies (Preziosa *et al.*, 2011; Rocca *et al.*, 2010c), WM tract analysis revealed that SPMS patients exhibited more severe microstructural alterations in the fornix, optic radiation, and posterior corona radiata compared to PPMS. Moreover, SPMS had higher occurrence of T2 lesions in in the corpus callosum, left superior corona radiata, left anterior corona radiata, bilateral posterior corona radiata, right superior longitudinal fasciculus and bilateral inferior longitudinal

fasciculus than PPMS. We also found a reduction of RS FC in the basal ganglia network in SPMS compared to PPMS, supporting the notion that different pathologic mechanisms might underlie MRI abnormalities and, consequently, clinical manifestations in these progressive phenotypes (Revesz *et al*, 1994).

No significant association was found between cognitive status and lesion distribution in either PPMS or SPMS. This result was expected since multiparametric MRI studies have consistently shown that WM lesions contribute to cognitive impairment only partially or together with damage to normal-appearing WM and GM (Rocca *et al.*, 2015b).

In PPMS, reduced NGMV accounted for a significant proportion of overall cognitive performance, suggesting that GM damage plays a crucial role in their cognitive dysfunction (Riccitelli *et al.*, 2011). Despite the pathogenic mechanism responsible for cortical atrophy is not fully understood, several studies have found a correlation between patterns of reduced GM volume and impairment in processing speed, working memory, attention, verbal memory and verbal fluency (Messina & Patti, 2014). GM atrophy can be detected even at the earliest stages of PPMS and progresses more rapidly than WM atrophy. An histopathologic study identified neuro-axonal loss and neuronal shrinkage as the possible cause for cortical atrophy in PPMS, which appears to be independent from WM demyelination (Popescu *et al*, 2015).

Consistent with previous research (Conti *et al.*, 2021a), decreased FA of the medial lemniscus was associated with worse global cognitive functioning in PPMS. The medial lemniscus pathway is essential for the analysis and integration of sensory-motor information, and microstructural damage within this tract is likely to affect integration processes, resulting in poor performance in neuropsychological tests (Conti *et al.*, 2021a).

In SPMS patients, lower NWMV was significantly associated with worse cognitive status, supporting the notion that a reduction of WM volume influences the speed of mental processing (Sanfilipo *et al*, 2006). The temporary storage and manipulation of new information demands rapid communication between different brain regions via WM tracts, which may decrease with disease progression (Sanfilipo *et al.*, 2006). However, regression analysis suggested that the integrity of the fornix might be the most critical anatomical correlate of cognitive functioning in SPMS. The fornix is the

major hippocampal efferent pathway and damage to this tract can cause episodic memory deficits in MS (Kern *et al*, 2012).

Interestingly, measures derived from the analysis of RS FC were not present among the predictors of overall cognitive performance neither in PPMS nor SPMS. Previous studies suggest that increased RS FC may be present at early disease stage to compensate structural disease-related burden, while RS FC depletion takes place in later phases contributing to a wide spectrum of clinical manifestations (Rocca *et al*, 2021b). However, the extent of structural damage accumulation might limit our sensitivity to assess the role of specific RS FC alterations for cognitive dysfunction in progressive MS (Cordani *et al*, 2022).

In PPMS and SPMS, distinct MRI abnormalities produced comparable levels of cognitive impairment. This suggests that different underlying neuropathological mechanisms may be responsible for the observed cognitive deficits in these two groups. Although there were no significant differences in the pattern of structural MRI alterations between PPMS and SPMS, subtle variations in the location or extent of damage could account for the differences in MRI predictors of global cognition. Furthermore, differences in the timing and progression of the disease could also contribute to the observed variability. Both demyelination and neurodegeneration are involved in the cognitive impairment observed in MS. However, the relative contribution of each of these mechanisms may differ between PPMS and SPMS.

This study is not without limitations. First, compared to PPMS, SPMS patients had longer disease duration. Matching these patients could be difficult since in SPMS the disease starts with a recognizable relapsing-remitting course, which is followed by a SP phase, while PPMS patients miss the relapsing-remitting stage and start with uninterrupted progression from disease onset. Second, we did not administer specific scales for the assessment of depression and anxiety, which may influence cognitive performance in MS patients (Rocca *et al.*, 2015b). Third, the study is cross-sectional, thus not allowing evaluating whether the observed MRI correlates are related to cognitive worsening. Fourth we adopted a strict definition of cognitive impairment that can neglect mild degrees of impairment. Finally, the selected demographical, clinical and MRI variables were able to explain only 36% and 37% of variance of the overall cognitive performance in PPMS and SPMS respectively. The inclusion of other

variables such as cognitive reserve could have increased the proportion of variance accounted (Rocca *et al.*, 2015b).

In conclusion, this study represents the largest multiparametric MRI exploration of the cognitive differences between PPMS and SPMS. Our results suggest that the different disease courses do not affect cognitive function in a detectably distinct manner. However, the degree of cognitive dysfunction in PPMS and SPMS patients resulted associated with different patterns of structural MRI abnormalities.

This implies that all patients with progressive MS should undergo the same routine cognitive screening and monitoring. However, the different structural substrates of cognitive dysfunction detected in PPMS and SPMS indicate a need for individualized diagnostic, treatment and management approaches.

Supplementary Material

MRI acquisition. Each subject underwent a brain MRI scan performed using two 3.0 T scanners (Scanner 1: Achieva, n=62 HCs/155 patients; Scanner 2: Ingenia, n=13 HCs/28 patients; Philips Medical Systems, Eindhoven, The Netherlands). Images acquired using the Achieva scanner included the following scans: 1) 200 sets of T2*-weighted echo planar imaging sequence for RS fMRI (TR=3000 ms; TE=35 ms, flip angle=90°, matrix size=128x128; FOV=240 mm^2; 30 contiguous axial slices, 4 mm thick); 2) dual-echo turbo spin echo (TR=2599; TE=16-80 ms; flip angle=90°; matrix=256×256; FOV=240mm^2; ETL=6; 44 contiguous axial slices, 3 mm thick); 3) 3D T1-weighted fast field echo (TR=25; TE=4.6 ms; flip angle=30°; matrix=256x256; FOV=230 mm^2; 220 contiguous axial slices, 0.8 mm thick); and 4) pulsed-gradient spin echo echo planar imaging (TR/TE=8938/58 ms, acquisition matrix=112x88, FOV=240x231 mm^2, slice thickness=2.3 mm, 55 contiguous axial slices) with SENSE (acceleration factor=2) and diffusion gradients applied in 35 non-collinear directions. Two optimized b factors were used: b1=0, b2=900 s/mm^2.

Images acquired using the Ingenia scanner included: 1) 320 sets of T2*-weighted echo planar imaging sequence for RS fMRI (TR=1567 ms; TE=35 ms, flip angle=70°; multi-band factor=2, matrix size=96×96; FOV=240 x 240 mm^2; 48 contiguous axial slices, 3 mm thick); 2) 3D FLAIR (TR=4800 ms; TE=270 ms; inversion time [TI]=1650 ms; matrix size=256 × 256; FOV=256 × 256 mm^2; ETL=167; 192 contiguous sagittal

slices, 1 mm thick); 3) 3D T2-weighted sequence (TR=2500 ms; TE=330 ms; matrix size=256 × 256; FOV=256 × 256 mm^2; ETL = 117; 192 contiguous sagittal slices, 1 mm thick); 4) 3D T1-weighted turbo field echo (TR=7 ms; TE=3.2 ms; TI=1000 ms; flip angle=8°; matrix size=256 × 256; FOV=256 × 256 mm^2; 204 contiguous sagittal slices, 1 mm thick); and 5) axial pulsed-gradient spin echo single shot diffusion-weighted echo planar imaging (TR=5900 ms, TE=78 ms; FOV=240×233 mm, pixel size=2.14×2.69 mm, 56 slices, 2.3 mm-thick, matrix=112×85). Three shells at b-value=700/1000/2855 s/mm^2 along 6/30/60 non-collinear directions and 10 b=0 volumes were acquired. Three additional b=0 volumes with reversed polarity of gradients for distortion correction.

Conventional MRI analysis. For MS patients acquired on Scanner 1, T2-hyperintense WM lesions were manually identified on dual-echo scans by an experienced observer, and LV was quantified using a semi-automatic local thresholding segmentation technique (Jim 7.0, Xinapse Systems Ltd, Colchester, UK). For patients acquired on Scanner 2, T2-hyperintense WM lesions were identified using a fully automated approach based on a cascade of two 3D patch-wise convolutional neural networks, using 3D fluid-attenuated inversion recovery and 3D T1-weighted MRI sequences as input images (Valverde *et al.*, 2017), and total T2-hyperintense LV was calculated. On both scanners, normalized brain, WM and GM volumes (NBV, NWMV and NGMV, respectively) were calculated using FSL SIENAx software on lesion-filled (Battaglini *et al.*, 2012) 3D T1-weighted images. The NDGMV was also calculated as a sum of the normalized volumes of bilateral thalamus, caudate, putamen, pallidum, amygdala and accumbens measured with the FIRST tool (Patenaude *et al.*, 2011a).

RS fMRI analysis.

Pre-processing. The main pre-processing steps were performed using SPM12 and REST software (http://resting-fmri.sourceforge.net). After discarding the first two timepoints, RS fMRI scans were rigid-body realigned to the mean of each session to correct for minor head movements. After rigid registration of realigned images to the lesion filled 3D T1-weighted scan, RS fMRI images were normalized to the MNI template using a standard affine transformation followed by non-linear warping. Linear detrending and band-pass filtering (0.01-0.08 Hz) were performed to partially remove

low-frequency drifts and physiological high-frequency noise. Finally, normalized images were smoothed using a 3D 6-mm Gaussian kernel.

Independent component analysis. RS FC was assessed using ICA and the GIFT software (https://trendscenter.org/software/gift) following three main steps: (i) data reduction, (ii) group ICA, and (iii) back reconstruction, as described in detail elsewhere (Calhoun *et al.*, 2001). The number of independent group components was 40, a dimension determined using the minimum description length criterion (Calhoun *et al.*, 2001). The statistical reliability of the independent components decomposition was tested using the ICASSO toolbox (Himberg *et al.*, 2004).

Supplementary Table 9.2.1. List of white matter regions studied.

White matter regions	
Inferior cerebellar peduncle	Body of corpus callosum
Middle cerebellar peduncle	Splenium of corpus callosum
Superior cerebellar peduncle	Anterior corona radiate
Medial lemniscus	Superior corona radiate
Corticospinal tract	Posterior corona radiate
Cingulum	Superior longitudinal fasciculus
Fornix	
Inferior longitudinal fasciculus	
Inferior fronto-occipital fasciculus	
Optic radiation	
Uncinate fasciculus	
Anterior thalamic radiation	
Genu of corpus callosum	

Supplementary Table 9.2.2. Diffusion tensor MRI-derived metrics of the different WM fiber bundles studied from healthy volunteers and patients with MS. Bold text indicates a statistically significant result ($p<0.05$). Unless otherwise specified, data are presented as mean (95% confidence interval).

	HC (n=75)	MS (n=183)	p: HC vs MS	PPMS (n=60)	SPMS (n=123)	p: PPMS vs SPMS
Inferior cerebellar peduncle average FA	**0.57 (0.56 – 0.58)**	**0.53 (0.52 – 0.54)**	**<0.001**[a]	0.53 (0.52 – 0.55)	0.53 (0.52 – 0.54)	0.53[b]
Middle cerebellar peduncle average FA	**0.58 (0.57 – 0.59)**	**0.55 (0.55 – 0.56)**	**<0.001**[a]	0.55 (0.54 - 0.56)	0.55 (0.55 – 0.56)	0.99[b]
Superior cerebellar peduncle average FA	**0.69 (0.68 – 0.70)**	**0.67 (0.66 – 0.68)**	**<0.001**[a]	0.68 (0.66 – 0.69)	0.69 (0.66 – 0.68)	0.26[b]
Medial lemniscus average FA	**0.60 (0.59 – 0.61)**	**0.57 (0.57 – 0.58)**	**<0.001**[a]	0.58 (0.57 – 0.59)	0.57 (0.56 - 0.58)	0.46[b]
Corticospinal tract average FA	**0.62 (0.61 – 0.62)**	**0.60 (0.60 – 0.61)**	**<0.001**[a]	0.60 (0.59 – 0.61)	0.60 (0.60 – 0.61)	0.96[b]
Cingulum average FA	**0.60 (0.59 – 0.61)**	**0.56 (0.55 – 0.57)**	**<0.001**[a]	0.57 (0.55 – 0.58)	0.56 (0.55 – 0.57)	0.31[b]
Fornix average FA	**0.56 (0.55 – 0.58)**	**0.47 (0.46 – 0.48)**	**<0.001**[a]	**0.49 (0.47 – 0.51)**	**0.46 (0.45 – 0.47)**	**0.01**[b]
Inferior longitudinal fasciculus average FA	**0.50 (0.49 – 0.51)**	**0.46 (0.45 – 0.46)**	**<0.001**[a]	0.46 (0.45 – 0.47)	0.45 (0.45 – 0.46)	0.61[b]
Inferior fronto-occipital fasciculus average FA	**0.51 (0.50 – 0.52)**	**0.47 (0.46 – 0.47)**	**<0.001**[a]	0.47 (0.46 – 0.48)	0.46 (0.46 – 0.47)	0.60[b]
Optic radiation average FA	**0.63 (0.62 – 0.65)**	**0.54 (0.53 – 0.55)**	**<0.001**[a]	0.56 (0.54 – 0.58)	0.54 (0.52 – 0.55)	0.08[b]
Uncinate fasciculus average FA	**0.51 (0.50 – 0.53)**	**0.48 (0.47 – 0.49)**	**<0.001**[a]	0.48 (0.46 – 0.49)	0.48 (0.47 – 0.49)	0.87[b]
Anterior thalamic radiation average FA	**0.58 (0.57 – 0.59)**	**0.57 (0.56 – 0.57)**	**0.003**[a]	0.57 (0.56 – 0.58)	0.57 (0.56 – 0.58)	0.51[b]
Genu of corpus callosum average FA	**0.72 (0.71 – 0.74)**	**0.68 (0.67 – 0.69)**	**<0.001**[a]	0.68 (0.66 - 0.70)	0.67 (0.66 – 0.69)	0.66[b]
Body of corpus callosum average FA	**0.73 (0.71 – 0.74)**	**0.64 (0.63 – 0.65)**	**<0.001**[a]	0.66 (0.64 – 0.68)	0.64 (0.62 – 0.65)	0.13[b]
Splenium of corpus callosum average FA	**0.79 (0.78 – 0.80)**	**0.74 (0.73 – 0.75)**	**<0.001**[a]	0.75 (0.73 - 0.77)	0.74 (0.72 – 0.75)	0.18[b]
Anterior corona radiata average FA	**0.46 (0.45 – 0.47)**	**0.42 (0.41 – 0.42)**	**<0.001**[a]	0.42 (0.41 – 0.43)	0.42 (0.41 – 0.43)	0.75[b]
Superior corona radiata average FA	**0.48 (0.48 – 0.49)**	**0.45 (0.45 – 0.46)**	**<0.001**[a]	0.45 (0.44 – 0.46)	0.45 (0.45 – 0.46)	0.57[b]
Posterior corona radiata average FA	**0.50 (0.49 – 0.51)**	**0.45 (0.44 – 0.45)**	**<0.001**[a]	0.45 (0.44 – 0.47)	0.44 (0.43 – 0.45)	0.38[b]
Superior longitudinal fasciculus average FA	**0.51 (0.50 – 0.52)**	**0.49 (0.49 – 0.50)**	**<0.001**[a]	0.49 (0.48 – 0.50)	0.49 (0.48 – 0.45)	0.87[b]

Inferior cerebellar peduncle average MD	**0.73 (0.72 – 0.74)**	**0.77 (0.77 – 0.78)**	**<0.001**[a]	0.76 (0.75 – 0.78)	0.77 (0.76 – 0.78)	0.20[b]
Middle cerebellar peduncle average MD	**0.70 (0.69 – 0.71)**	**0.73 (0.72 – 0.74)**	**<0.001**[a]	0.73 (0.71 – 0.74)	0.73 (0.72 – 0.74)	0.52[b]
Superior cerebellar peduncle average MD	**0.80 (0.79 – 0.81)**	**0.83 (0.82 – 0.84)**	**<0.001**[a]	0.82 (0.80 – 0.84)	0.84 (0.83 – 0.85)	0.10[b]
Medial lemniscus average MD	**0.70 (0.69 – 0.71)**	**0.73 (0.72 – 0.74)**	**<0.001**[a]	0.72 (0.71 – 0.74)	0.73 (0.72 – 0.74)	0.55[b]
Corticospinal tract average MD	**0.71 (0.71 – 0.72)**	**0.74 (0.74 – 0.75)**	**<0.001**[a]	0.74 (0.72 – 0.75)	0.75 (0.74 – 0.75)	0.25[b]
Cingulum average MD	**0.73 (0.72 – 0.74)**	**0.75 (0.75 – 0.76)**	**<0.001**[a]	0.75 (0.74 – 0.76)	0.76 (0.75 – 0.76)	0.31[b]
Fornix average MD	**0.92 (0.82 – 0.96)**	**1.11 (1.08 – 1.14)**	**<0.001**[a]	**1.05 (0.99 – 1.11)**	**1.14 (1.10 – 1.18)**	**0.02**[b]
Inferior longitudinal fasciculus average MD	**0.79 (0.77 – 0.80)**	**0.86 (0.85 – 0.87)**	**<0.001**[a]	0.85 (0.83 – 0.86)	0.86 (0.85 – 0.87)	0.25[b]
Inferior fronto-occipital fasciculus average MD	**0.78 (0.77 – 0.79)**	**0.84 (0.83 – 0.85)**	**<0.001**[a]	0.83 (0.82 – 0.85)	0.85 (0.83 – 0.86)	0.24[b]
Optic radiation average MD	**0.78 (0.76 – 0.80)**	**0.91 (0.89 – 0.92)**	**<0.001**[a]	**0.88 (0.85 – 0.91)**	**0.92 (0.90 – 0.94)**	**0.03**[b]
Uncinate fasciculus average MD	**0.78 (0.76 – 0.81)**	**0.84 (0.82 – 0.85)**	**<0.001**[a]	0.84 (0.81 – 0.87)	0.83 (0.81 – 0.85)	0.65[b]
Anterior thalamic radiation average MD	**0.73 (0.72 – 0.73)**	**0.75 (0.74 – 0.76)**	**<0.001**[a]	0.75 (0.74 – 0.77)	0.75 (0.74 – 0.76)	0.98[b]
Genu of corpus callosum average MD	**0.79 (0.77 – 0.81)**	**0.86 (0.85 – 0.88)**	**<0.001**[a]	0.85 (0.82 – 0.88)	0.87 (0.85 – 0.89)	0.39[b]
Body of corpus callosum average MD	**0.76 (0.73 – 0.79)**	**0.88 (0.86 – 0.90)**	**<0.001**[a]	0.86 (0.82 – 0.90)	0.89 (0.87 – 0.92)	0.20[b]
Splenium of corpus callosum average MD	**0.72 (0.69 – 0.74)**	**0.82 (0.80 – 0.83)**	**<0.001**[a]	0.80 (0.76 – 0.83)	0.83 (0.81 – 0.85)	0.12[b]
Anterior corona radiata average MD	**0.77 (0.76 – 0.79)**	**0.84 (0.83 – 0.85)**	**<0.001**[a]	0.83 (0.81 – 0.85)	0.84 (0.83 – 0.85)	0.49[b]
Superior corona radiata average MD	**0.71 (0.70 – 0.73)**	**0.78 (0.77 – 0.79)**	**<0.001**[a]	0.77 (0.75 – 0.79)	0.79 (0.77 – 0.80)	0.18[b]
Posterior corona radiata average MD	**0.77 (0.74 – 0.80)**	**0.92 (0.90 – 0.94)**	**<0.001**[a]	**0.89 (0.85 – 0.93)**	**0.94 (0.92 – 0.97)**	**0.03**[b]
Superior longitudinal fasciculus average MD	**0.73 (0.72 – 0.74)**	**0.78 (0.77 – 0.79)**	**<0.001**[a]	0.77 (0.75 – 0.78)	0.78 (0.77 – 0.79)	0.09[b]

Note. Average MD is expressed in units of [$\times 10^{-3}$ mm^2 s^{-1}], FA is dimensionless.

[a]Age-, sex- and scanner-adjusted linear models; [b]Age-, sex-, disease duration- and scanner-adjusted linear models.

Abbreviations: FA=fractional anisotropy; HC=healthy controls; MD=mean diffusivity; MS=multiple sclerosis; PPMS=primary progressive multiple sclerosis; SPMS=secondary progressive multiple sclerosis; WM=white matter.

Supplementary Table 9.2.3. Main demographic, clinical, neuropsychological and structural MRI characteristics of the subgroup of subjects acquired on Scanner 1. Bold text indicates a statistically significant result ($p<0.05$).

	HC (n=62)	MS (n=150)	p: HC vs MS	PPMS (n=51)	SPMS (n=99)	p: PPMS vs SPMS
Demographic and clinical variables						
Number of women / men (%)	35 (56) / 27 (44)	82 (55) / 68 (45)	0.93[a]	25 (49) / 26 (51)	57 (58) / 42 (42)	0.41[a]
Mean Age (SD) [years]	48.8 (10.6)	51.2 (9.6)	0.12[b]	53.3 (10.6)	50.1 (8.8)	0.07[b]
Median Education (IQR) [years]	-	13.0 (8.0 – 13.0)	-	12.0 (8.0 – 13.0)	13.0 (8.0 – 14.0)	0.14[c]
Median Disease Duration (IQR) [years]	-	16.0 (12.0 – 24.0)	-	**13.0 (7.6 – 19.0)**	**17.0 (13.0 – 26.0)**	**0.004[c]**
Median EDSS (IQR)	-	6.0 (5.5 – 6.5)	-	6.0 (5.5 – 6.5)	6.0 (5.5 – 6.5)	0.56[c]
Mean MFIS (SD)	-	41.8 (16.2)	-	40.1 (20.0)	42.7 (13.5)	0.45[b]
Cognitive performance						
Number of cognitively impaired subjects (%)	-	57 (38.0)	-	16 (31.4)	41 (41.4)	0.31[a]
Mean z-BRB-N (SD)	-	-1.0 (1.1)	-	-1.0 (1.2)	-1.1 (1.0)	0.46[b]
Mean z-Verbal memory (SD)	-	-1.3 (1.1)	-	-1.4 (1.1)	-1.3 (1.1)	0.60[b]
Mean z-Visuospatial memory (SD)	-	-1.0 (1.0)	-	-1.0 (1.0)	-1.0 (1.1)	0.95[b]
Mean z-Attention/processing speed (SD)	-	-1.2 (1.3)	-	-1.0 (1.3)	-1.3 (1.3)	0.12[b]
Mean z-Verbal fluency (SD)	-	-0.8 (1.1)	-	-0.8 (1.2)	-0.8 (1.1)	0.88[b]
MRI measures						
Median T2-LV (CI) [mL]	**0.3 (0.0 – 2.4)**	**9.4 (8.1 – 10.8)**	**<0.001[d]**	8.2 (5.5 – 10.9)	10.2 (8.3 – 12.1)	0.14[e]
Median T1-LV (CI) [mL]	**0.3 (0.0 – 3.0)**	**12.9 (11.3 – 14.6)**	**<0.001[d]**	11.6 (8.3 – 14.8)	13.8 (11.5 – 16.1)	0.19[e]
Mean NBV (CI) [mL]	**1526 (1503 – 1550)**	**1435 (1421 – 1450)**	**<0.001[f]**	1445 (1418 – 1472)	1427 (1409 – 1445)	0.29[g]
Mean NGMV (CI) [mL]	**702 (687 – 717)**	**636 (627 – 646)**	**<0.001[f]**	647 (629 – 665)	629 (617 – 641)	0.12[g]
Mean NWMV (CI) [mL]	**824 (811 – 838)**	**799 (791 – 807)**	**0.002[f]**	798 (783 – 814)	798 (788 – 809)	0.98[g]
Mean NDGMV (CI) [mL]	**51 (50 – 52)**	**45 (44 – 46)**	**<0.001[f]**	46 (44 – 47)	44 (43 – 45)	0.054[g]

[a]Chi-squared test; [b]Two-sample t test; [c]Mann-Withney U test; [d]Age- and sex-adjusted linear models performed on log-scale; [e]Age-, sex- and disease duration-adjusted linear models performed on log-scale; [f]Age- and sex-adjusted linear models; [g]Age-, sex- and disease duration-adjusted linear models.

Abbreviations: BRB-N=Brief Repeatable Battery of Neuropsychological Tests; EDSS=Expanded Disability Status Scale; IQR=interquartile range; HC=healthy controls; LV=lesion volume; MFIS=modified fatigue impact scale; MS=multiple sclerosis; NBV=normalized brain volume; NDGMV=normalized deep gray matter volume; NGMV=normalized gray matter volume; NWMV=normalized white matter volume; PPMS=primary progressive multiple sclerosis; S=scanner; SD=standard deviation; SPMS=secondary progressive multiple sclerosis; z=z-score.

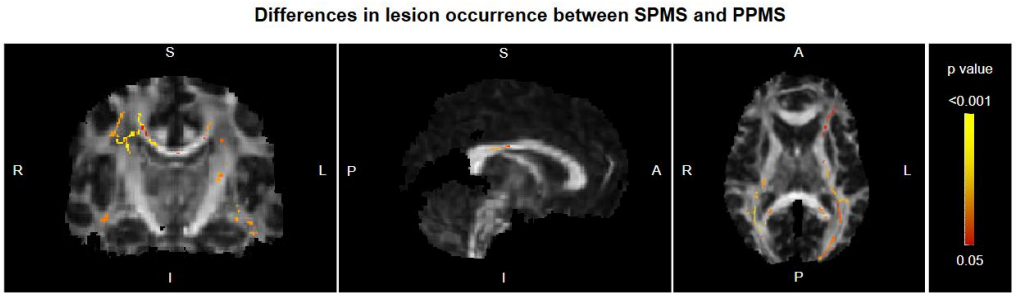
Supplementary Table 9.2.4. Diffusion tensor MRI-derived metrics of the different WM fiber bundles studied from PPMS and SPMS acquired on Scanner 1. Bold text indicates a statistically significant result ($p<0.05$). Unless otherwise specified, data are presented as mean (95% confidence interval).

	PPMS (n=51)	**SPMS (n=99)**	**p**
Optic radiation average FA	**0.56 (0.54 – 0.58)**	**0.54 (0.52 – 0.55)**	**0.05**[a]
Superior cerebellar peduncle average MD	**0.82 (0.80 – 0.83)**	**0.84 (0.83 – 0.85)**	**0.04**[a]
Corticospinal tract average MD	**0.73 (0.72 – 0.74)**	**0.75 (0.74 – 0.75)**	**0.02**[a]
Superior longitudinal fasciculus average MD	**0.77 (0.75 – 0.78)**	**0.79 (0.78 – 0.80)**	**0.05**[a]

Note. Average MD is expressed in units of [$\times 10^{-3}$ mm^2 s^{-1}], FA is dimensionless.
[a]Age-, sex- and disease duration-adjusted linear models.

Abbreviations: FA=fractional anisotropy; MD=mean diffusivity; PPMS=primary progressive multiple sclerosis; SPMS=secondary progressive multiple sclerosis; WM=white matter.

Supplementary Figure 9.2.1. Voxelwise differences of T2-hyperintense white matter lesions between PPMS and SPMS patients. The image shows voxels with a significantly (red for p value, FWE-corrected) higher frequency of white matter lesions in SPMS patients compared to PPMS patients. Background image is the mean FA map derived from all subjects.



Abbreviations: FA=fractional anisotropy; FWE=family-wise error; PPMS=primary progressive multiple sclerosis; SPMS=secondary progressive multiple sclerosis.

9.3 Cognitive phenotypes in multiple sclerosis: mapping the spectrum of impairment

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ORIGINAL COMMUNICATION

Cognitive phenotypes in multiple sclerosis: mapping the spectrum of impairment

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Abstract

Background Available criteria for cognitive phenotypes in multiple sclerosis (MS) do not consider the severity of impairment.

Objectives To identify cognitive phenotypes with varying degrees of impairment in MS patients and describe their demographic, clinical and MRI characteristics.

Methods Two hundred and forty-three MS patients and 158 healthy controls underwent neuropsychological tests to assess memory, attention, and executive function. For each domain, mild impairment was defined as performing 1.5 standard deviations below the normative mean on two tests, while the threshold for significant impairment was 2 standard deviations. Patients were classified into cognitive phenotypes based on severity of the impairment (mild/significant) and number of domains affected (one/more).

Results Five cognitive phenotypes emerged: Preserved cognition (PC; 56%), Mild Single-Domain Impairment (MSD; 15%), Mild Multi-Domain Impairment (MMD; 9%), Significant Single-Domain Impairment (SSD; 12%), Significant Multi-Domain Impairment (SMD; 8%). Compared with PC, MSD patients were older, had longer disease duration (DD) and higher T2-hyperintense lesion volume (LV; all $p \leq 0.02$); MMD patients were older, had longer DD, higher disability, higher T2 LV and lower thalamic volume (all $p \leq 0.01$); SSD patients had longer DD and lower gray matter cortical volume, thalamic, caudate, putamen and accumbens volumes (all $p \leq 0.04$); and SMD patients were older, had longer DD, higher disability and more extensive structural damage in all brain regions explored (all $p \leq 0.03$), except white matter and amygdala volumes.

Conclusions We identified five cognitive phenotypes with graded levels of impairment. These phenotypes were characterized by distinct demographic, clinical and MRI features, indicating potential variations in the neural substrates of dysfunction throughout disease stages.

Keywords Cognitive dysfunction · Multiple sclerosis · Magnetic resonance imaging · Cognitive phenotypes

Introduction

Up to 65% (Amato *et al*, 2010; Benedict *et al.*, 2020) of MS patients exhibit deficits in at least one cognitive domain. Speed of information processing (Amato *et al.*, 2010; Benedict *et al.*, 2020), complex aspects of attention (Amato *et al.*, 2010), episodic memory (Amato *et al.*, 2010; Benedict *et al.*, 2020), working-memory (Amato *et al.*, 2010) and executive function (Amato *et al.*, 2010; Benedict *et al.*, 2020; Marchesi *et al*, 2021) are commonly affected. Deficit in visuospatial processing and social cognition have also been reported but less frequently (Benedict *et al.*, 2020; Chalah & Ayache, 2017). Linguistic abilities, semantic memory and attention span are usually spared (Amato *et al.*, 2010; Benedict *et al.*, 2020).

Cognitive impairment is present in all disease stages and clinical phenotypes although patients with progressive MS (PMS) (i.e., primary and secondary progressive MS) generally have more severe deficits than those with RR MS (Benedict *et al.*, 2020). Cognitive dysfunction may be subtle, particularly in the early phases, and the trajectory of cognitive worsening is characterized by great inter-patient variability (Amato *et al.*, 2010). Although the precise sequence of decline of different cognitive domains remains unknown, previous research has suggested that verbal fluency (Achiron *et al*, 2005; De Meo *et al.*, 2021a) and verbal memory (Achiron *et al.*, 2005; Amato *et al*, 2001; De Meo *et al.*, 2021a) were likely the first cognitive functions to be impaired, followed by a decrease in information processing speed (Achiron *et al.*, 2005; De Meo *et al.*, 2021a), visuospatial memory (Achiron *et al.*, 2005; Amato *et al.*, 2001; De Meo *et al.*, 2021a), and executive function (De Meo *et al.*, 2021a). Conversely, Wojcik and colleagues (Wojcik *et al*, 2022) suggested a different sequence of decline. According to their findings IPS is the first cognitive function to be impaired in MS patients, followed by visual learning, verbal learning, working memory/attention, and executive function. However, other studies found that impaired IPS, executive function, and non-verbal memory can be detected from the early stages of MS (Deloire *et al*, 2011; Feuillet *et al*, 2007; Schulz *et al*, 2006).

In MS patients, cognitive performance is often dichotomized into two categories (impaired, not impaired), and such categorization may not fully capture the complexity of cognitive manifestations in MS. Recent studies have attempted to better characterize the heterogeneity of cognitive deficits in MS by identifying groups of patients who

share similar cognitive profiles termed “cognitive phenotypes”. Two investigations (De Meo *et al.*, 2021a; Podda *et al.*, 2021) adopted an empirical approach by using latent profile analysis to define patterns of cognitive performance examining neuropsychological data. Through this method, multiple profiles of impairment characterized by distinct demographic, clinical and MRI features have been extracted (De Meo *et al.*, 2021a; Podda *et al.*, 2021). Despite being appealing, this methodology does not generate operational criteria that would be applicable by clinicians and researchers. To overcome this issue, other studies (Hancock *et al*, 2022a; Leavitt *et al*, 2018) used an a priori classification approach whereby cognitive phenotypes are designated by specific impairment criteria. Hancock et al. (Hancock *et al.*, 2022a) defined impairment as the presence of at least two test scores that fell below a cut-off value (<1.0 or <1.5 standard deviations [SD] below the normative mean) within a given cognitive domain. The authors then categorized MS patients into phenotypes based on the number of impaired domains (“intact”, “single domain”, “bi-domain”, and “multi-domain”). Leavitt et al. (Leavitt *et al.*, 2018) established cognitive phenotypes according to memory and processing speed capabilities. Individuals who scored 1.0 SD below the normative value on at least one memory measure were classified as memory-impaired, while those who scored 1.0 SD below the normative mean on two processing speed measures were classified as processing speed-impaired. These criteria were then used to classify MS patients into one of four cognitive phenotypes: “not impaired”, “memory-impaired”, “processing speed-impaired”, or “memory and processing speed-impaired”.

These studies provided two sets of operational criteria which allow to identify four cognitive phenotypes ranging from preserved cognition to multi-domain impairment (Hancock *et al.*, 2022a; Leavitt *et al.*, 2018). However, these investigations did not take into account the degree of impairment and did not provide MRI features of cognitive phenotypes. MRI studies have greatly helped to identify the neuroanatomical substrates of cognitive dysfunction, including the presence, extent and location of white matter (WM) lesions (Rocca *et al.*, 2015b), gray matter (GM) atrophy (Preziosa *et al.*, 2016), normal-appearing WM damage (Preziosa *et al.*, 2016) and abnormal functional activation patterns (Rocca *et al*, 2017).

The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) (2013) was the first major attempt to bring cohesion in the field of neurocognitive disorders. The Neurocognitive Disorders Work Group for DSM-5 described specific cognitive domains for assessment (learning and memory, complex attention, executive function, language, perceptual-motor function, and social cognition) and developed a taxonomy of cognitive disorders that lies on a continuum of cognitive impairment from normality to significant impairment (Sachdev *et al.*, 2014). Nonetheless, these terms are not frequently used in the MS research field.

The one exception is a recent study (Hancock *et al*, 2022b) that explored the prevalence of Mild and Major Neurocognitive Disorder (NCD) in 210 MS patients. According to the DSM-5 criteria, 66.7% of the sample was diagnosed with Mild NCD, while 20.5% had Major NCD. The Authors categorized MS patients as having Mild NCD or Major NCD according to the level of cognitive decline and the loss of independence, however, clear cut-off thresholds were not established.

Against this background, the aim of this study was to apply the classification criteria proposed by the Neurocognitive Disorders Work Group for DSM-5 (Sachdev *et al.*, 2014), amended for MS (Eijlers *et al*, 2017; Hancock *et al.*, 2022a), to assess mild and significant impairments in learning and memory, complex attention and executive function in a relatively large cohort of MS patients. We then identified unique cognitive phenotypes according to the degree of impairment (i.e., mild *vs* significant) and the number of affected domains (i.e., single-domain *vs* multidomain). To gain additional insights into the mechanisms underlying the distinction between the main cognitive phenotypes, we also explored their main demographic, clinical and structural MRI features, including T2 LV and atrophy.

Methods

Study population. We enrolled 243 MS patients and 158 age-, sex-, education-matched HCs. Inclusion criteria were: 1) age ≥18 years; 2) Italian native speaking; 3) no major systemic, psychiatric or neurological diseases (other than MS); and 4) a score ≤9 on the Montgomery-Åsberg Depression Rating Scale (MADRS) (Hawley *et al*, 2002) (i.e., no depressive symptoms). MS patients had also to be relapse- and steroid-free for

at least three months before cognitive assessment and have a stable disease-modifying treatment from at least 6 months.

Clinical and neuropsychological assessment. All participants were administered the BRB-N (Amato *et al.*, 2006) and the computerized version of the WCST (Heaton *et al*, 1993b) to assess cognitive function across three domains (Sachdev *et al.*, 2014): learning and memory (SRT-lts, (Amato *et al.*, 2006) SRT-cltr, (Amato *et al.*, 2006) SRT-delayed recall, (Amato *et al.*, 2006) SPART (Amato *et al.*, 2006) and SPART-delayed recall (Amato *et al.*, 2006)), complex attention (PASAT 3" (Amato *et al.*, 2006), PASAT 2" (Amato *et al.*, 2006) and SDMT (Amato *et al.*, 2006)) and executive function (WLG (Amato *et al.*, 2006) and number of perseverative errors and number of perseverative responses on the WCST (Heaton *et al.*, 1993b)). Age-, sex-, and education-adjusted scores were calculated based on normative data, and z-scores were obtained for each neuropsychological measure (Amato *et al.*, 2006; Heaton *et al.*, 1993b). According to the traditional dichotomous classification of cognitive dysfunction, participants who scored below the 5th percentile of the normative sample on tests assessing at least two different cognitive domains were classified as cognitively impaired (Amato *et al*, 2018a).

On the same day, MS patients underwent a neurological examination with EDSS (Kurtzke, 1983) score rating and definition of clinical phenotype (RR or progressive) (Polman *et al*, 2011). Patients with a RR phenotype were further divided into early- (disease duration <5 years) and late-RRMS (disease duration ≥5 years) (Debernard *et al*, 2013).

Cognitive phenotyping. Each cognitive domain was considered mildly impaired if a minimum of two cognitive tests in that domain were more than 1.5 SD below the normative mean (Eijlers *et al.*, 2017; Fischer *et al*, 2014; Sachdev *et al.*, 2014), whereas if two or more cognitive tests fell below 2 SD the domain was defined significantly impaired (Eijlers *et al.*, 2017; Fischer *et al.*, 2014; Sachdev *et al.*, 2014). The number of impaired cognitive domains (i.e., none, one or more than one) and the severity of the impairment (i.e., not impaired, mild or significant) were examined to determine cognitive phenotypes.

Five phenotypes were considered: 1. Mild Single-Domain Impairment (MSD), defined as having mild impairment in one of the three cognitive domains; 2. Mild Multi-

Domain Impairment (MMD), defined as having mild impairment in two or more of the three cognitive domains; 3. Significant Single-Domain Impairment (SSD), defined as having significant impairment in one of the three cognitive domains; 4. Significant Multi-Domain Impairment (SMD), defined as having significant impairment in two or more of the three cognitive domains; and 5. PC included patients with no impairment in any of the three cognitive domains.

The same criteria were also applied to HCs in order to assess the probability of false positive results.

Cognitive phenotypes were generated for participants who completed at least two tests in each cognitive domain (78 HC and 243 MS).

MRI acquisition and analysis. Within 2 days of clinical and neuropsychological assessment, 210 MS patients and 139 HCs underwent brain MRI scans for brain T2-hyperintense WM lesions and atrophy quantification using two 3.0 T scanners (Philips Medical Systems, Eindhoven, The Netherlands; Scanner 1: 92 MS patients and 70 age-, sex-, education-matched HCs; Scanner 2: 118 MS patients and 69 age-, sex-, education-matched HCs). By using the Achieva scanner, we acquired dual-echo turbo spin echo (TR=2599 ms; TE=16-80 ms; flip angle=90°; matrix=256 × 256; FOV=240 × 240 mm2; ETL=6; 44 contiguous axial slices, 3 mm thick) and 3D T1-weighted fast field echo (TR=25; TE=4.6 ms; flip angle=30°; matrix=256x256; FOV=230 mm2; 220 contiguous axial slices, 0.8 mm thick).

Images acquired using the Ingenia scanner included: 1) 3D FLAIR (TR=4800 ms; TE=270 ms; TI=1650 ms; matrix size=256 × 256; FOV=256 × 256 mm2; ETL=167; 192 contiguous sagittal slices, 1 mm thick); 2) 3D T2-weighted sequence (TR=2500 ms; TE=330 ms; matrix size=256 × 256; FOV=256 × 256 mm2; ETL=117; 192 contiguous sagittal slices, 1 mm thick); and 3) 3D T1-weighted turbo field echo (TR=7 ms; TE=3.2 ms; TI=1000 ms; flip angle=8°; matrix size=256 × 256; FOV=256 × 256 mm2; 204 contiguous sagittal slices, 1 mm thick).

For MS patients acquired on Scanner 1, T2-hyperintense WM lesions were manually identified on dual-echo scans by an experienced observer, and LV was quantified using a semi-automatic local thresholding segmentation technique (Jim 7.0, Xinapse Systems Ltd, Colchester, UK). For patients acquired on Scanner 2, T2-hyperintense WM lesions were identified using a fully automated approach based on a cascade of two 3D patch-

wise convolutional neural networks, using 3D fluid-attenuated inversion recovery and 3D T1-weighted MRI sequences as input images (Valverde *et al.*, 2017), and total T2-hyperintense LV was calculated after a visual check of the results of the automated method. On both scanners, normalized brain, WM and cortical GM volumes (NBV, NWMV and NCGMV, respectively) were calculated using FSL SIENAx software on lesion-filled (Battaglini *et al.*, 2012) 3D T1-weighted images. The normalized volumes of bilateral hippocampus, thalamus, caudate, putamen, pallidum, amygdala and accumbens were measured using the FIRST tool (Patenaude *et al.*, 2011a).

Statistical analysis. All statistical analyses were performed with R-4.2.2 and SPSS, version 26.0 (IBM, Armonk, NY, USA).

Demographic and neuropsychological variables were compared between MS patients and HC using the Chi-squared test, Mann-Whitney U test or two-sample t test. Demographic and clinical features were compared between cognitive phenotypes using the Chi-squared test, Mann-Whitney U test or linear models as appropriate. Benjamini-Hochberg FDR correction was applied to adjust for multiple tests.

For easier interpretation of MRI measures, normalized brain volumes were converted to z-scores by subtracting the mean and dividing by the SD of the HCs, separately for each scanner. Brain T2 LVs were log-transformed and converted to z-scores according to the overall distribution in MS patients. MRI variables were compared between HC and cognitive phenotypes and between cognitive phenotypes using age- and sex-adjusted linear models. FDR correction was applied to adjust for multiple tests.

Results

Demographic, clinical and neuropsychological features. Table 9.3.1 summarizes the main demographic, clinical and neuropsychological characteristics of the study population. MS patients had higher MADRS scores than HCs ($p = 0.006$), without reaching the cut-off to be classified as depressed.

Patients with MS exhibited higher rates of cognitive impairment ($p < 0.001$) and poorer performance than controls in learning and memory (z SRT-lts, z SRT-cltr, z SRT-recall, z SPART and z SPART-recall), complex attention (z SDMT, z PASAT 3”

Supplementary Table 9.3.1 summarizes the main demographic and clinical features of MS patients grouped according to disease clinical phenotype: 175 patients were diagnosed with RRMS (72 early-RRMS and 103 late-RRMS), and 68 with PMS. Demographic and clinical features of the subgroups of participants acquired on Scanner 1 and Scanner 2 are reported in Supplementary Table 9.3.2.

Table 9.3.1. Main demographic, clinical and neuropsychological features of patients with MS and healthy controls (HC). Bold text indicates a statistically significant result.

	HC (n=158)	**MS (n=243)**	**P**
N. of females (%)	87 (55)	138 (57)	0.81[a]
Median Age (IQR) [years]	41.9 (29.3 – 52.7)	42.2 (34.3 – 49.6)	0.80[b]
Median Education (IQR) [years]	13.0 (13.0 – 17.0)	13.0 (13.0 – 16.0)	0.14[b]
Median Disease Duration (IQR) [years]	-	10.0 (3.0 – 18.0)	-
Median EDSS (IQR)	-	2.0 (1.0 – 4.0)	-
MS phenotype (%)			
Early-RRMS	-	72 (30)	-
Late-RRMS	-	103 (42)	-
PMS	-	68 (28)	-
No. of subjects scanned with S 1/ S 2 (%)	70 (50)/ 69 (50)	92 (66)/ 118 (34)	0.27[a]
Mean MADRS (SD)	3.4 (2.7)	4.6 (2.6)	**0.006[c]**
Mean z SRT-lts (SD)	-0.2 (0.9)	-0.9 (1.2)	**<0.001[c]**
Mean z SRT-cltr (SD)	-0.2 (0.9)	-0.8 (1.0)	**<0.001[c]**
Mean z SRT-recall (SD)	0.0 (0.9)	-0.6 (1.2)	**<0.001[c]**
Mean z SPART (SD)	0.3 (0.9)	-0.1 (1.1)	**<0.001[c]**
Mean z SPART-recall (SD)	0.4 (0.8)	-0.1 (1.0)	**<0.001[c]**
Mean z SDMT (SD)	0.4 (1.3)	-0.6 (1.5)	**<0.001[c]**
Mean z PASAT 3" (SD)	0.2 (0.8)	-0.6 (1.3)	**<0.001[c]**
Mean z PASAT 2" (SD)	-0.1 (0.8)	-0.6 (1.1)	**<0.001[c]**
Mean z WLG (SD)	0.4 (0.9)	-0.2 (1.2)	**<0.001[c]**
Mean z WCST perseverative errors (SD)	0.3 (1.3)	-0.2 (1.2)	**0.004[c]**
Mean z WCST perseverative responses (SD)	0.3 (1.3)	0.0 (1.3)	0.10[c]
N. of cognitively impaired subjects (%)	1 (1)	69 (28)	**<0.001[a]**

[a]Chi-squared test; [b]Mann-Whitney U test; [c]Two-sample t test.

Abbreviations: cltr=consistent long-term retrieval; EDSS=Expanded Disability Status Scale; IQR=interquartile range; lts=long term storage; MADRS=Montgomery-

Åsberg Depression Rating Scale; RRMS=relapsing-remitting multiple sclerosis; PMS=progressive multiple sclerosis; S=scanner; SD=standard deviation; SDMT=symbol digit modalities test; SPART=10/36 spatial recall test; SRT=selective reminding test; WCST=Wisconsin card sorting test; WLG=word list generation; z=z-score

Demographic and clinical characteristics of cognitive phenotypes. Table 9.3.2 summarizes demographic and clinical variables across cognitive phenotypes. Patients with PC were younger than patients with MSD ($p = 0.001$), MMD ($p = 0.01$), and SMD ($p = 0.003$); had higher years of education than patient with SSD ($p = 0.01$); had the shortest disease duration (all $p \leq 0.01$); had less severe disability than patients with MMD ($p = 0.01$), and SMD ($p < 0.001$); and had the lowest frequency of cognitive dysfunction according to the dichotomous classification of cognitive impairment ($p < 0.001$).

Figure 9.3.2b shows the distribution of MS clinical phenotypes within each cognitive phenotype. The PC phenotype had the highest proportion of early-RRMS (39%), late-RRMS accounted for 40% of the cases and PMS represented 21% of the total number. In the MSD phenotype, 30% were early-RRMS, 35% late-RRMS and 35% PMS. In the MMD phenotype, late-RRMS and PMS were 45% and 41% respectively. The majority of patients with SSD were late-RRMS (69%). The SMD phenotype had the highest proportion of PMS (63%) and the lowest percentage of early-RRMS (5%).

Figure 9.3.1 Percent representation of cognitive phenotypes in (a) patients with multiple sclerosis and (b) healthy controls

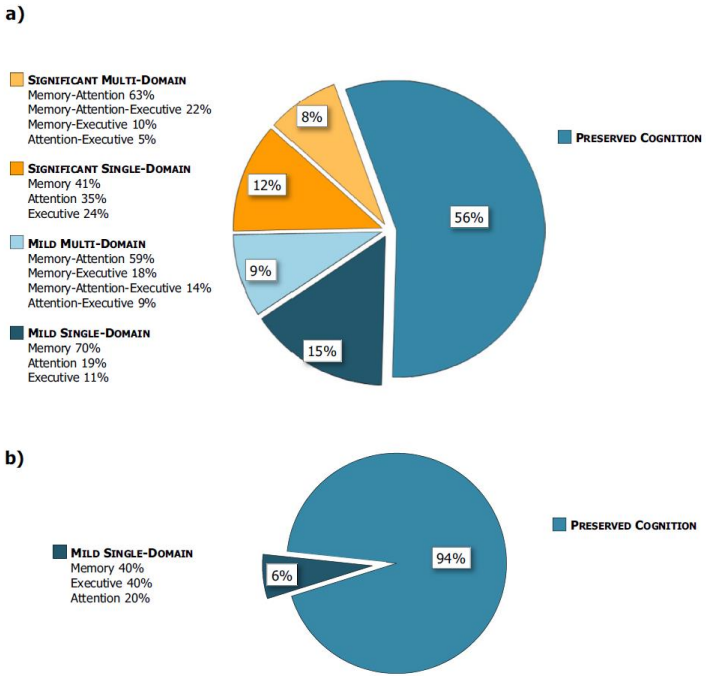
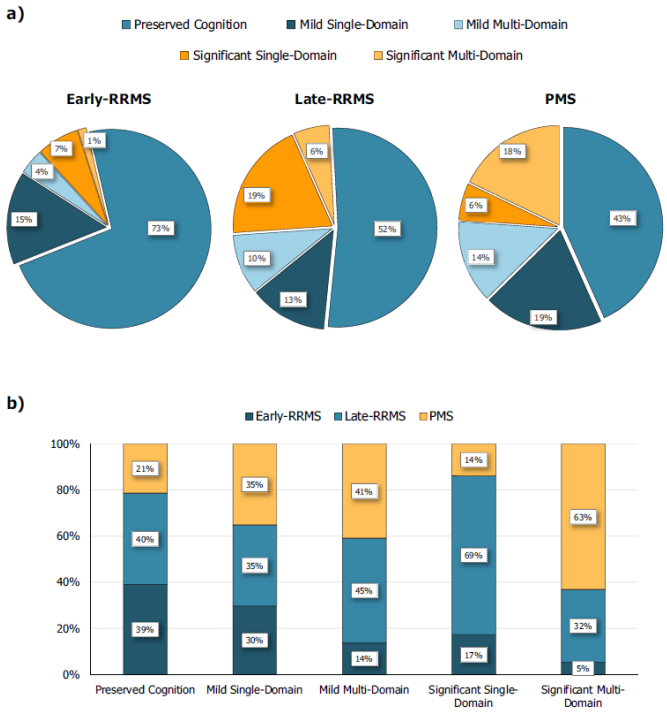


Figure 9.3.2 (a) Pie chart representation of percentage of cognitive phenotypes in each multiple sclerosis clinical phenotype. (b) Stacked bar graph showing the percentage distribution of multiple sclerosis' clinical phenotypes across cognitive phenotypes



Abbreviations: RRMS=relapsing-remitting multiple sclerosis; PMS=progressive multiple sclerosis

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Table 9.3.2. Main demographic and clinical characteristics of cognitive phenotypes. Unless otherwise specified data are presented as median (interquartile range).

	PC (n=136)	**MSD (n=37)**	**MMD (n=22)**	**SSD (n=29)**	**SMD (n=19)**	**p**
N. of females (%)	74 (54)	25 (68)	14 (64)	19 (65)	6 (32)	≥0.17
Age, years	40.3 (30.6 – 47.2)	46.2 (40.1 – 52.7)	47.3 (38.3 – 58.2)	41.4 (38.0 – 45.9)	48.1 (39.2 – 56.2)	**≤0.01**[a,b,c]
Education, years	13.0 (13.0 – 17.0)	13.0 (13.0 – 15.0)	13.0 (12.0 – 13.0)	13.0 (8.0 – 13.0)	13.0 (13.0 – 18.0)	**0.01**[d]
Disease Duration, years	7.5 (2.0 – 14.0)	15.0 (3.7 – 24.0)	14.0 (10.0 – 22.0)	14.0 (6.7 – 21.2)	14.0 (7.6 – 27.7)	**≤0.01**[a,b,c,d]
EDSS	1.5 (1.0 – 3.5)	2.2 (1.5 – 4.5)	2.5 (1.5 – 6.0)	1.5 (1.3 – 4.0)	4.0 (2.5 – 6.5)	**≤0.01**[b,c]
Mean MADRS (SD)	4.7 (2.6)	5.1 (2.4)	3.5 (2.8)	4.8 (2.4)	3.8 (3.0)	≥0.23
N. of cognitively impaired subjects (%)	4 (3)	14 (38)	20 (90)	12 (41)	19 (100)	**≤0.01**[a,b,c,d]

Comparisons performed by Chi-squared test (sex and frequency of cognitive impairment), linear models (age) and Mann-Whitney U test (education, disease duration, EDSS and MADRS). FDR correction was applied to account for the overall number of tests.

Letters indicate significant differences as follows: [a]PC *vs* MSD; [b]PC *vs* MMD; [c]PC *vs* SMD; [d]PC *vs* SSD.

Abbreviations: DI=domain impairment; EDSS=Expanded Disability Status Scale; FDR=false discovery rate; MADRS=Montgomery-Åsberg Depression Rating Scale; MMD=mild multi-domain-impairment; MSD=mild single-domain impairment; NS=not significant; PC=patients with preserved cognition; SMD=significant multi-domain impairment; SSD=significant single-domain impairment; SD=standard deviation.

MRI features of cognitive phenotypes. Table 9.3.3 summarizes lesional and atrophy findings of the five cognitive phenotypes. Two hundred ten of 243 MS patients (86%) and 139 of 158 healthy controls (88%) underwent MRI, representing 87% of the study population.

Compared with HCs, patients with PC had lower volumes in all the investigated brain structures (all $p \leq 0.005$), except for the amygdala, whereas patients with MSD had lower NBV and normalized volume of hippocampus, thalamus, caudate, putamen and accumbens (all $p \leq 0.03$). Patients with MMD, SSD and SMD showed reduced volumes across all the analysed structures (all $p \leq 0.01$).

Compared with patients with PC, those with MSD exhibited higher T2 LV ($p = 0.02$), those with MMD showed higher T2 LV ($p = 0.007$) and lower thalamic volume ($p = 0.008$), those with SSD were characterized by lower NCGMV and normalized volume of thalamus, caudate, putamen and accumbens (all $p \leq 0.04$), those with SMD were characterized by higher T2 LV and lower volumes in all the analysed brain structures (all $p \leq 0.008$), except for NWMV and amygdala.

Table 9.3.3. Pairwise comparison of MRI z-scores between cognitive phenotypes. Data are presented as estimated marginal means (standard error). Bold text indicates a statistically significant result.

	HC (n=139)	**PC (n=119)**	**p *vs* HC**	**MSD (n=30)**	**p *vs* HC**	**p *vs* PC**	**MMD (n=20)**	**p *vs* HC**	**p *vs* PC**	**SSD (n=27)**	**p *vs* HC**	**p *vs* PC**	**SMD (n=14)**	**p *vs* HC**	**p *vs* PC**
T2 LV	-	0.2 (0.1)	-	-0.2 (0.2)	-	**0.02**	-0.4 (0.2)	-	**0.007**	-0.1 (0.2)	-	0.09	-0.7 (0.2)	-	**0.001**
NBV	0.0 (0.1)	-0.7 (0.1)	**<0.001**	-0.8 (0.2)	**<0.001**	0.90	-1.2 (0.2)	**<0.001**	0.07	-1.1 (0.2)	**<0.001**	0.07	-2.1 (0.3)	**<0.001**	**<0.001**
NCGMV	0.0 (0.1)	-0.3 (0.1)	**0.005**	-0.4 (0.2)	**0.03**	0.67	-0.5 (0.2)	**0.03**	0.43	-0.8 (0.2)	**<0.001**	**0.03**	-1.5 (0.2)	**<0.001**	**<0.001**
NWMV	0.0 (0.1)	-0.8 (0.1)	**<0.001**	-0.4 (0.2)	0.12	0.21	-1.4 (0.3)	**<0.001**	0.11	-1.1 (0.2)	**<0.001**	0.24	-1.6 (0.4)	**<0.001**	0.06
NHippV	0.0 (0.1)	-0.5 (0.1)	**<0.001**	-0.6 (0.2)	**0.02**	0.83	-0.9 (0.3)	**0.002**	0.17	-0.7 (0.2)	**0.004**	0.39	-1.9 (0.3)	**<0.001**	**<0.001**
NThalV	0.0 (0.1)	-1.2 (0.1)	**<0.001**	-1.1 (0.2)	**<0.001**	0.88	-2.1 (0.3)	**<0.001**	**0.008**	-1.9 (0.2)	**<0.001**	**0.01**	-3.0 (0.3)	**<0.001**	**<0.001**
NCaudV	0.0 (0.1)	-0.6 (0.1)	**<0.001**	-0.9 (0.2)	**<0.001**	0.28	-0.8 (0.3)	**0.006**	0.43	-1.1 (0.2)	**<0.001**	**0.04**	-1.6 (0.3)	**<0.001**	**0.008**
NPutV	0.0 (0.1)	-0.8 (0.1)	**<0.001**	-0.9 (0.2)	**0.001**	0.75	-1.2 (0.3)	**<0.001**	0.21	-1.6 (0.2)	**<0.001**	**0.004**	-2.7 (0.3)	**<0.001**	**<0.001**
NPallV	0.0 (0.1)	-0.4 (0.1)	**0.003**	-0.4 (0.2)	0.11	0.85	-0.9 (0.3)	**0.005**	0.17	-0.8 (0.2)	**0.003**	0.21	-1.6 (0.3)	**<0.001**	**0.001**
NAmyV	0.0 (0.1)	-0.2 (0.1)	0.11	0.0 (0.2)	0.92	0.40	-0.7 (0.3)	**0.01**	0.10	-0.6 (0.2)	**0.01**	0.12	-0.9 (0.3)	**0.01**	0.07
NAccV	0.0 (0.1)	-0.6 (0.1)	**<0.001**	-0.6 (0.2)	**0.01**	0.84	-0.9 (0.2)	**0.001**	0.32	-1.2 (0.2)	**<0.001**	**0.009**	-1.7 (0.3)	**<0.001**	**<0.001**

Comparisons performed by age- and sex-adjusted linear models. FDR correction was applied to account for the overall number of tests.

Abbreviations: DI=domain impairment; FDR=false discovery rate; HC=healthy controls; LV=lesion volume; MMD=mild multi-domain-impairment; MSD=mild single-domain impairment; NAccV=normalized accumbens volume; NAmyV=normalized amygdala volume; NBV=normalized brain volume; NCaudV=normalized caudate volume; NCGMV=normalized cortical gray matter volume; NHippV=normalized hippocampal volume; NPallV=normalized pallidum volume; NPutV=normalized putamen volume; NThalV=normalized thalamic volume; NWMV=normalized white matter volume; PC=patients with preserved cognition; SMD=significant multi-domain impairment; SSD=significant single-domain impairment.

Compared with patients with PC, those with mild learning and memory impairment exhibited higher T2 LV ($p < 0.001$), while those with significant learning and memory impairment exhibited higher T2 LV and lower volumes in all the examined brain structures (all $p \leq 0.01$), except for NWMV (Supplementary Table 9.3.3).

Compared with patients with PC, those with mild complex attention impairment showed higher T2 LV and lower NBV, NWMV, thalamic, caudate and putamen volume (all $p \leq 0.02$). In comparison to patients with PC, those with significant complex attention impairment were characterized by higher T2 LV and lower volumes across all the examined brain structures (all $p \leq 0.03$), except for NWMV and pallidum (Supplementary Table 9.3.4).

Compared with patients with PC, those with mild executive function impairment showed no differences, while those with significant impairment had higher T2 LV ($p = 0.01$) and lower NWMV ($p = 0.01$) (Supplementary Table 9.3.5).

Discussion

In this study, we used the criteria proposed by the Neurocognitive Disorders Work Group for DSM-5 (Sachdev *et al.*, 2014), adapted for MS (Fischer *et al.*, 2014; Hancock *et al.*, 2022a), to detect mild and significant impairments in learning and memory, complex attention, and executive function in a large sample of patients with MS. Based on the severity of the dysfunction and the number of affected domains, we identified five distinct cognitive phenotypes, representing a graded degree of impairment: 56% of patients fell into the PC phenotype, 15% in the MSD phenotype, 9% in the MMD phenotype, 12% in the SSD phenotype and 8% in the SMD phenotype. In line with previous studies reporting a prevalence of cognitive impairment in MS patients between 34% and 65% (Benedict *et al.*, 2020), we found that 44% of patients exhibited cognitive dysfunction. In MS, cognition has traditionally been dichotomized (impaired vs preserved), neglecting the heterogeneity of cognitive manifestations within these patients. Recent attempts to develop new taxonomies of cognitive phenotypes using specific impairment criteria (Hancock *et al.*, 2022a; Leavitt *et al.*, 2018) represent an advance in characterizing the patterns of impairment in this condition. However, the application of non-stringent cut-off criteria (Fischer *et al.*, 2014) (i.e., test performance

1 SD below the normative mean) (Hancock *et al.*, 2022a; Leavitt *et al.*, 2018) and the lack of MRI data (Hancock *et al.*, 2022a; Leavitt *et al.*, 2018) have limited their impact in the MS research literature and their applicability in the clinical setting. In this study, we attempted to overcome these limitations by applying strict impairment criteria and integrating the analysis with MRI data. As recommended by the Neurocognitive Disorders Work Group for DSM-5 (Sachdev *et al.*, 2014), in each cognitive domain, significant impairment was defined as performing 2 SD below the normative value. Mild impairment, however, was defined using the cut-off 1.5 SD rather than 1 SD, as evidence from multiple studies suggests a possible increase in false-positive diagnoses of cognitive impairment when using the 1 SD threshold (Fischer *et al.*, 2014; Hancock *et al.*, 2022a). In addition, a domain was considered impaired if two tests within that domain fell below the designated cut-off (Hancock *et al.*, 2022a). By doing so, we were able to distinguish five cognitively homogeneous groups of patients, each characterized by unique demographic and clinical features. MRI analysis allowed the identification of distinct neuroanatomical substrates associated with each profile of impairment and provided biological evidence to support the proposed classification system.

Patients with PC were generally young individuals, with RRMS and a shorter disease duration than patients with other cognitive phenotypes. Compared with HCs, they had reduced volume across all brain compartments except the amygdala. However, due to the early stage of the disease, structural damage may be relatively limited (De Meo *et al.*, 2021a; Eshaghi *et al.*, 2018). In addition, patients belonging to this cognitive phenotype are likely to benefit from protective factors, such as high cognitive reserve, which mitigate MS-related structural damage through adaptive functional compensation (Rocca *et al.*, 2015b). Furthermore, it is possible that patients with higher cognitive reserve, which contributes to the preservation of cognitive abilities, fell into the PC phenotype (Podda *et al.*, 2021; Rocca *et al.*, 2018a).

The second phenotype included patients with mild impairment in a single cognitive domain. Compared to PC patients, those with MSD were older and had longer disease duration. In line with this finding, large cross-sectional studies have shown that age and disease duration are often associated and that cognitive function tends to decline as disease progresses (Aktas *et al*, 2013; Ruano *et al.*, 2017). This group was characterized by a higher T2 LV compared to the PC phenotype, consistent with research linking

higher lesion burden to poorer performance on measures of memory (Sacco *et al*, 2015), processing speed (Summers *et al*, 2008), sustained attention (Sacco *et al.*, 2015) and executive function (Sacco *et al.*, 2015; Summers *et al.*, 2008).

The third cognitive phenotype included patients with mild impairment in multiple domains. Compared to the PC group, these patients had a more severe clinical profile characterized by older age, longer disease duration, greater physical disability, and a higher prevalence of PMS. As mentioned above, the presence of cognitive deficits may be associated with more advanced disease stage (Aktas *et al.*, 2013; Brochet & Ruet, 2019; Ruano *et al.*, 2017). Consistent with this, patients with mild impairment in multiple domains had higher T2 LV and more severe thalamic atrophy compared to PC ones. The thalamus plays a key role in several cognitive processes (Saalmann & Kastner, 2015). Its extensive connections with different brain regions makes it vulnerable to degeneration due to demyelination and axonal loss in brain WM (Houtchens *et al*, 2007). Previous MRI studies have consistently demonstrated a robust correlation between thalamic atrophy with a wide range of clinical manifestations including cognitive decline, motor deficits and fatigue in patients with MS (Bisecco *et al*, 2019; Minagar *et al*, 2013; Rocca *et al.*, 2015b).

The fourth phenotype, SSD, included patients with both a longer disease duration and lower level of education in comparison to those with PC. Since educational attainment is often considered a proxy of cognitive reserve (Pettigrew & Soldan, 2019), it is tempting to speculate that this group of were more prone to the development of MS-related cognitive deficits. The relative frequency of patients with late-RRMS was highest within the SSD phenotype (69%), suggesting that cognitive problems in the later stages of RRMS are confined to a specific cognitive function rather than affecting multiple cognitive domains. Cortical atrophy and reduced volumes of the thalamus, caudate, putamen and accumbens emerged as the distinctive MRI features of this cognitive phenotype. The proximity of these deep GM structures with the periventricular WM, which is more likely to develop focal lesions, makes them more susceptible to atrophy since the early stages of the disease. Volume reduction of deep GM nuclei has been frequently associated with cognitive deficits in MS (Eijlers *et al*, 2019). Interestingly, a longitudinal investigation suggested that the pathologic substrates of cognitive dysfunction may gradually shift from WM lesions and deep GM atrophy, in

the inflammatory RRMS phase, towards cortical atrophy in the more advanced stage of the disease (Eijlers *et al.*, 2019).

The fifth phenotype included MS patients exhibiting significant impairments in multiple cognitive functions. In terms of their clinical features, these patients had longer disease duration and a higher degree of physical disability compared to patients with PC. Moreover, 63% of patients with SMD were diagnosed with a progressive form of MS, consistently with previous studies indicating that cognitive dysfunction is more severe in PMS than in RRMS (Brochet & Ruet, 2019). The clinical and cognitive profile of this phenotype is consistent with the extensive brain atrophy observed on MRI affecting all tissue compartments examined except NWMV and amygdala.

In line with previous research (Benedict *et al.*, 2020), in our sample of MS patients, memory and complex attention were the most commonly affected cognitive areas, while executive function showed lower frequency of impairment. The pairwise comparisons of MRI metrics revealed that patients with mild and significant impairments in specific cognitive domains exhibited distinct patterns of brain structural changes. More in detail, compared to PC patients, those with mild learning and memory deficits showed a higher T2 LV, suggesting an association between mild memory impairment and increased lesion burden (Randolph *et al*, 2005). On the other hand, compared to patients with PC, those with significant learning and memory dysfunction showed higher T2 LV and decreased volumes in all brain regions studied except NWMV. This result may indicate that more widespread neurodegenerative processes contribute to the severity of this cognitive deficit (Preziosa *et al*, 2017). Similar patterns were observed in the complex attention domain, with higher T2 LV and decreased volumes across multiple brain regions in the mild impairment subgroup, and a greater volume loss in all brain structures examined, except NWMV and pallidum, in the severe impairment subgroup. These results suggest that severe impairment in complex attention may be associated with broader neurodegenerative changes affecting various cognitively relevant brain networks (Azzimonti *et al*, 2023). Finally, compared with patients with PC, those with significant executive impairment had higher T2 LV and lower NWMV, suggesting that focal WM lesions and WM atrophy contribute to executive dysfunction in MS (Marchesi *et al.*, 2021). Clearly, the relatively small number of patients presenting this deficit may have limited the statistical power of this latter analysis.

Notably, 21% of patients exhibited deficits in complex attention (7% mild and 14% significant impairment), while this cognitive area was preserved in a large proportion of impaired patients. This result further confirms the limitations of the use of a single cognitive test, such as SDMT, to detect cognitive impairment at the individual level (Kalb *et al*, 2018).

This study has a few limitations. First, the cross-sectional design does not allow for the examination of both the stability and temporal changes of cognitive phenotypes over time. Second, cognitive functions such as visuospatial processing and social cognition were not assessed. Moreover, despite their good psychometric properties (Amato *et al.*, 2006; Benedict *et al.*, 2017) the SDMT (Sandry *et al.*, 2021) and PASAT (Tombaugh, 2006a) may tap into cognitive abilities outside complex attention. Third, despite the use of a standardized neuropsychological battery, our results may have been influenced by the inclusion of a broader range of memory and learning measures compared to other cognitive domains. Additionally, the fact that PASAT 3" and PASAT 2" are closely related tasks may have contributed to potential overdiagnosis of complex attention deficits. Future studies should evaluate the applicability of the proposed criteria to different neuropsychological batteries and cohorts of MS patients as was done with the IC-CoDiMS criteria (Hancock *et al.*, 2022a). Fourth, most participants, though not all, underwent MRI at the time of cognitive assessment. Nevertheless, their distribution into cognitive phenotypes closely resembled that of the entire sample. Finally, the division of the MS sample in multiple cognitive phenotypes might have limited statistical power.

In conclusion, this study presents a new approach that allows to identify five distinct cognitive phenotypes in MS in both clinical and research settings.

Supplementary Material

Supplementary Table 9.3.1. Main demographic and clinical characteristics of patients with MS, grouped according to disease clinical phenotype. Unless otherwise specified data are presented as median (interquartile range). Bold text indicates a statistically significant result.

	Early-RRMS (n=72)	Late-RRMS (n=103)	PMS (n=68)	p
N. of females (%)	50 (69)	62 (60)	26 (38)	**≤0.02[a,b]**
Age, years	32.2 (25.7 – 40.9)	43.5 (37.5 – 48.5)	49.1 (43.1 – 56.8)	**<0.001[a,b,c]**
Education, years	13.0 (13.0 – 16.5)	13.0 (13.0 – 16.7)	13.0 (13.0 – 13.5)	≥0.08
Disease Duration, years	1.1 (1.0 – 2.5)	14.0 (9.0 – 20.7)	16.5 (12.0 – 24.0)	**<0.001[a,c]**
EDSS	1.0 (1.0 – 1.5)	1.5 (1.5 -2.5)	6.0 (4.5 -6.5)	**<0.001[a,b,c]**
No. of subjects scanned with S 1/ S 2 (%)	16 (28)/ 42 (72)	45 (48)/ 49 (52)	31 (53)/ 27 (47)	**≤0.046[a,c]**
Mean MADRS (SD)	4.1 (2.4)	4.6 (2.8)	5.3 (2.4)	**0.049[a]**
Mean z SRT-lts (SD)	-0.5 (1.0)	-1.0 (1.1)	-1.3 (1.2)	**≤0.008[a,c]**
Mean z SRT-cltr (SD)	-0.4 (1.0)	-0.9 (0.9)	-1.2 (1.1)	**≤0.004[a,c]**
Mean z SRT-recall (SD)	-0.1 (1.0)	-0.7 (1.1)	-1.2 (1.3)	**≤0.03[a,b,c]**
Mean z SPART (SD)	0.2 (0.9)	-0.1 (1.0)	-0.5 (1.2)	**≤0.04[a,c]**
Mean z SPART-recall (SD)	0.2 (0.9)	-0.2 (1.1)	-0.3 (1.0)	**≤0.049[a,c]**
Mean z SDMT (SD)	0.2 (1.7)	-0.7 (1.2)	-1.4 (1.4)	**≤0.006[a,b,c]**
Mean z PASAT 3" (SD)	-0.4 (1.1)	-0.6 (1.2)	-0.8 (1.5)	≥0.11
Mean z PASAT 2" (SD)	-0.6 (1.0)	-0.9 (1.1)	-1.0 (1.1)	≥0.06
Mean z WLG (SD)	-0.2 (1.2)	-0.1 (1.1)	-0.6 (1.1)	**0.049[b]**
Mean z WCST perseverative errors (SD)	0.0 (1.2)	-0.4 (1.1)	-0.2 (1.3)	≥0.11
Mean z WCST perseverative responses (SD)	0.1 (1.3)	-0.2 (1.3)	0.1 (1.3)	≥0.36
N. of cognitively impaired subjects (%)	11 (15)	28 (27)	30 (44)	**0.001[a]**

Comparisons performed by Chi-squared test (sex, scanner, frequency of cognitive impairment), linear models (age) and Mann-Whitney U test (education, disease duration, EDSS, MADRS and cognitive z-scores). FDR correction was applied to account for the overall number of tests.

Letters indicate significant differences as follows: [a]Early-RRMS *vs* PMS; [b]Late-RRMS *vs* PMS; [c]Early-RRMS *vs* Late-RRMS.

Abbreviations: cltr=consistent long-term retrieval; EDSS=Expanded Disability Status Scale; FDR=false discovery rate; lts=long term storage; MADRS=Montgomery-Åsberg Depression Rating Scale; MS=multiple sclerosis; NS=not significant; RRMS=relapsing-remitting multiple sclerosis; PMS=progressive multiple sclerosis; S=scanner; SD=standard deviation; SDMT=symbol digit modalities test; SPART=10/36

spatial recall test; SRT=selective reminding test; WCST=Wisconsin card sorting test; WLG=word list generation; z=z-score.

Supplementary Table 9.3.2. Main demographic, clinical and neuropsychological features of participants scanned with Scanner 1 and Scanner 2. Bold text indicates a statistically significant result.

	Scanner 1			**Scanner 2**		
	HC (n=70)	**MS (n=92)**	**p**	**HC (n=69)**	**MS (n=118)**	**p**
N. of females (%)	39 (56)	49 (53)	0.88[a]	36 (52)	68 (58)	0.57[a]
Median Age (IQR) [years]	46.6 (33.2 – 57.0)	41.1 (34.8 – 49.6)	0.15[b]	38.3 (27.2 – 51.2)	41.7 (33.5 – 48.3)	0.38[b]
Median Education (IQR) [years]	13.0 (13.0 – 17.0)	13.0 (13.0 – 16.0)	0.18[b]	13.0 (13.0 – 17.0)	13.0 (13.0 – 17.0)	0.88[b]
Median Disease Duration (IQR) [years]	-	12.0 (7.0 – 18.5)	-	-	8.5 (2.0 – 17.0)	-
Median EDSS (IQR)	-	2.0 (1.5 – 4.5)	-	-	1.5 (1.0 – 3.5)	-
MS subtype (%)						
Early-RRMS	-	16 (17)	-	-	42 (36)	-
Late-RRMS	-	45 (49)	-	-	49 (41)	-
PMS	-	31 (34)	-	-	27 (23)	-
Mean MADRS (SD)	**2.3 (0.6)**	**5.0 (2.8)**	**<0.001[c]**	3.7 (2.7)	4.4 (2.5)	0.09[c]
Mean z SRT-lts (SD)	**-0.2 (1.0)**	**-1.0 (1.1)**	**0.01[c]**	**-0.3 (0.9)**	**-0.8 (1.1)**	**<0.001[c]**
Mean z SRT-cltr (SD)	**-0.4 (0.7)**	**-1.1 (1.0)**	**0.01[c]**	**-0.2 (0.8)**	**-0.7 (1.0)**	**<0.001[c]**
Mean z SRT-recall (SD)	**-0.1 (1.0)**	**-0.8 (1.1)**	**0.03[c]**	**0.0 (0.9)**	**-0.5 (1.2)**	**0.002[c]**
Mean z SPART (SD)	**0.2 (0.9)**	**-0.4 (1.1)**	**0.03[c]**	**0.3 (0.9)**	**0.0 (1.0)**	**0.02[c]**
Mean z SPART-recall (SD)	0.1 (0.8)	-0.3 (1.1)	0.14	**0.4 (0.7)**	**0.0 (1.0)**	**0.003[c]**
Mean z SDMT (SD)	**0.0 (1.2)**	**-1.1 (1.4)**	**0.005[c]**	**0.5 (1.2)**	**-0.3 (1.4)**	**<0.001[c]**
Mean z PASAT 3" (SD)	**0.3 (0.9)**	**-0.9 (1.4)**	**<0.001[c]**	**0.1 (0.8)**	**-0.4 (1.0)**	**<0.001[c]**
Mean z PASAT 2" (SD)	**-0.2 (0.8)**	**-1.1 (1.2)**	**<0.001[c]**	**-0.2 (0.8)**	**-0.7 (0.9)**	**<0.001[c]**
Mean z WLG (SD)	0.0 (0.1)	-0.4 (1.3)	0.10[c]	**0.5 (0.9)**	**-0.1 (1.0)**	**<0.001[c]**
Mean z WCST perseverative errors (SD)	0.5 (1.0)	0.2 (1.2)	0.13[c]	**0.3 (1.2)**	**-0.4 (1.1)**	**<0.001[c]**
Mean z WCST perseverative responses (SD)	0.5 (1.0)	0.6 (1.4)	0.61 [c]	**0.2 (1.3)**	**-0.4 (1.1)**	**<0.001[c]**

[a]Chi-squared test; [b]Mann-Whitney U test; [c]Two-sample t test.

Abbreviations: cltr=consistent long-term retrieval; EDSS= Expanded Disability Status Scale; IQR= interquartile range; HC= healthy controls; lts=long term storage; MADRS= Montgomery-Åsberg Depression Rating Scale; MS= multiple sclerosis; RRMS= relapsing-remitting multiple sclerosis; PMS= progressive multiple sclerosis; SD= standard deviation; SDMT=symbol digit modalities test; SPART=10/36 spatial recall test; SRT=selective reminding test; WCST=Wisconsin card sorting test; WLG=word list generation; z=z-score.

Supplementary Table 9.3.3. Pairwise comparison of MRI z-scores between HC and patients with PC, Mild and Significant learning and memory impairment. Data are presented as estimated marginal means (standard error). Bold text indicates a statistically significant result.

	HC (n=139)	**PC (n=119)**	**p *vs* HC**	**Mild (n=38)**	**p *vs* HC**	**p *vs* PC**	**Significant (n=26)**	**p *vs* HC**	**p *vs* PC**	**p *vs* Mild**
T2 LV	-	0.2 (0.2)	-	-0.4 (0.1)	-	**<0.001**	-0.4 (0.2)	-	**0.002**	0.97
NBV	0.0 (0.1)	-0.7 (0.1)	**<0.001**	-1.0 (0.2)	**<0.001**	0.21	-1.5 (0.2)	**<0.001**	**<0.001**	0.07
NCGMV	0.0 (0.1)	-0.3 (0.1)	**0.004**	-0.6 (0.1)	**<0.001**	0.15	-0.9 (0.2)	**<0.001**	**0.008**	0.25
NWMV	0.0 (0.1)	-0.8 (0.1)	**<0.001**	-0.7 (0.2)	**0.004**	0.76	-1.3 (0.2)	**<0.001**	0.07	0.08
NHippV	0.0 (0.1)	-0.5 (0.1)	**<0.001**	-0.9 (0.2)	**<0.001**	0.14	-1.3 (0.2)	**<0.001**	**0.004**	0.19
NThalV	0.0 (0.1)	-1.2 (0.1)	**<0.001**	-1.6 (0.2)	**<0.001**	0.09	-2.5 (0.2)	**<0.001**	**<0.001**	**0.01**
NCaudV	0.0 (0.1)	-0.6 (0.1)	**<0.001**	-0.9 (0.2)	**<0.001**	0.24	-1.3 (0.2)	**<0.001**	**0.003**	0.11
NPutV	0.0 (0.1)	-0.8 (0.1)	**<0.001**	-1.1 (0.2)	**<0.001**	0.20	-2.2 (0.2)	**<0.001**	**<0.001**	**0.001**
NPallV	0.0 (0.1)	-0.4 (0.1)	**0.003**	-0.6 (0.2)	**0.01**	0.53	-1.5 (0.2)	**<0.001**	**<0.001**	**0.003**
NAmyV	0.0 (0.1)	-0.2 (0.1)	0.11	-0.2 (0.2)	0.27	0.97	-0.8 (0.2)	**0.006**	**0.01**	**0.04**
NAccV	0.0 (0.1)	-0.6 (0.1)	**<0.001**	-0.7 (0.2)	**<0.001**	0.62	-1.5 (0.2)	**<0.001**	**<0.001**	**0.008**

Comparisons performed by age- and sex-adjusted linear models. FDR correction was applied to account for the overall number of tests.

Abbreviations: HC= healthy controls; FDR=false discovery rate; LV= lesion volume; NAccV= normalized accumbens volume; NAmyV= normalized amygdala volume; NBV= normalized brain volume; NCaudV= normalized caudate volume;

NCGMV= normalized cortical gray matter volume; NHippV= normalized hippocampal volume; NPallV= normalized pallidum volume; NPutV= normalized putamen volume; NThalV= normalized thalamic volume; NWMV= normalized white matter volume; PC= patients with preserved cognition.

Supplementary Table 9.3.4. Pairwise comparison of MRI z-scores between HC and patients with PC, Mild and Significant complex attention impairment. Data are presented as estimated marginal means (standard error). Bold text indicates a statistically significant result.

	HC (n=139)	**PC (n=119)**	**p *vs* HC**	**Mild (n=14)**	**p *vs* HC**	**p *vs* PC**	**Significant (n=30)**	**p *vs* HC**	**p *vs* PC**	**p *vs* Mild**
T2 LV	-	0.2 (0.1)	-	-0.4 (0.2)	-	**0.008**	-0.2 (0.2)	-	**0.01**	0.52
NBV	0.0 (0.1)	-0.7 (0.1)	**<0.001**	-1.7 (0.3)	**<0.001**	**0.001**	-1.6 (0.2)	**<0.001**	**<0.001**	0.90
NCGMV	0.0 (0.1)	-0.3 (0.1)	**0.005**	-0.7 (0.2)	**0.004**	0.12	-1.2 (0.2)	**<0.001**	**<0.001**	0.15
NWMV	0.0 (0.1)	-0.8 (0.1)	**<0.001**	-1.7 (0.3)	**<0.001**	**0.01**	-1.1 (0.2)	**<0.001**	0.30	0.13
NHippV	0.0 (0.1)	-0.5 (0.1)	**0.001**	-1.1 (0.3)	**0.002**	0.15	-1.5 (0.2)	**<0.001**	**0.005**	0.19
NThalV	0.0 (0.1)	-1.1 (0.1)	**<0.001**	-2.3 (0.3)	**<0.001**	**0.003**	-2.3 (0.2)	**<0.001**	**<0.001**	0.92
NCaudV	0.0 (0.1)	-0.6 (0.1)	**<0.001**	-1.4 (0.3)	**<0.001**	**0.02**	-1.3 (0.2)	**<0.001**	**0.004**	0.92
NPutV	0.0 (0.1)	-0.7 (0.1)	**<0.001**	-1.7 (0.3)	**<0.001**	**0.009**	-2.1 (0.2)	**<0.001**	**<0.001**	0.50
NPallV	0.0 (0.1)	-0.4 (0.1)	**0.003**	-1.1 (0.3)	**0.001**	0.06	-0.9 (0.2)	**<0.001**	0.09	0.68
NAmyV	0.0 (0.1)	-0.2 (0.1)	0.12	-0.7 (0.3)	**0.02**	0.12	-0.7 (0.2)	**0.002**	**0.03**	0.92
NAccV	0.0 (0.1)	-0.6 (0.1)	**<0.001**	-0.9 (0.3)	**0.003**	0.38	-1.4 (0.2)	**<0.001**	**<0.001**	0.12

Comparisons performed by age- and sex-adjusted linear models. FDR correction was applied to account for the overall number of tests.

Abbreviations: HC= healthy controls; FDR=false discovery rate; LV= lesion volume; NAccV= normalized accumbens volume; NAmyV= normalized amygdala volume; NBV= normalized brain volume; NCaudV= normalized caudate volume;

NCGMV= normalized cortical gray matter volume; NHippV= normalized hippocampal volume; NPallV= normalized pallidum volume; NPutV= normalized putamen volume; NThalV= normalized thalamic volume; NWMV= normalized white matter volume; PC= patients with preserved cognition.

Supplementary Table 9.3.5. Pairwise comparison of MRI z-scores between HC and patients with PC, Mild and Significant executive function impairment. Data are presented as estimated marginal means (standard error). Bold text indicates a statistically significant result.

	HC (n=139)	**PC (n=119)**	**p *vs* HC**	**Mild (n=8)**	**p *vs* HC**	**p *vs* PC**	**Significant (n=15)**	**p *vs* HC**	**p *vs* PC**	**p *vs* Mild**
T2 LV	-	0.3 (0.1)	-	-0.1 (0.3)	-	0.35	-0.4 (0.2)	-	**0.01**	0.47
NBV	0.0 (0.1)	-0.7 (0.1)	**<0.001**	-0.8 (0.4)	**0.048**	0.73	-1.3 (0.3)	**<0.001**	0.07	0.44
NCGMV	0.0 (0.1)	-0.3 (0.1)	**0.004**	-0.2 (0.3)	0.52	0.79	-0.7 (0.2)	**0.002**	0.09	0.22
NWMV	0.0 (0.1)	-0.8 (0.1)	**<0.001**	-1.1 (0.5)	0.052	0.61	-1.8 (0.3)	**<0.001**	**0.01**	0.35
NHippV	0.0 (0.1)	-0.5 (0.1)	**0.001**	-1.1 (0.4)	**0.03**	0.29	-0.6 (0.2)	0.08	0.73	0.29
NThalV	0.0 (0.1)	-1.1 (0.1)	**<0.001**	-1.7 (0.4)	**0.001**	0.33	-1.8 (0.3)	**<0.001**	0.11	0.90
NCaudV	0.0 (0.1)	-0.6 (0.1)	**<0.001**	-0.7 (0.4)	0.14	0.79	-1.4 (0.3)	**0.01**	0.47	0.79
NPutV	0.0 (0.1)	-0.7 (0.1)	**<0.001**	-0.6 (0.4)	0.22	0.87	-1.1 (0.3)	**0.003**	0.39	0.50
NPallV	0.0 (0.1)	-0.4 (0.1)	**0.004**	-0.9 (0.4)	0.08	0.43	-0.6 (0.3)	0.10	0.73	0.65
NAmyV	0.0 (0.1)	-0.2 (0.1)	0.16	-1.0 (0.4)	**0.03**	0.10	-0.6 (0.3)	0.08	0.28	0.49
NAccV	0.0 (0.1)	-0.6 (0.1)	**<0.001**	-0.9 (0.4)	**0.03**	0.46	-1.1 (0.3)	**<0.001**	0.13	0.80

Comparisons performed by age- and sex-adjusted linear models. FDR correction was applied to account for the overall number of tests.

Abbreviations: HC= healthy controls; FDR=false discovery rate; LV= lesion volume; NAccV= normalized accumbens volume; NAmyV= normalized amygdala volume; NBV= normalized brain volume; NCaudV= normalized caudate volume; NCGMV= normalized

cortical gray matter volume; NHippV= normalized hippocampal volume; NPallV= normalized pallidum volume; NPutV= normalized putamen volume; NThalV= normalized thalamic volume; NWMV= normalized white matter volume; PC= patients with preserved cognition.

9.4 Monoaminergic network dysfunction and development of depression in multiple sclerosis: a longitudinal investigation

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ORIGINAL COMMUNICATION



Monoaminergic network dysfunction and development of depression in multiple sclerosis: a longitudinal investigation

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Abstract

Background Monoaminergic network dysfunction is thought to underpin depression in multiple sclerosis (MS) patients. However, longitudinal studies are lacking.

Objectives Here, we investigated the association between development of depressive symptoms in MS and changes of resting-state functional connectivity (RS FC) within monoaminergic networks.

Methods Forty-nine MS patients without depression [Montgomery–Asberg Depression Scale (MADRS) ≤ 9] and 27 healthy controls underwent clinical and 3.0 T RS FC assessment at baseline and after a median follow-up of 1.6 years (interquartile range 1.0–2.1 years). Monoamine-related RS FC was derived by independent component analysis, constrained to PET atlases for dopamine, noradrenaline and serotonin transporters. Longitudinal changes of RS FC within monoaminergic networks and their correlations with MADRS scores were assessed.

Results At baseline, MS patients showed decreased RS FC vs healthy controls in all PET-guided monoaminergic networks in frontal, cingulate and cerebellar cortices, and increased RS FC in parieto-occipital regions. Fourteen (29%) MS patients developed depressive symptoms (MADRS > 9) at follow-up (D-MS) and exhibited widespread RS FC decrease over time in the PET-guided dopamine network, mainly in orbitofrontal, occipital, anterior cingulate and precuneal cortices compared to patients who did not develop depressive symptoms. In D-MS, decreased RS FC over time was also observed in parahippocampal and occipital regions of the PET-guided noradrenaline network. Decreased RS FC over time in dopamine and noradrenaline PET-guided networks correlated with concomitant increased MADRS scores (r = range − 0.65/− 0.61, $p < 0.001$).

Conclusions The development of depressive symptoms in MS patients was associated with specific RS FC changes within the dopamine and noradrenaline networks.

Keywords Depression · Multiple sclerosis · Functional networks · Connectivity

Introduction

Approximately 25-50% of MS patients develop a form of major depression during their lifetime, which is 2-5 times higher than the rate in the general population (Feinstein *et al*, 2014). Depressive symptoms, including feelings of sadness, loss of interest, irritability, sleep disturbances and weight gain/loss, are important determinants of patients' quality of life and may negatively impact adherence to disease-modifying therapy in these patients (Solaro *et al*, 2018). However, the neurobiological mechanisms that lead to such a high prevalence of depression in MS patients are still unclear.

Several studies applied structural and functional MRI techniques to improve the understanding of the association between MS-related brain abnormalities and depressive symptoms (Feinstein *et al.*, 2014). There is consistent evidence in the literature that structural damage in the temporal and frontal lobes, including T2-hyperintense lesion burden, GM atrophy, and extensive changes in NAWM, are associated with depression in MS (Solaro *et al.*, 2018). In addition, RS fMRI studies detected decreased RS FC between the amygdala and the prefrontal cortex (Riccelli *et al*, 2016), and decreased RS FC between the hippocampus and frontal, temporal and parietal regions, in depressed compared to not-depressed MS patients (Rocca *et al*, 2015e).

According to the monoamine deficiency hypothesis, depressive symptoms may be due to insufficient levels of monoamine neurotransmitters (Hamon & Blier, 2013). In line with this, previous positron emission tomography (PET) studies detected abnormalities in the concentration of presynaptic noradrenaline and serotonin transporters in frontal and limbic areas of MS patients (Hesse *et al*, 2014b; Schmidt *et al*, 2020b). The importance of monoaminergic networks derangements also emerged from a RS FC study that using graph theory analysis found an association between reorganization of network topography in serotonergic and noradrenergic systems and depressive symptoms in 29 patients with MS (Carotenuto *et al*, 2020).

A recent study (Carotenuto *et al*, 2023) investigated RS FC abnormalities in dopamine, noradrenaline and serotonin networks, reconstructed through an ICA using PET-based atlases of monoamine receptor levels as constraints. Higher levels of depression resulted associated with decreased RS FC within PET-guided monoaminergic networks in frontal areas, decreased RS FC in the hippocampus within the PET-guided noradrenaline network, and decreased RS FC in the right precuneus

within the PET-guided serotonin network. These findings were interpreted as possible evidence that depressive symptoms in MS are related to the disconnection between fronto-limbic regions and the DMN caused by derangements in monoaminergic networks (Carotenuto *et al.*, 2023).

Taken together these results suggest that diffuse dysregulation in the monoaminergic networks is a pathologic marker for depression. However, it is still not clear whether the onset of depressive symptoms in MS is accompanied by RS FC changes within PET-guided monoamine networks.

In this study, we explored the relationship between the development of depressive symptoms in patients with MS and concomitant longitudinal changes of RS FC within noradrenaline, serotonin and dopamine networks reconstructed using a PET-based constrained ICA analysis, previously validated (Carotenuto *et al.*, 2023). Since fatigue and depression often overlap, we excluded patients with fatigue to be able to disentangle specific RS FC changes associated with depressive symptoms.

Methods

Participants. Overall, 113 MS patients and 27 HCs were screened for enrolment. Inclusion criteria were: 1) right-handedness (Oldfield, 1971); 2) no substance abuse; 3) no major systemic, psychiatric or neurological diseases other than MS; 4) no ongoing chronic treatment with drugs impacting monoaminergic levels, such as levodopa, amantadine, fluoxetine, paroxetine, chronic antipsychotics, etc. Patients with MS had also to be relapse and steroid free for at least 3 months before MRI acquisition and have a stable disease-modifying treatment from at least 6 months. In addition, MS patients were excluded if they were found to be depressed at baseline evaluation, or fatigued at baseline or follow-up evaluation (see below). Fatigue and depression often co-occur in MS and by excluding patients with fatigue, we aimed to reduce the confounding factors, thereby increasing the likelihood that any observed changes in RS FC were associated only with the development of depressive symptoms.

Clinical and neuropsychological assessment. At baseline and follow-up visits, all patients underwent a clinical and neuropsychological assessment. The clinical evaluation included the EDSS (Kurtzke, 1983) and recording of disease modifying treatments. Depressive symptoms were assessed using the MADRS (Hawley *et al.*,

2002) and fatigue was evaluated with the Modified Fatigue Impact Scale (MFIS) (Marchesi *et al*, 2020). Patients with an MFIS score ≥ 37 (Marchesi *et al.*, 2020) at baseline or follow-up, or with a MADRS score > 9 (Hawley *et al.*, 2002) at baseline were excluded. At follow-up, MS patients with a MADRS score > 9 were classified as developing depressive symptoms. To examine the impact of the occurrence of depressive symptoms on cognitive function, a cognitive assessment was performed both at baseline and at follow-up. All patients were administered the BRB-N (Amato *et al.*, 2006), alternate versions A and B of the BRB-N were used at each examination. Longitudinal changes in cognitive performance on each neuropsychological test were assessed by calculating a reliable change index (RCI) with correction for measurement error and practice effects. The z-score cut-off identifying a reliable deterioration in each cognitive test was RCI < -1.645 (Portaccio *et al*, 2010).

MRI acquisition. Within 48 hours from baseline and follow-up clinical evaluations, participants underwent brain MRI scans, which were performed using two 3.0 T scanners (same scanner used for baseline and follow-up in the same subject; Scanner 1: Achieva, n=15 HCs/25 MS patients; Scanner 2: Ingenia, n=12 HCs/24 MS patients; Philips Medical Systems, Eindhoven, The Netherlands).

Images acquired using the Achieva scanner included the following scans: 1) 200 sets of T2*-weighted echo planar imaging sequence for RS fMRI (TR=3000 ms; echo time TE=35 ms, flip angle=90°, matrix size=128x128; FOV=240 mm2; 30 contiguous axial slices, 4 mm thick); 2) dual-echo turbo spin echo (TR=2599; TE=16-80 ms; flip angle=90°; matrix=256×256; FOV=240mm2; ETL=6; 44 contiguous axial slices, 3 mm thick); and 3) 3D T1-weighted fast field echo (TR=25; TE=4.6 ms; flip angle=30°; matrix=256x256; FOV=230 mm2; 220 contiguous axial slices, 0.8 mm thick).

Images acquired using the Ingenia scanner included: 1) 320 sets of T2*-weighted echo planar imaging sequence for RS fMRI (TR=1567 ms; TE=35 ms, flip angle=70°; multi-band factor=2, matrix size=96×96; FOV=240 x 240 mm2; 48 contiguous axial slices, 3 mm thick); 2) 3D FLAIR (TR=4800 ms; TE=270 ms; TI=1650 ms; matrix size=256 × 256; FOV=256 × 256 mm2; ETL=167; 192 contiguous sagittal slices, 1 mm thick); 3) 3D T2-weighted sequence (TR=2500 ms; TE=330 ms; matrix size=256 × 256; FOV=256 × 256 mm2; ETL = 117; 192 contiguous sagittal slices, 1 mm thick); and 4) 3D T1-weighted turbo field echo (TR=7 ms; TE=3.2 ms; TI=1000 ms; flip angle=8°;

matrix size=256 × 256; FOV=256 × 256 mm2; 204 contiguous sagittal slices, 1 mm thick).

Structural MRI analysis. For MS patients acquired on Scanner 1, T2-hyperintense WM lesions were manually identified on dual-echo scans by an experienced observer, and LV was quantified using a semi-automatic local thresholding segmentation technique (Jim 7.0, Xinapse Systems Ltd, Colchester, UK). For patients acquired on Scanner 2, T2-hyperintense WM lesions were identified using a fully automated approach based on a cascade of two 3D patch-wise convolutional neural networks, using 3D FLAIR and 3D T1-weighted MRI sequences as input images (Valverde *et al.*, 2017), and total T2-hyperintense LV was calculated. At follow-up, the number of new T2-hyperintense lesions was also counted. On both scanners, baseline NBV was calculated using FSL SIENAx software on lesion-filled (Battaglini *et al.*, 2012) 3D T1-weighted images, while PBVC during the follow-up was calculated using FSL SIENA software (Jenkinson *et al.*, 2012).

RS fMRI pre-processing. RS fMRI data processing was performed using the CONN toolbox (https://web.conn-toolbox.org/). RS fMRI images were realigned to the mean of each session using a rigid-body transformation to correct for head movements. After rigid registration of realigned images to the lesion filled 3D T1-weighted scan, RS fMRI images were normalized to the MNI space using a non-linear transformation (spatial resolution of normalized scans=2x2x2 mm3). After detection of outliers (using the ART tool), images were smoothed with a 6-mm3 Gaussian filter. For denoising, the first five cerebro-spinal fluid and white matter principal components were used as nuisance covariates in accordance with the anatomical component-based noise correction method (aCompCor). The six rigid motion parameters and their first temporal derivatives were also regressed out from data. Finally, RS fMRI time series were linearly detrended and band-pass filtered (0.01-0.1 Hz).

Constrained ICA analysis. After RS fMRI pre-processing, patterns of RS FC within monoaminergic networks were reconstructed using constrained ICA, as implemented in the GIFT software (https://trendscenter.org/software/gift). Constrained ICA is a data-driven tool using prior spatial information to reconstruct spatial distribution of intrinsically connected voxels related to a priori given sources of signal (Lin *et al*, 2009). The following steps were performed: 1) group ICA and 2) back-reconstruction.

This procedure provides maps of RS FC within PET-guided networks by reconstructing components based on prior spatial information with a weighting of signal according to prior atlas signal intensity. We passed to the ICA algorithm three PET-based monoamine transporter atlases as priors. Specifically, the atlases for serotonin and dopamine transporters were publicly available and obtained by averaging [^{11}C]3-amino-4-(2-dimethylaminomethylphenylsulfanyl)-benzonitrile and [^{18}F]DOPA PET images from HCs, respectively (García Gómez *et al*, 2018; Hesse *et al*, 2014a). Atlas for noradrenaline was not publicly available and therefore we constructed the atlas based on previously published data for [^{11}C](S,S)-Methylreboxetine brain PET imaging reporting the binding potential values in cortical and subcortical GM areas for 12 HCs.(Schmidt *et al*, 2020a) Image intensity of all atlases was normalised by scaling image values from 0 to 1 for non-zero voxels, by dividing intensity of each voxel for the maximum intensity value.

Statistical analysis. Baseline and follow-up between-group comparisons of demographic, clinical, and structural MRI variables were performed using using χ^2, Mann-Whitney U and Student's t-tests, and analysis of variance (ANOVA) with post-hoc Bonferroni correction, as appropriate (R-4.2.2 and SPSS software version 22.0). MRI variables were compared between HC and MS patients as well as between MS patients who did not develop depressive symptoms at follow-up (ND-MS) and patients who developed depressive symptoms at follow-up (D-MS) using age-, sex- and acquisition scanner-adjusted linear models. Within- and between-group longitudinal changes of clinical and MRI variables in MS patients were assessed with the χ^2 and Mann-Whitney U test. Differences of monoamine-related RS FC in the PET-guided networks at baseline were assessed between HCs and MS patients, as well as among HCs, ND- and D-MS, using SPM12 and ANOVA models, adjusted for age, sex and scanner. Conjunction analyses of D- and ND-MS patients *vs* HC were performed to determine which baseline RS FC abnormalities were characteristic of these two patients' groups. Longitudinal RS FC changes in the same networks within each group were assessed using ANOVA models for repeated measures, adjusted for age, sex and scanner. The same models were used to evaluate time-by-group interaction analyses. Results were assessed at $p<0.001$, uncorrected, cluster extent $K_E=10$ voxels, for explorative purposes.

Finally, age-, sex- and scanner-adjusted multiple regression models were used to test associations between: 1) baseline monoamine-related RS FC abnormalities and clinical/structural MRI variables; 2) changes over time of monoamine-related RS FC and concomitant MADRS changes.

Results

Demographic, clinical, neuropsychological and structural MRI features. The final sample included 49 MS patients and 27 HCs. Table 9.4.1 summarizes the main demographic, clinical and structural MRI characteristics of study participants.

At baseline, 4 MS patients had a stable treatment with interferon-beta from at least 6 months and were neither depressed nor fatigued.

At baseline, MS patients had lower NBV than HCs ($p = 0.02$). ND- and D-MS were matched in terms of demographic, clinical and structural MRI variables (Table 1). No significant difference in cognitive performance was observed between ND- and D-MS (Table 9.4.2). The median follow-up duration was 1.6 years (IQR = 1.0-2.0 years) for MS patients and 1.9 years (IQR = 1.2 - 2.4 years) for HCs ($p = 0.07$). Changes of clinical and MRI variable during the follow-up are summarized in Table 9.4.1. No significant changes of EDSS score ($p=0.62$) and T2 LV were detected over time. PBVC did not differ between MS patients and HCs ($p = 0.10$).

At follow-up, 14 (29%) MS patients developed depressive symptoms, while 35 (71%) patients did not. The mean number of neuropsychological tests with a deteriorating performance, based on RCI, did not differ between ND- and D-MS (Table 9.4.2).

Table 9.4.1. Main demographic, clinical and structural MRI characteristics of healthy controls (HC) and multiple sclerosis (MS) patients. Patients are first considered as a whole, and then divided into MS patients who developed depressive symptoms at follow-up (D-MS) and MS patients who did not develop depressive symptoms follow-up (ND-MS). Significant results are highlighted in **bold**.

	HCs (n=27)	**MS patients (n=49)**	**p HC *vs* MS**	**ND-MS (n=35)**	**D-MS (n=14)**	**p ND *vs* D**
Number of women / men (%)	16 (59) / 11 (41)	28 (57) / 21 (43)	0.99[a]	21 (60) / 14 (40)	7 (50) / 7 (50)	0.75[a]
Median Age (IQR) [years]	30.6 (27.4 to 40.8)	38.0 (29.7 to 43.7)	0.11[b]	37.9 (31.7 to 46.4)	39.9 (27.4 to 42.4)	0.63[b]
Median Education (IQR) [years]	**18.0 (13.7 to 18.0)**	**13.0 (13.0 to 16.0)**	**<0.001[b]**	13.0 (13.0 - 16.0)	14.0 (11.5 - 16.0)	0.75[b]
Median baseline MADRS (IQR)	-	4.0 (2.0 to 7.0)	-	4.0 (2.0 to 6.0)	4.5 (3.2 to 7.0)	0.43[b]
Median baseline MFIS (IQR)	-	19.0 (12.0 to 27.0)	-	19.0 (11.5 to 26.0)	22.0 (12.2 to 29.5)	0.45[b]
Median baseline disease duration (IQR) [years]	-	7.6 (2.0 to 13.3)	-	5.7 (2.0 to 13.8)	9.1 (2.4 to 10.8)	0.91[b]
Clinical phenotype RRMS/ Progressive MS (%)	-	43 (88) / 6 (12)	-	30 (86) / 5 (14)	13 (93) / 1 (7)	0.83[a]
Median FU duration (IQR) [years]	1.9 (1.2 to 2.4)	1.6 (1.0 to 2.0)	0.07[b]	1.5 (1.0 to 2.0)	1.7 (1.0 to 1.9)	0.45[b]
Median baseline EDSS (IQR)	-	1.5 (1.0 to 2.0)	-	1.5 (1.0 to 2.0)	1.5 (1.0 to 2.0)	0.79[b]
Median FU EDSS (IQR)	-	1.5 (1.0 to 2.0)	-	1.5 (1.0 to 2.5)	1.5 (1.0 to 2.0)	0.92[a]
Baseline DMT: No treatment/ 1st line/ 2nd line (%)	-	3 (6) / 23 (47) / 23 (47)	-	1 (2) / 17 (49) / 17 (49)	2 (14) / 6 (43) / 6 (43)	-
DMT change: yes (%) / no (%)	-	10 (20) / 39 (80)	-	7 (20) / 28 (80)	3 (21) / 11 (79)	0.99[a]
Annualized relapse rate during the FU (range)	-	0.06 (0.00 to 0.80)	-	0.06 (0.00 to 0.80)	0.07 (0.00 to 0.52)	0.69[b]
No. of subjects scanned with S 1/ S 2 (%)	15 (56) / 12 (44)	25 (51) / 24 (49)	0.89[a]	16 (64) / 19 (36)	9 (46) / 5 (54)	0.39[a]
Median baseline T2 LV (IQR) [mL]	**0.0 (0.0 to 0.0)**	**2.4 (1.0 to 5.1)**	**<0.001[c]***	2.2 (1.0 to 4.7)	2.7 (1.4 to 6.9)	0.99[e#]

Median FU T2 LV (IQR) [mL]	**0.0 (0.0 to 0.0)**	**2.6 (1.2 to 8.4)**	**<0.001[c]***	2.6 (1.2 to 5.8)	2.9 (1.8 to 9.2)	0.99[e#]
Median new T2-hyperintense lesion number (IQR)	-	1.0 (0.0 – 3.0)	-	1.0 (0.0 – 3.0)	1.0 (0.0 – 2.7)	0.62[b]
Mean baseline NBV (95% CI) [mL]	**1578 (1552-1604)**	**1540 (1521-1558)**	**0.02[d]***	1535 (1517-1552)	1540 (1514-1567)	0.99[f]*
Median PBVC (IQR) [%]	-0.30 (-0.59 to -0.01)	-0.68 (-1.21 to -0.20)	0.10[b]	-0.50 (-1.22 to -0.17)	-0.80 (-1.08 to -0.28)	0.99[b]

[a]Chi-squared test; [b]Mann-Withney U test; [c]Age-, sex- and scanner-adjusted linear models performed on log-scale; [d]Age-, sex- and scanner-adjusted linear models; [e]Age-, sex- and scanner-adjusted linear models performed on log-scale with p values adjusted for multiple comparisons (Bonferroni method); [f]Age-, sex- and scanner-adjusted linear models with p values adjusted for multiple comparisons (Bonferroni method); [g]Mann-Withney U test performed on log-scale

Statistically significant covariates: *Age; [#]Age and Scanner number

Abbreviations: D= MS patients who developed depressive symptoms at follow-up; DMT= disease modifying treatment; EDSS= Expanded Disability Status Scale; FU= follow-up; HC= healthy controls; IQR= interquartile range; LV= lesion volume; MADRS= Montgomery-Asberg Depression Scale; MFIS= Modified Fatigue Impact Scale; MS= multiple sclerosis; NBV= normalized brain volume; ND= MS patients who did not develop depressive symptoms at follow-up; PBVC= percentage brain volume change; RRMS= relapsing-remitting MS; S= scanner.

Table 9.4.2. Patients' cognitive performance at baseline and follow-up (FU) evaluations. Data are presented as mean (standard deviation).

	MS patients at baseline (n=49)	ND-MS at FU (n=35)	D-MS at FU (n=14)	p
Baseline z SRT lts	-0.4 (0.9)	-0.5 (0.8)	-0.2 (0.9)	0.29[a]
Baseline z SRT cltr	-0.5 (0.9)	-0.4 (0.8)	-0.5 (1.1)	0.98[a]
Baseline z SRT recall	-0.3 (1.2)	-0.4 (1.1)	-0.2 (1.4)	0.71[a]
Baseline z SPART	0.3 (0.9)	0.2 (0.9)	0.3 (0.9)	0.94[a]
Baseline z SPART recall	0.3 (0.8)	0.2 (0.7)	0.4 (1.0)	0.73[a]
Baseline z SDMT	-0.2 (1.7)	-0.1 (1.8)	-0.4 (1.5)	0.57[a]
Baseline z PASAT 3"	-0.4 (1.2)	-0.3 (1.2)	-0.5 (1.1)	0.56[a]
Baseline z PASAT 2"	-0.6 (1.1)	-0.6 (1.1)	-0.8 (1.1)	0.52[a]
Baseline z WLG	-0.1 (1.0)	0.0 (1.0)	-0.5 (1.1)	0.13[a]
FU z SRT lts	-0.4 (1.1)	-0.4 (1.1)	-0.6 (1.3)	0.49[a]
FU z SRT cltr	-0.3 (1.0)	-0.3 (0.9)	-0.4 (1.2)	0.82[a]
FU z SRT recall	-0.2 (1.1)	-0.1 (1.0)	-0.3 (1.4)	0.59[a]
FU z SPART	0.1 (1.1)	0.2 (1.0)	-0.2 (1.3)	0.29[a]
FU z SPART recall	0.1 (1.1)	0.3 (0.9)	-0.2 (1.3)	0.26[a]
FU z SDMT	-0.1 (1.6)	0.1 (1.5)	-0.6 (1.6)	0.14[a]
FU z PASAT 3"	-0.1 (1.2)	-0.1 (1.3)	-0.3 (0.9)	0.50[a]
FU z PASAT 2"	-0.5 (1.1)	-0.4 (1.1)	-0.7 (1.2)	0.49[a]
FU z WLG	0.0 (1.1)	0.2 (1.1)	-0.5 (1.1)	0.06[a]
Number of tests with a deteriorating performance at FU	0.0 (0.02)	0.0 (0.02)	0.0 (0.03)	0.58[a]

[a]Two-sample t-test.

Abbreviations: cltr= consistent long-term retrieval; D= MS patients who developed depressive symptoms at follow-up; FU= follow-up; lts= long term storage; ND= MS patients who did not develop depressive symptoms at follow-up; PASAT= paced auditory serial addition test; SDMT= symbol digit modalities test; SPART= 10/36 spatial recall test; SRT= selective reminding test; WLG= word list generation; z= z-score.

Baseline RS FC in monoamine-related networks. Baseline abnormalities of monoamine-related RS FC in MS patients compared to HC are summarized in Table 9.4.3 and shown in Figure 9.4.1.

Compared with HCs, within the three PET-guided monoaminergic networks, MS patients showed decreased RS FC in several frontal regions, including the bilateral inferior and middle frontal gyrus, the left supplementary motor area and the bilateral anterior cingulate cortex. They also had decreased monoamine-related RS FC in cerebellar cortices. They also showed increased RS FC in left inferior parietal and postcentral cortices, as well as in bilateral occipital regions within the three PET-guided monoaminergic networks. When comparing baseline RS FC in D-MS *vs* HCs and ND-MS patients *vs* HCs, separately, patterns of detected RS FC abnormalities mirrored those identified in the whole MS group, and consisting in decreased RS FC in frontal, cingulate and cerebellar cortices, and increased RS FC in parieto-occipital regions (data not shown). However, the conjunction analysis showed a peculiar RS FC decrease in D-MS *vs* both HCs and ND-MS in the left middle temporal gyrus within the PET-guided dopamine network (MNI space coordinates: -48 -60 24, K_E=11, T=3.8), and in the right calcarine cortex within the PET-guided noradrenaline network (MNI space coordinates: 60 -90 0, K_E=39, T=4.3).

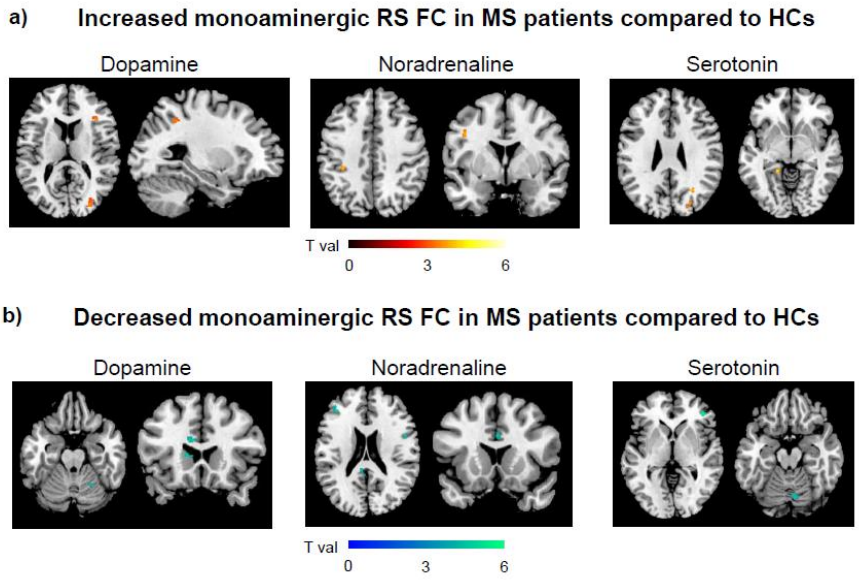
Table 9.4.3. Brain regions showing significant differences of baseline resting state (RS) functional connectivity (FC) between HCs and MS patients within the examined PET-guided monoaminergic networks (SPM12 two-sample t test adjusted for age, sex and acquisition scanner, $p<0.001$ uncorrected, cluster extent K_E=10).

Contrast	Areas	MNI space coordinates	K_E	T value
Dopamine-related RS FC				
MS patients < HCs	L Caudate nucleus	-12 22 6	13	4.54
	R MFG	34 28 32	15	4.25
	R Cerebellum vermis IX	2 -60 -38	21	4.24
	L ACC	-10 20 22	14	4.12
	R Cerebellum lobule VI	22 -58 -24	13	3.82
MS patients > HCs	R IFG	36 26 12	18	4.25
	R Cerebellum crus I	10 -82 -18	24	4.10
	L IPL	-28 -50 42	27	4.03

	R MOG	30 -86 12	34	3.98
Noradrenaline-related RS FC				
MS patients < HCs	L MFG	-40 36 22	28	5.05
	R IFG	50 6 22	34	4.63
	L SMA	6 0 58	14	4.61
	L Precentral gyrus	-34 8 46	19	4.30
	L Cerebellum lobule VI	-24 -50 -28	15	4.06
	R IFG	44 30 14	15	3.89
	R ACC	2 16 28	13	3.87
MS patients > HCs	L Postcentral gyrus	-34 -34 42	11	3.94
	L Precentral gyrus	-42 0 34	10	3.79
Serotonin-related RS FC				
MS patients < HCs	R Cerebellum vermis VI	6 -70 -20	29	4.38
	R IFG	42 40 0	22	4.43
	L IFG	-46 20 28	12	4.08
	L Precuneus	-6 -54 28	14	3.58
MS patients > HCs	L Lingual gyrus	-18 -42 -6	10	4.32
	R SOG	26 -78 30	25	3.82
	R Pallidum	18 6 0	16	3.77

Abbreviations: L=left; R=right; ACC=anterior cingulate cortex; IFG=inferior frontal gyrus; IPL=inferior parietal lobule; MOG=middle occipital gyrus; MFG=middle frontal gyrus; SMA=supplementary motor area; SOG=superior occipital gyrus.

Figure 9.4.1. Baseline resting state (RS) functional connectivity (FC) abnormalities in monoaminergic networks in patients with multiple sclerosis (MS) compared to healthy controls (HCs) (SPM12 two-sample t test adjusted for age, sex and scanner, $p<0.001$ uncorrected). Increased RS FC (panel A) is color-coded in red-yellow, while decreased RS FC (panel B) is color-coded in blue-lightblue. Images are in neurological convention.



Longitudinal changes of RS FC in monoamine-related networks. No significant changes over time of monoamine-related RS FC were detected in HCs. Longitudinal changes of monoamine-related RS FC in D- and ND-MS are summarized in Table 9.4.4 and shown in Figure 9.4.2.

Within the PET-guided dopamine network, ND-MS showed decreased RS FC in the left amygdala. Conversely, D-MS showed a widespread decrease of RS FC, mainly involving the bilateral middle occipital gyrus, right orbitofrontal cortex, left anterior cingulate cortex and right precuneus (all significant at time-by-group interaction analysis), as well as in some occipital regions. In this group, some clusters of increased RS FC in parietal regions were also detected.

Within the PET-guided noradrenaline network, ND-MS showed increased RS FC in the anterior cingulate cortex (significant at time-by-group interaction analysis) and in

the right precentral and angular gyri. Decreased noradrenaline-related RS FC was found in parietal and medial frontal regions for ND-MS, and in occipital/parahippocampal cortices and in the putamen for D-MS.

Finally, within the PET-guided serotonin network, ND-MS showed decreased RS FC in the putamen and in parieto-temporal regions, whereas D-MS showed increased RS FC in the anterior cingulate cortex.

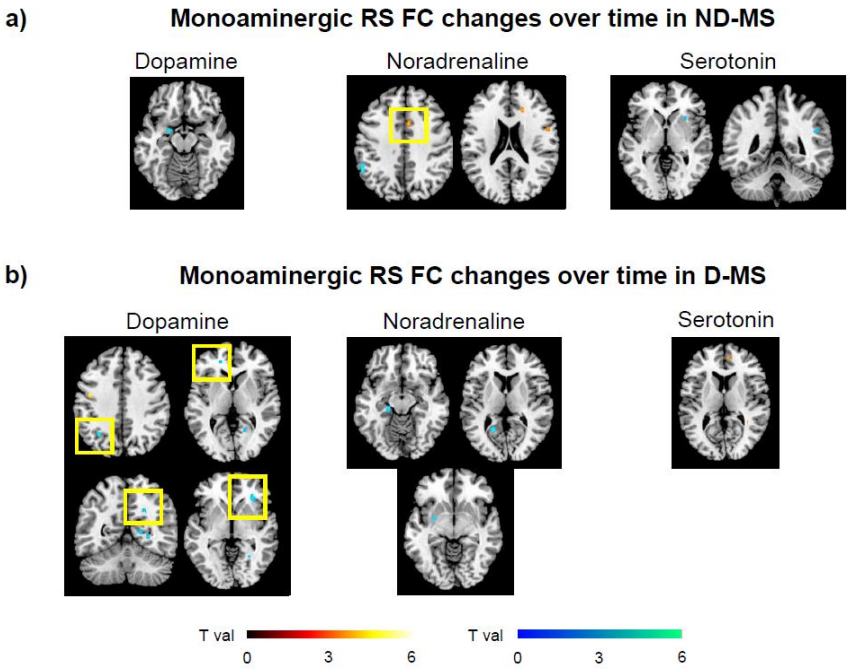
Table 9.4.4. Brain regions showing significant changes of resting state (RS) functional connectivity (FC) at follow-up vs baseline in patients with multiple sclerosis (MS) according to the presence or absence of depressive symptoms at follow-up (SPM12 repeated measure ANOVA, adjusted for age, sex and scanner, $p<0.001$ uncorrected, cluster extent $K_E=10$). Clusters significant at the time-by-group interaction analysis are highlighted with *.

Group	Contrast	Areas	MNI space coordinates	K_E	T value
Dopamine-related RS FC					
ND-MS	FU > Baseline	-	-	-	-
	Baseline > FU	L Amygdala	-20 -4 -14	15	3.85
D-MS	FU > Baseline	L Precentral gyrus	-42 -8 38	20	4.12
		R Angular gyrus	48 -58 42	16	3.76
		R IFG	54 22 14	10	3.69
	Baseline > FU	R OFC*	32 30 -2	39	4.85
		R MOG*	36 -76 24	16	4.84
		R Calcarine cortex	16 -58 6	21	4.45
		R Lingual gyrus	26 -58 0	14	4.34
		L MOG*	-30 -60 38	21	4.33
		R Precentral gyrus	20 -30 62	28	4.30
		L ACC*	-18 44 4	15	4.25
		R Precuneus*	22 -56 32	12	3.80
Noradrenaline-related RS FC					
ND-MS	FU> Baseline	R Angular gyrus	50 -64 32	27	4.21
		R ACC*	6 10 40	20	3.99
		R Precentral gyrus	52 2 22	19	3.91
	Baseline > FU	L IPL	-54 -50 40	29	4.57
		L Medial SFG	-2 48 30	41	4.24
D-MS	FU> Baseline	-	-	-	-
	Baseline > FU	L Calcarine cortex	-18 -56 8	37	4.69
		L Parahippocampal gyrus	-20 -28 -12	25	4.14

		L Putamen	-28 -2 -6	16	4.13
		L MOG	-32 -60 4	21	3.86
Serotonin-related RS FC					
ND-MS	FU > Baseline	-	-	-	-
	Baseline > FU	R Putamen	28 16 2	16	4.06
		R Angular gyrus	48 -46 26	15	3.73
		R Fusiform gyrus	20 -52 -14	21	3.70
D-MS	FU > Baseline	R ACC	6 50 12	19	3.80
	Baseline > FU	-	-	-	-

Abbreviations: D= MS patients who developed depressive symptoms at follow-up; ND= MS patients who did not develop depressive symptoms at follow-up; FU= follow-up; L=left; R=right; ACC=anterior cingulate cortex; IFG=inferior frontal gyrus; IPL=inferior parietal lobule; OFG=orbitofrontal cortex; MOG=middle occipital gyrus; SFG=superior frontal gyrus.

Figure 9.4.2. Longitudinal changes of resting state (RS) functional connectivity (FC) in monoaminergic networks in multiple sclerosis (MS) patients who did not develop depressive symptoms at follow-up (ND) (panel A), and in MS patients developing depressive symptoms (D) at follow-up (panel B) (SPM12 full-factorial model for repeated measures, adjusted for age, sex and acquisition scanner, p<0.001 uncorrected). Increased RS FC over time is color-coded in red-yellow, while decreased RS FC over time is color-coded in blue-lightblue. Yellow boxes highlight clusters significant at the time-by-group interaction analysis. Images are in neurological convention.



Correlation analysis. Baseline findings. In MS patients, a higher baseline EDSS score was associated with increased dopamine-related RS FC in right cerebellum (crus I) ($r = 0.59$, $p < 0.001$), and decreased noradrenaline-related RS FC in right inferior frontal gyrus ($r = -0.55$, $p < 0.001$), while a lower EDSS score correlated with increased serotonin-related RS FC in left lingual gyrus ($r = -0.57$, $p < 0.001$). A higher T2 LV correlated with decreased dopamine-related RS FC in left caudate nucleus ($r = -0.67$, $p < 0.001$) and decreased dopamine-related RS FC in right middle frontal gyrus ($r = -0.65$,

$p < 0.001$). No significant correlations were found between monoamine-related RS FC abnormalities and disease duration or NBV.

Longitudinal findings. In MS patients, an increase of MADRS score over time (i.e., ΔMADRS) was significantly associated with a concomitant decrease of dopamine-related RS FC over time in the right precuneus ($r = -0.65$, $p < 0.001$), and with a decrease of noradrenaline-related RS FC over time in the left parahippocampal gyrus ($r = -0.61$, $p < 0.001$).

Discussion

This is the first study that has investigated the association between RS FC changes within PET-guided dopamine, noradrenaline and serotonin networks and development of depressive symptoms in patients with MS. Our results showed that compared to HC, MS patients had decreased RS FC within all three monoaminergic networks in the frontal, cingulate, and cerebellar cortices, and increased RS FC in parieto-occipital regions. After a median follow-up of 1.6 years 29% of the MS patients developed depressive symptoms and showed widespread RS FC decrease within the dopamine network and decreased RS FC in limbic and occipital regions within the noradrenaline network compared to ND-MS. Of note, MADRS scores resulted associated with decrease over time of RS FC in the precuneus within the dopamine network and in the parahippocampal gyrus within the noradrenaline network. In this study, we minimized the potential confounding effects of fatigue and cognitive impairment (ND-MS and D-MS demonstrated similar cognitive performance at baseline and follow-up) in order to disentangle the specific RS FC changes associated with the development of depressive symptoms in MS.

In MS, dysfunction of the monoamine pathways results from both inflammation and structural changes (Carandini *et al*, 2021a). Only a few studies explored the structural alteration of monoaminergic networks in MS. Polak et al. (Polak *et al*, 2011) detected inflammation and focal lesions in the locus coeruleus of MS patients. Moreover, one diffusion-tractography study (Carandini *et al*, 2021c) identified axonal damage in fiber tracts originating from the brainstem monoaminergic nuclei. In addition, recent investigations in MS patients have found altered RS FC among the striato-fronto-limbic areas within monoaminergic networks (Carandini *et al*, 2021b; Carotenuto *et al.*, 2020;

Martino *et al,* 2020). Consistently, our study found a reduced monoamine-related RS FC in frontal regions and cerebellar cortices in MS patients compared to HCs at baseline. A higher lesion burden in MS patients resulted correlated with decreased RS FC in the middle frontal gyrus and caudate nucleus within the PET-guided dopamine network supporting the hypothesis of a disrupted communication within the monoaminergic systems (Carandini *et al.*, 2021b; Carandini *et al.*, 2021c; Carotenuto *et al.*, 2023; Cercignani *et al,* 2021). At baseline, we detected increased RS FC in inferior parietal and occipital regions within the three PET-guided monoaminergic networks. It is tempting to speculate that this finding may be related to he presence of compensatory mechanisms (Carotenuto *et al.*, 2020; Rocca *et al,* 2022; Rocca *et al,* 2012).

The most intriguing and novel results of our investigation come from the longitudinal analysis of the association between the development of depressive symptoms and RS FC changes within PET-guided monoaminergic networks. Reward processing is a neural and psychological multistep process that underlies the ability to experience pleasure in response to an event. The first step is the formation of a link between a stimulus and a reward, which generates interest and anticipation. Then motivation is formed based on the "cost-benefit calculation" between the expected value of the reward and the expected effort required to obtain it. Finally, a hedonic response is generated once the goal is achieved. These concatenated steps are influenced by feedback integration and reward-based learning that shape future behavioural responses to reward-related stimuli (Höflich *et al,* 2019). Previous studies have led to the hypothesis that dysfunctional reward processing, in which dopamine acts as a central neurotransmitter, represents a significant pathophysiological feature underlying anhedonia (i.e., the inability to feel pleasure in normally pleasurable situations) (Rolls *et al*, 2020), a core symptom of MS-related depression (Heitmann *et al*, 2022). In line with this hypothesis, we observed a widespread RS FC reduction within the dopamine network (orbitofrontal cortex, anterior cingulate cortex, precuneus, and occipital regions) in D-MS compared to ND-MS and a correlation between reduced precuneal RS FC within the dopamine network and increased MADRS score over time in MS patients. Multiple studies (Höflich *et al.*, 2019; Rolls *et al.*, 2020) have reported an attenuated activation of the orbitofrontal cortex and anterior cingulate cortex in individuals diagnosed with major depressive disorder. These two highly interconnected

regions are involved in the representation of reward value and guiding actions based on rewards (Rolls *et al.*, 2020). Reduced activation is associated with diminished response to reward (Höflich *et al.*, 2019; Rolls *et al.*, 2020). Altered RS FC patterns involving the orbitofrontal cortex and cingulate cortex within the limbic system have also been detected in MS patients with depression (Nigro *et al*, 2014; Riccelli *et al.*, 2016). The occipital lobe contains most of the anatomical regions of the visual cortex, contributes to visual information processing, and plays also a role in the perception of facial emotions (Teng *et al*, 2018). The decreased activation of the middle occipital gyrus in individuals with major depressive disorder is associated with dysfunctional visual processing, contributing to a cognitive bias toward negative emotional information (Teng *et al.*, 2018). Consistent with this finding, a previous work found a distributed pattern of GM atrophy, including the occipital lobe, in MS patients with depressive symptoms (Gobbi *et al*, 2013). The precuneus is a key hub of a neural network involved in integration of information, self-referential processing, and emotion regulation (Bendall, 2017; Cavanna & Trimble, 2006). Abnormal RS FC in the precuneus has been identified in individuals with depression (Peng *et al*, 2015), and similarly, an association between reduced RS FC in the precuneus and depressive symptoms has been reported in MS patients (Rocca *et al.*, 2015e).

In D-MS, we observed a decreased RS FC within the PET-guided noradrenaline network in the parahippocampal gyrus, compared to ND-MS. This aligns with the association found between increase over time of MADRS scores and concomitant decrease of RS FC in the parahippocampal gyrus of the noradrenaline network in the whole sample of MS patients. The parahippocampal gyrus plays an important role in memory and emotion regulation, and functional abnormalities of this region have been identified in individuals with major depressive disorder during RS and negative emotion processing (Zang *et al*, 2013). In MS patients, atrophy of the parahippocampal cortex has been associated with depressed mood (Hanken *et al*, 2016). In addition, altered hippocampal RS FC to the main hubs of the DMN is thought to be involved in the cognitive and emotional disturbances associated with depression (Rocca *et al.*, 2015e).

It is interesting to note that decreased RS FC over time within monoaminergic networks in D-MS occurred in brain areas such as the prefrontal cortex, temporal regions, and precuneal cortices, which are known to be components of the DMN.

Altered connectivity of the DMN is associated with development of depression in patients with MS (Rocca *et al.*, 2015e; Rocca *et al.*, 2022) possibly because it is involved in introspection, self-referential thinking, and rumination that are processes common in individuals with depression (Hamilton *et al*, 2015; Sheline *et al*, 2009; Zhou *et al*, 2020). In addition, abnormal between-network integration seems to play a key role in depressive symptoms as demonstrated by previous studies that found altered RS FC among striato-fronto-limbic cortical areas and DMN in depressed MS patients (Carotenuto *et al.*, 2023; Rocca *et al.*, 2012). Our findings suggest the presence of derangements within the monoaminergic networks underlying depressive symptoms (Carotenuto *et al.*, 2023).

Our study is not without limitations. First, the use of PET-constrained ICA provides only an indirect measure of monoaminergic RS FC. Second, the PET atlases used were derived from HCs, potentially disregarding the progressive loss of the presynaptic monoamine transporter observed in patients with MS. In addition, although the atlases were constructed by using PET tracers that are highly selective, they may be partially overlapped due to marginal binding of the PET tracers with other receptors. Third, study participants were scanned using two different scanners. Notably, each group of participants (HC vs MS and ND-MS vs D-MS) had a similar proportion of subjects scanned with Scanner 1 and Scanner 2, and the scanner was considered a covariate in all models. Nevertheless, the possible influence of different imaging parameters may not have been completely eliminated. Fourth, depressive symptoms were only assessed using MADRS. Finally, the study included a relatively small sample size. Clearly, while excluding fatigued MS patients was beneficial for examining RS FC changes associated with the development of depressive symptoms, it may have limited the statistical power of our analysis and the generalizability of our results given the frequent coexistence of depression and fatigue in MS.

In conclusion, our study provided evidence of an association between RS FC abnormalities within dopamine and noradrenaline networks and development of depressive symptoms in MS, highlighting the key role of dysfunctional reward processing and the disconnection between fronto-limbic structures and the DMN.

9.5 Other studies

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