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# INVESTIGATION OF THE PREVALENCE, EPIDEMIOLOGICAL AND CLINICAL CHARACTERISTICS OF FAMILIAL OCCURRENCE OF MULTIPLE SCLEROSIS IN THE POPULATION OF BELGRADE

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# ISTRAŽIVANJE PREVALENCIJE, EPIDEMIOLOŠKIH I KLINIČKIH KARAKTERISTIKA PORODIČNE MULTIPLE SKLEROZE U POPULACIJI BEOGRADA

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**INVESTIGATION OF THE PREVALENCE, EPIDEMIOLOGICAL AND CLINICAL CHARACTERISTICS OF FAMILIAL OCCURRENCE OF MULTIPLE SCLEROSIS IN THE POPULATION OF BELGRADE**

Abstract

**Introduction:** Familial multiple sclerosis (fMS) comprises about 12% of all multiple sclerosis (MS) cases.

**Aims:** To estimate the prevalence of fMS in the MS population in Belgrade, Serbia; to determine if any differences exist in the risk factor profile between fMS and sporadic MS (sMS); to determine if there is any difference in prognosis between fMS and sMS; and to evaluate rare gene variants in the fMS Serbian population.

**Methods**: A prevalence study, two case control studies, and a retrospective cohort study were performed at the Clinic of Neurology, University Clinical Center of Serbia, and Clinical Institute for Medical Genetics, University Medical Centre Ljubljana, Ljubljana, Slovenia in the period 2021-2023.

**Results:** The prevalence of fMS in the Belgrade MS population is 6.4%. Using logistic regression analysis adjusted for confounders revealed exclusive breastfeeding for 7-9 months (adjusted odds ratio 0.47, 95% confidence interval 0.28-0.80) and seafood usage (adjusted odds ratio 0.50, 95% confidence interval 0.26-0.95) to be protective factors for fMS when compared with sMS. On the other hand, use of cow's milk (adjusted odds ration 1.97, 95% confidence interval 1.13-3.44) and infant formula (adjusted odds ratio 2.07, 95% confidence interval 1.07-4.02) in infanthood increased the risk of fMS. Prognosis in terms of multiple sclerosis severity score did not differ between fMS and sMS. Whole exome sequencing analysis revealed 9 rare or uncommon gene variants predicted pathogenic in the fMS group.

C**onclusion:** Our overall findings suggest that certain differences exist between fMS and sMS; however, confirmatory studies are necessary to accurately determine their implications for prevention, treatment, and research of MS.

**Key words:** multiple sclerosis, familial occurence, prevalence, risk factors, risk profile, prognosis, disability progression, genetics, burden analysis, rare gene variants

**Scientific field:** Medicine

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**ISTRAŽIVANJE PREVALENCIJE, EPIDEMIOLOŠKIH I KLINIČKIH KARAKTERISTIKA PORODIČNE MULTIPLE SKLEROZE U POPULACIJI BEOGRADA**

Sažetak

**Uvod:** Porodična multipla skleroza (fMS) čini oko 12% svih slučajeva multiple skleroze (MS).

**Cilj:** Proceniti prevalenciju fMS u MS populaciji u Beogradu, Srbija; utvrditi da li postoje razlike u profilu faktora rizika između fMS i sporadične MS (sMS); odrediti da li postoje razlike u prognozi između fMS i sMS; i evaluirati retke genske varijante u srpskoj fMS populaciji.

**Metod**: Studija prevalencije, dve studije slučajeva i kontrola, kao i retrospektivna kohortna studija sprovedene su u Klinici za neurologiju, Univerzitetski klinički centar Srbije i Kliničkom institutu za medicinsku genetiku, Univerzitetski medicinski centar Ljubljana, Ljubljana, Slovenija u periodu 2021-2023. godine.

**Rezultati:** Prevalencija fMS u beogradskoj MS populaciji iznosi 6.4%. Nalazi logističke regresione analize korigovane za konfaunding varijable ukazuju da dojenje kao jedini izvor hrane u prvih 7-9 meseci života (korigovani odnos šansi 0,47, 95% interval poverenja 0,28-0,80) i konzumacija morskih plodova (korigovani odnos šansi 0,50, 95% interval poverenja 0,26-0,95) imaju protektivni efekat za nastanak fMS u poređenju sa sMS. S druge strane, upotreba kravljeg mleka (korigovani odnos šansi 1,97, 95% interval poverenja 1,13-3,44) i mleka u prahu (korigovani odnos šansi 2,07, 95% interval poverenja 1,07-4,02) u uzrastu odojčeda povećavaju rizik od fMS. Prognoza procenjena pomoću *multiple sclerosis severity score* se nije razlikovala između fMS i sMS. Analiza celokupnog egzoma otkrila je 9 retkih genskih varijanti koje su po predikciji patogene u grupi fMS.

**Zaključak:** Naši rezultati ukazuju da postoje izvesne razlike između fMS i sMS; međutim, potrebne su dalja istraživanja kako bi se tačno definisale njihove implikacije za prevenciju, lečenje i istraživanje MS.

**Ključne reči:** multipla skleroza, porodično javljanje bolesti, prevalencija, faktori rizika, profil faktora rizika, prognoza, progresija onesposobljenosti, genetika, analiza opterećenja genetskim varijantama, retke varijante gena

**Naučna oblast:** Medicina

**Uža naučna oblast:** Epidemiologija

**UDK broj:**_____

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# 1. INTRODUCTION

Multiple sclerosis (MS) is a chronic, immune-mediated disease of the central nervous system (CNS), with inflammation, demyelination, and neurodegeneration occurring in the CNS of the affected individuals (Filippi et al., 2018). Multiple sclerosis has an important place in contemporary medicine, due to it being the leading cause of non-traumatic disability in young adults (Kobelt et al., 2017). The last estimation published in the MS Atlas is that there are 2.9 million persons living with MS worldwide (Multiple Sclerosis International Federation, 2020). In Europe, an estimated MS prevalence is about 133/100,000 (Multiple Sclerosis International Federation, 2020).

Patients with MS present with neurological deficits or magnetic resonance imaging (MRI) findings indicative of CNS demyelination, and are diagnosed using the revised 2017 McDonald criteria (Thompson et al., 2018a). These criteria require evidence of dissemination in time and space, either clinically or radiologically, often including oligoclonal bands in cerebrospinal fluid (Thompson et al., 2018a). Multiple sclerosis presents in several phenotypes. Clinically isolated syndrome (CIS) occurs when the initial neurologic event doesn't meet dissemination in space or dissemination in time criteria. Over 80% of patients have relapsing-remitting MS (RRMS), marked by relapses and remissions. About 30% of RRMS cases transition to secondary progressive MS (SPMS) within 15-20 years, characterized by increasing disability. Primary progressive MS (PPMS), seen in about 15% of patients, involves continuous disability progression from onset without relapses (Lublin et al., 2014). MS phenotypes are further classified by disease activity and progression.

There are still gaps in understanding the etiology of MS, however, it is apparent that both environmental factors and genetic susceptibility influence its development. Attempts to pinpoint the exact role of the genetic background have been made, with genome-wide association studies (GWAS) finding over 200 genes, loci and single nucleotide polymorphisms (SNPs) linked with MS, although each one of them participating only slightly in the etiology of the disease (International Multiple Sclerosis Genetics Consortium, 2019).

In the majority of cases, MS presents as sporadic multiple sclerosis (sMS), with only one person in the family affected. It has been estimated that around 12% of patients have familial multiple sclerosis (fMS), with two or more close family members affected (Ehtesham, Rafie and Mosallaei, 2021; Harirchian et al., 2018). Depending on the definition of fMS, the estimates of prevalence can also vary, but the most commonly used definitions of fMS are: a) a person with MS (PwMS) that has one or more first-degree relatives with MS; and b) a PwMS with one or more first-, second-, or third-degree relatives with MS (O'Gorman et al., 2011; Steenhof et al., 2019a). It is still unclear to which extent the demographic characteristics, clinical presentation, radiographic findings, disease course and prognosis vary between fMS and sMS, with studies providing disparate findings (Aljumah et al., 2020; Andrijauskis et al., 2019; Ceccarelli et al., 2020; Katsavos et al., 2018; Mokhtari et al., 2023; Regal et al., 2018; Steenhof et al., 2019a). The studies investigating the degree in which the risk factors for fMS and sMS differ have scarcely been performed.

## *1.1. Pathophysiology of multiple sclerosis*

Pathological evidence indicates that inflammation drives tissue damage throughout all disease stages. Focal inflammatory infiltrates in the meninges and perivascular spaces produce soluble factors that cause demyelination or neurodegeneration, either directly or via microglia activation. The specific nature of these soluble factors in the sera and cerebrospinal fluid of patients

remains unidentified. Oxidative injury and mitochondrial damage ultimately result in demyelination and neurodegeneration, leading to "virtual hypoxia" (Lassmann, 2018).

Traditionally, it was established that the key event in the pathogenesis of MS is demyelination due to inflammation, which appears as relapse in the clinical presentation of the patient, and as lesions on MRI (Fisniku et al., 2008; Kutzelnigg et al., 2005). These lesions occur as a consequence of peripheral immune cells infiltrating the CNS (Zéphir, 2018). They are multifocal, and develop in both the white and gray matter of the CNS early in the disease and accumulate over time (Dunham and Mahajan, 2021). Additionally, active lesions involve demyelination, oligodendrocyte destruction, axonal injury, gliosis, microglial/macrophage activation, and infiltration of immune cells including macrophages and T- and B-cells. B-cells have an important role in MS pathogenesis, with oligoclonal bands production frequently present in PwMS (about 80% of patients) (Ghasemi, Razavi and Nikzad, 2017).

Macrophagal activation alongside microglial cells leads to the development of plaques in which myelin sheaths and oligodendrocytes are damaged (Dendrou, Fugger and Friese, 2015). The inflammation occurs throughout the course of the disease, but it is more apparent during the acute phases of the disease as opposed to the chronic ones (Dendrou, Fugger and Friese, 2015).

Secondary pathogenic mechanisms, such as oxidative stress and mitochondrial deficits, may contribute to neurodegeneration (Dunham and Mahajan, 2021). The accumulation of axonal injury leads to irreversible damage, resulting in disability of the patient. This neurodegeneration process apparently develops at the onset of the disease in patients with RRMS, and manifests as SPMS once the compensatory capacities of neuronal injury have been breached (Friese, Schattling and Fugger, 2014).

Oligodendrocytes are a type of glial cells in the CNS which have an important role in creating the myelin sheath which enables saltatory conduction of action potential of the neurons, ensuring energetic efficiency and speed of nerve conduction (Fünfschilling et al., 2012). What differs in MS when compared with other diseases that cause focal lesions in both gray and white matter is the existence of oligodendrocyte loss alongside selective perivenous and confluent primary demyelination (Lassmann, 2018) Loss of oligodendrocytes is one of the hallmarks of MS, however, recent findings have elucidated some of their qualitative differences in PwMS and persons without MS (Jäkel et al., 2019). These findings signal that there exist qualitative differences, in addition to the quantitative ones in oligodendrocytes in persons with and without MS.

## *1.2. Clinical presentation of multiple sclerosis*

The most distinct feature of MS is its variable neurological clinical presentation which reflects the wide diversity of predilection places in the CNS where the disease might occur. On the other hand, the symptoms and signs may drastically fluctuate in a single patient throughout the course of the disease (Ghasemi, Razavi and Nikzad, 2017). Besides the neurological symptoms, another important aspect of the disease are also the consequences of immobility, inactivity, bladder disfunction, social problems, depression, and other common comorbidities in MS (Gelfand, 2014; Maric et al., 2021). The numerous symptoms of MS can negatively affect both the everyday life and the quality of life of PwMS, especially bearing in mind that they can progress and precipitate the occurrence of new symptoms (Dačković, Stojsavljević and Mesaroš, 2013).

### 1.2.1. Symptoms and signs of multiple sclerosis

Since the pathological process in MS can affect any part of the CNS, the symptoms and signs that PwMS present with are very diverse, and can manifest as sensory, motor, visual, cerebellar symptoms, brainstem syndromes, cognitive symptoms, fatigue, sphincter and sexual

dysfunction, or as a combination of these. In order for a clinical attack to be considered related with MS, it must last longer than 24 hours, keeping in mind the inflammatory nature of MS (Ford, 2020).

Motor symptoms and signs are a consequence of affection of multiple pathways, including corticobulbar, corticospinal tracks, and cerebellar and sensitive pathways (Dačković, Stojsavljević and Mesaroš, 2013). The corticospinal tract affection occurs in the first attack of MS in 32-41% patients, and in 62% cases during the disease course. Signs of corticospinal tract affection may range from minimal, such as pathological reflexes, to severe such as spastic paraparesis. Muscle weakness also occurs due to corticospinal tract involvement, and can be accompanied by other symptoms of the upper motor neuron syndrome: spasticity, hyperreflexia, and positive Babinski sign. Spasticity, which is defined as the increased resistance to passive stretching of a muscle can accompany muscle weakness, but it can also occur when weakness is not present (Gelfand, 2014).

Cerebellar symptoms are traditionally associated with the triad of nystagmus, scanning speech, and tremor, which were initially described by Jean-Martin Charcot in 1877 (Waraich and Shah 2018). Besides these three symptoms, PwMS might also experience truncal ataxia, gait, slurred speech, ocular dysmetria and hypotonia as a result of cerebellum being affected by inflammation and demyelination (Weier et al., 2015). Along with brainstem involvement, cerebellar symptoms are a marker of poor prognosis in PwMS (Yang et al., 2022). Balance impairment can occur as a consequence of a variety of different mechanisms, and the clinical presentation will differ depending on the affected area responsible for it (cerebellum, vestibular system, sensory system) (Gelfand, 2014).

Sensory symptoms, such as paresthesias and numbness are very common in PwMS (Gelfand, 2014). They occur in 87% of PwMS, while they appear as presenting symptoms in 34% of patients (Swingler and Compston, 1992). Depending on their duration and intensity, they can be recognized as a part of an acute attack if they last for hours or days and are intense, or not related to an acute demyelination event if they are lasting only for minutes at a time (Gelfand, 2014). In the case there exists myelitis of the dorsal columns of the spinal cord, the patient may experience a “MS hug” - a squeezing feeling around the abdomen or chest (Gelfand, 2014). Horizontal sensory level can often be present, as well as the Lhermitte’s sign, which is characterized by sensations similar to electric shock travelling down the spine upon flexion of the neck (Ford, 2020). On physical examination, the patient may have reduced vibratory sense, joint position sense, and fine touch (Ford, 2020). Pain is also a frequently present symptom in PwMS, affecting from 44% to 80%, and even up to 92% in specific populations such as veterans with MS (Ehde, Osborne and Jensen, 2005; Hirsh et al., 2009). This pain can be mild, but also moderate or debilitating, impacting the functioning of PwMS (Hirsh et al., 2009). It usually presents in the form of neuropathic pain, with burning, electrical or stabbing sensations (Gelfand, 2014).

Optic neuritis is a consequence of acute demyelination of the optic nerve. It appears in about 50% of PwMS, while in a fifth of the cases, it is the initial symptom on presentation (Balcer, 2006). It is unilateral in the majority of PwMS. Patients complain of blurring or loss of vision, blind spots, trouble discerning colors, and/or pain behind the eye. The loss of vision has a spectrum of presentation anywhere from mild to complete (Optic Neuritis Study Group, 2008). It develops gradually, over the course of two weeks, and starts receding within the first month, however, the vision may not fully recover to the levels before the attack (Ford, 2020; Kale, 2016). The pain in optic neuritis is retrobulbar, occurs with eye movements, and usually precedes the loss of vision (Kale, 2016). Impaired color vision can often occur in optic neuritis, with patients complaining of red color being less intense than usual, and they can be identified by using color plates such as Ishihara color plates (Kale, 2016). Contrast sensitivity deterioration can be an important finding, seeing how its degree can be a good parameter of disease progression, and it is tested by finding the minimum contrast level where a patient can still discern large letters (Kale, 2016). On physical examination, an unequal response of pupils to light can often be found (a Marcus Gunn pupil)

(Balcer, 2006). This relative afferent pupillary defect can be identified by performing the swinging flashlight test, where pupils are being exposed to direct light alternately, with the reflex in the contralateral pupil being reduces when the affected pupil is exposed (Kale, 2016).

Brainstem syndromes are another common finding in MS. Depending on the nerves that are affected, they can manifest as diplopia (cranial nerves III, IV, VI), facial sensory loss (cranial nerve V), weakness of facial muscles (cranial nerve VII), vertigo (cranial nerve VIII), dysartria, weakness of tongue muscles, and dysphagia (cranial nerves IX, X, XII), or internuclear ophthalmoplegia if medial longitudinal fasciculus is affected (Gelfand, 2014).

Cognitive impairment occurs in about 50-60% of PwMS (Meca-Lallana et al., 2021). PwMS experience reduced information processing speed, decrease of complex attention, visuospatial ability, and executive functions, and deterioration of working memory (Chiaravalloti and DeLuca, 2008; Labiano-Fontcuberta et al., 2014; Rocca et al., 2015). Cognitive impairment correlates with MRI findings to a certain degree, but not entirely, and it has been proposed that different cognitive reserves between individuals are the reason for the heterogenous levels of cognitive impairment present in PwMS (Benedict and Zivadinov, 2011; Sumowski et al., 2018).

Mood disorders and behavioral disorders are very frequent in MS, and they occur in about 95% of PwMS. Depression is present in 79% of cases, agitation in 40%, anxiety in 37%, apathy in 20%, euphoria in 13%, behavioral disinhibition in 13%, hallucinations in 10% (Haussleiter, Brüne and Juckel, 2009).

Sphincter dysfunction is a common complaint among PwMS. The patients commonly exhibit an overactive neurogenic bladder, with about 60% of patients presenting with symptoms like urinary frequency, urgency and incontinence (Gelfand, 2014). On the other hand, bowel impairment is less frequent, with patients most frequently complaining of constipation when it is present. Sexual dysfunction is another common symptom in MS, presenting as loss of libido in women and erectile dysfunction in men (Demirkiran et al., 2006).

Most of the PwMS experience fatigue (83%) (Minden et al., 2006). Fatigue in MS usually presents as the sense of having a lack of energy in, and it can be very detrimental to functionality and well-being of PwMS. However, it is not always easy to discern whether the fatigue is part of the clinical presentation of MS, or a symptom of a comorbidity, such as depression. The substrate of fatigue in MS is still inadequately understood, although several explanations have been proposed such as inflammatory processes, structural damage of grey and white matter, metacognition, and disturbance of networks initiating cognitive operations (Manjaly et al., 2019).

### 1.2.2. Clinical phenotypes in multiple sclerosis

Most of the cases of MS (about 85%) can be categorized as RRMS (Klineova and Lublin, 2018). The mean age of onset of these patients is 30 years, with a distinct predominance of female sex (2-3:1) (Compston and Coles, 2008). The spectrum of disease in RRMS is wide, with some patients having many debilitating relapses, and rapidly progressing towards disability, while some patients have benign MS with few relapses that are mild in nature, affecting only the sensory system (Prajjwal et al., 2023). Patients with RRMS most frequently initially present with optic neuritis, numbness or tingling in the extremities, spasticity or weakness (Dobson and Giovannoni, 2019). The RRMS is characterized by the existence of relapses or disease exacerbations between which are the periods of complete or partial remission, in which the neurological status is stationary (Thompson et al., 2018a). Disease modifying therapy (DMT) can influence the outcomes of patients with RRMS by reducing the number of relapses, and slowing the progression of disability (Robertson and Moreo, 2016).

Patients with RRMS progress to SPMS if they are untreated, with the median time to progression of 19 years after MS onset (Rovaris et al., 2006). Establishing the exact point in which the progression to SPMS is not always simple and the diagnosis is performed retrospectively in the majority of the cases (Katz Sand et al., 2014).

A small proportion of cases of MS (~15%) can be classified as PPMS (Klineova and Lublin, 2018). These patients have a mean age of onset of 40 years, with an approximately equal female to male ratio (Compston and Coles, 2008). The vast majority of these patients present with spinal cord involvement (Compston and Coles, 2008). PPMS presents differently when compared to RRMS, with gradual deterioration of symptoms in PwMS, and with a lack of clear relapses and periods of remissions (Prajjwal et al., 2023). On the other hand, similar to RRMS, the spectrum of disease in PPMS is wide, with some patients experiencing rapid progression of the disease, and others having a very slow deterioration of symptoms (Montalban et al., 2017).

Clinically isolated syndrome is a MS disease course that signifies a first clinical event indicating a high likelihood of a demyelinating disease of the CNS, but without the dissemination in time criteria fulfilled for the diagnosis of clinically definitive MS (Klineova and Lublin, 2018).

On the other hand, radiologically isolated syndrome (RIS) is not classified as a separate MS disease course, but it is an important entity, signifying MRI findings indicating MS, with the absence of clinical attacks of the disease (Thompson et al., 2018a).

## *1.3. Diagnosis of multiple sclerosis*

Diagnosing MS can be challenging due to the variety of non-specific symptoms it can produce, and the wide spectrum of the disease present in PwMS. The diagnosis has shifted from primarily clinical criteria to a combination of clinical, radiological, and laboratory criteria (Schumacher et al., 1965; Thompson et al., 2018a). Essential part of MS diagnosis is dissemination in space – meaning that the lesions have encompassed multiple foci in the CNS; and dissemination in time – meaning that the lesions have occurred at separate times in a person (Thompson et al., 2018a). Another important step in diagnosing MS is the exclusion of alternative diagnoses (Miller et al., 2008; Thompson et al., 2018b). “Red flags” are a concept used in MS diagnostics signifying findings that are more or less inconsistent with MS and imply a possibility of a different diagnosis (Filippi et al., 2019). Bearing in mind the diversity and non-specificity of MS symptoms and MRI findings, misdiagnosis is one important issue, while diagnostic delays are another, occurring in about 50% of patients, and leading to increased burden of the disease and poorer prognosis (Thompson et al., 2018a; Uher et al., 2023).

### 1.3.1. Diagnostic criteria

Diagnostic criteria for MS have undertaken several iterations during the last few decades, the most commonly used being Poser, and McDonald criteria developed in 2001, and undergoing revisions in 2005, 2010 and 2017 (McDonald et al., 2001; Polman et al., 2005; Polman et al., 2011; Poser et al., 1983; Thompson et al., 2018a). The 2017 McDonald criteria are most commonly used in contemporary practice (Thompson et al., 2018a). However, it is important to note that these criteria are made for use in patients with a typical CIS, and that previous meticulous exclusion of other likely diagnoses needs to be performed prior to establishing a MS diagnosis (Thompson et al., 2018a). The idea of the 2017 revised McDonald criteria is to facilitate diagnosing MS in a patient presenting with a typical CIS, utilizing available data, the final aim being earlier introduction of the DMT, possibly improving the patient’s prognosis (Thompson et al., 2018a).

Besides clinical findings, MRI is the most important tool in establishing diagnosis of MS, with MRI findings frequently being used to fulfill the dissemination in time or space criteria to diagnose MS (Thompson et al., 2018a). Brain MRI is recommended in all patients considered for

MS diagnosis, while spine MRI is recommended in cases where there is likely spinal cord involvement, as well as in populations that are uncommonly diagnosed with MS, and when PPMS is being considered (Thompson et al., 2018a). When considering dissemination in space, it is noteworthy that not all lesions in the CNS are characteristic of MS, with T2-hyperintense lesions in periventricular, cortical or juxtacortical, infratentorial brain regions, and the spinal cord being considered characteristic of MS, and lesions in ≥2 of these regions signifying dissemination in space. The dissemination in time criteria can be fulfilled if the MRI findings show either the simultaneous presence of gadolinium-enhancing and non-enhancing lesions, or a new T2-hyperintense or gadolinium-enhancing lesion on a MRI performed on follow-up, after comparing with a baseline scan (Thompson et al., 2018a).

Cerebrospinal fluid (CSF) examination has had diminishing importance in diagnosing MS throughout history, however, it is still relevant, with the contemporary 2017 McDonald criteria incorporating the presence of oligoclonal bands in the CSF as a substitute for dissemination in time in case other likely diagnosis have been excluded, and there is a lack of dissemination in time in the clinical or radiological findings (Thompson et al., 2018a). This recommendation has been integrated into the revised criteria due to evidence obtained from studies that the presence of oligoclonal bands in CSF is an independent predictor of a second attack in persons with CIS (Andreadou et al., 2013; Dobson et al., 2013a). The advantage of CSF analysis is its high sensitivity, being positive in cases when the pathological process in not underwent enough to have significant clinical or MRI presentations, but a significant limitation is its low specificity, with oligoclonal bands being positive in any condition with chronic CNS inflammation (Becker et al., 2015; Yang et al., 2022).

### *1.4. Treatment of multiple sclerosis*

The treatment of MS is an emerging field, with significant breakthroughs achieved in previous years. Various approaches are required in the management of MS. Immunomodulatory therapy with DMTs is the key treatment, but therapy for symptom relief and immunosuppressive therapy for acute relapses also have an important place in the management of PwMS. The main goals of DMTs are reducing relapse rates, slowing down disability progression, and enhancing overall outcomes. On the other hand, DMTs have minimal impact on tissue repair or myelin recovery (Buzzard et al., 2017; Hauser and Cree, 2020). As the number of improved DMTs for MS treatment grows, the evidence-based guidelines for MS treatment are regularly updated to reflect these changes (Montalban et al., 2018; Rae-Grant et al., 2018; Wiendl et al., 2021). The DMTs in MS can be classified by efficacy into moderately effective and highly effective therapies, which can be associated with less favorable safety profiles (Bowen, 2019; Klocke and Hahn, 2019).

The European Medicines Agency and the Food and Drug Administration have approved multiple agents for treating various forms of MS, including RRMS, active SPMS, and PPMS. Two primary treatment approaches are utilized: the escalation strategy and the induction strategy. The escalation strategy involves starting with moderately effective DMTs to avoid the potential toxicities of highly effective DMTs. If the response is inadequate, treatment escalates to highly effective DMTs (Klocke and Hahn, 2019; Ontaneda et al., 2019). Conversely, the induction strategy employs an initial aggressive treatment with highly effective DMTs to achieve substantial immune suppression, followed by maintenance therapy with moderately effective DMTs (Giovannoni, 2018; Ruggieri et al., 2018).

Switching between DMTs is another important issue in MS treatment, with guidelines recommending washout periods when changing therapies. The changing DMTs can be 1) vertical, when patients are changed from a first-line agent to a highly effective DMT; and 2) horizontal, when the patients change between two agents that have similar efficacy but different safety profiles after experiencing adverse events (Filipi and Jack, 2020; Kołtuniuk and Chojdak-Łukasiewicz, 2022).

The importance of early initiation of DMTs in RRMS cannot be overstated since it leads to long-term benefits for the patient (Montalban et al., 2018; Rae-Grant et al., 2018; Wiendl et al., 2021). Recent studies indicate that patients that were inducted early onto DMTs have lesser disability accumulation compared with those that are initiated later (Amato et al., 2020; Portaccio et al., 2022). However, recent studies have signaled that the strategy of initiating the treatment with drugs of moderate efficacy might not be optimal for PwMS, seeing how disability accumulation is higher in patients that receive moderate efficacy drugs compared to those that receive high efficacy therapy (Kappos et al., 2020). The need for an earlier initiation of highly effective DMTs is increasingly advocated (Giovannoni, 2018; Ruggieri et al., 2018).

Treatment of PwMS is complex, considering the diverse patient and disease factors, along with the pharmacological profiles and monitoring needs of DMTs. Personalized treatments based on prognostic scores have recently been suggested as a way of risk stratification of patients for optimal distribution of DMTs (Van Wijmeersch et al., 2022). A shared decision-making process is crucial, involving active counseling that takes into account various patient factors, including quality of life, lifestyle, adherence, disease and treatment history, comorbidities, and family planning. Additionally, clinical and drug factors like disease activity, drug safety profile, and monitoring requirements should be considered to improve patient outcomes (Madsen, 2017; Wiendl et al., 2021).

### *1.5. Prognosis of multiple sclerosis*

In MS, assessement of prognosis is essential for personalized treatment, allowing individuals to be categorized based on demographic and environmental factors, clinical characteristics, MRI findings, and biomarkers (Rotstein and Montalban, 2019).

It has been demonstrated that demographic and environmental factors that lead to poorer prognosis in MS are older age at onset, male sex, non-European descent, low levels of vitamin D, smoking, and coexistence of comorbidities (Rotstein and Montalban, 2019).

Among clinical factors, a significant predictive value was obtained for PPMS phenotype, high relapse rate, shorter interval between the first and second relapses, poor recovery from the first relapse, presence of cerebellar, brainstem or spinal cord symptoms at onset, polysymptomatic onset, higher Expanded Disability Status Scale (EDSS) score at diagnosis, and early cognitive impairment (Comi et al., 2001; Weinshenker et al., 1991).

Imaging studies identified the following MRI characteristics as potential prognostic factors in MS: a high number of T2 lesions, high T2 lesion volume, the presence of gadolinium-enhancing lesions, especially with infratentorial localization, the finding of spinal cord lesions, whole brain atrophy, and grey matter atrophy (Kappos et al., 1999; Rotstein and Montalban, 2019).

It has been found in recent studies that the presence of different biomarkers could have potential predictive value in MS. These are: the presence of IgM and IgG oligoclonal bands in the CSF, high levels of neurofilament light chain in the CSF and serum, high levels of chitinase in the CSF, and retinal nerve fibre layer thinning detected with optical coherence tomography (Comabella et al., 2010; Kuhle et al., 2016).

Histopathological studies have shown that axonal damage in MS is present on the very beginning of the disease, even when neurological disability doesn't exist or is minimal (Trapp et al., 1998). Clinical parameters that are implemented for estimating the progression of disability in PwMS are the EDSS and Multiple Sclerosis Severity Score (MSSS). Kurtzke's EDSS is the most frequently used instrument to measure disability in PwMS, with scores ranging from 0 (full health) to 10 (death from MS), with the scale having intervals of 0.5 (Kurtzke, 1983). The scale encompasses the degree of disability of pyramidal, visual, sensory, brainstem, cerebral, and bowel

and bladder functions, alongside ambulation score which reflects the degree in which the patient has impaired walking (Kurtzke, 1983). MSSS is derived from EDSS, and it also incorporates the dimension of time into the score (Roxburgh et al., 2005). It is based on analysis of almost 10,000 European patients, and provides a different score for all combinations of each EDSS value 0-9.5 and disease duration 1-30 years (Roxburgh et al., 2005) (Figure 1).

| | 0 | 1 | 1.5 | 2 | 2.5 | 3 | 3.5 | 4 | 4.5 | 5 | 5.5 | 6 | 6.5 | 7 | 7.5 | 8 | 8.5 | 9 | 9.5 | EDSS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.67 | 2.44 | 4.30 | 5.87 | 7.08 | 7.93 | 8.64 | 9.09 | 9.35 | 9.50 | 9.63 | 9.74 | 9.84 | 9.90 | 9.94 | 9.97 | 9.98 | 9.98 | 9.99 | |
| 2 | 0.53 | 2.01 | 3.69 | 5.24 | 6.46 | 7.27 | 7.98 | 8.58 | 8.95 | 9.18 | 9.38 | 9.59 | 9.79 | 9.88 | 9.93 | 9.97 | 9.99 | 9.99 | 9.99 | |
| 3 | 0.45 | 1.77 | 3.34 | 4.82 | 6.00 | 6.81 | 7.54 | 8.14 | 8.55 | 8.83 | 9.07 | 9.35 | 9.63 | 9.77 | 9.86 | 9.92 | 9.97 | 9.98 | 9.99 | |
| 4 | 0.35 | 1.45 | 2.87 | 4.27 | 5.41 | 6.24 | 6.98 | 7.65 | 8.12 | 8.42 | 8.70 | 9.08 | 9.47 | 9.68 | 9.80 | 9.88 | 9.95 | 9.98 | 9.99 | |
| 5 | 0.30 | 1.28 | 2.60 | 3.90 | 4.95 | 5.79 | 6.58 | 7.26 | 7.75 | 8.08 | 8.38 | 8.83 | 9.32 | 9.60 | 9.76 | 9.86 | 9.95 | 9.98 | 9.99 | |
| 6 | 0.25 | 1.13 | 2.33 | 3.54 | 4.55 | 5.38 | 6.14 | 6.81 | 7.33 | 7.66 | 7.98 | 8.50 | 9.08 | 9.45 | 9.68 | 9.81 | 9.93 | 9.97 | 9.99 | |
| 7 | 0.24 | 1.04 | 2.10 | 3.17 | 4.13 | 4.96 | 5.75 | 6.46 | 6.98 | 7.32 | 7.65 | 8.24 | 8.91 | 9.33 | 9.59 | 9.76 | 9.90 | 9.95 | 9.99 | |
| 8 | 0.21 | 0.94 | 1.92 | 2.93 | 3.81 | 4.57 | 5.36 | 6.10 | 6.61 | 6.95 | 7.32 | 7.97 | 8.71 | 9.21 | 9.55 | 9.74 | 9.89 | 9.96 | 9.99 | |
| 9 | 0.21 | 0.88 | 1.76 | 2.65 | 3.45 | 4.17 | 4.93 | 5.64 | 6.14 | 6.50 | 6.90 | 7.65 | 8.53 | 9.09 | 9.47 | 9.70 | 9.87 | 9.95 | 9.99 | |
| 10 | 0.19 | 0.78 | 1.53 | 2.34 | 3.10 | 3.79 | 4.55 | 5.28 | 5.77 | 6.14 | 6.58 | 7.39 | 8.31 | 8.92 | 9.34 | 9.61 | 9.83 | 9.94 | 9.99 | |
| 11 | 0.17 | 0.71 | 1.40 | 2.13 | 2.82 | 3.46 | 4.21 | 4.94 | 5.42 | 5.82 | 6.30 | 7.18 | 8.15 | 8.79 | 9.24 | 9.52 | 9.78 | 9.92 | 9.98 | |
| 12 | 0.16 | 0.64 | 1.28 | 1.98 | 2.64 | 3.25 | 3.94 | 4.63 | 5.13 | 5.54 | 6.03 | 6.92 | 7.93 | 8.63 | 9.13 | 9.43 | 9.71 | 9.88 | 9.97 | |
| 13 | 0.13 | 0.57 | 1.14 | 1.80 | 2.44 | 3.05 | 3.70 | 4.38 | 4.91 | 5.32 | 5.80 | 6.74 | 7.83 | 8.55 | 9.03 | 9.34 | 9.65 | 9.85 | 9.96 | |
| 14 | 0.11 | 0.49 | 1.03 | 1.70 | 2.33 | 2.91 | 3.55 | 4.26 | 4.82 | 5.23 | 5.70 | 6.56 | 7.59 | 8.34 | 8.86 | 9.20 | 9.57 | 9.82 | 9.95 | |
| 15 | 0.10 | 0.45 | 0.99 | 1.64 | 2.26 | 2.82 | 3.44 | 4.14 | 4.68 | 5.09 | 5.51 | 6.33 | 7.41 | 8.17 | 8.70 | 9.11 | 9.51 | 9.78 | 9.94 | |
| 16 | 0.09 | 0.38 | 0.85 | 1.42 | 1.99 | 2.56 | 3.17 | 3.86 | 4.41 | 4.81 | 5.18 | 6.00 | 7.14 | 7.97 | 8.54 | 9.04 | 9.49 | 9.75 | 9.94 | |
| 17 | 0.05 | 0.32 | 0.76 | 1.28 | 1.77 | 2.30 | 2.95 | 3.65 | 4.17 | 4.55 | 4.94 | 5.74 | 6.89 | 7.77 | 8.38 | 8.99 | 9.52 | 9.79 | 9.96 | |
| 18 | 0.04 | 0.26 | 0.66 | 1.12 | 1.57 | 2.09 | 2.70 | 3.37 | 3.89 | 4.27 | 4.62 | 5.43 | 6.62 | 7.54 | 8.23 | 8.94 | 9.51 | 9.78 | 9.96 | |
| 19 | 0.05 | 0.28 | 0.63 | 1.00 | 1.39 | 1.89 | 2.50 | 3.19 | 3.72 | 4.12 | 4.49 | 5.35 | 6.59 | 7.51 | 8.22 | 8.98 | 9.57 | 9.81 | 9.96 | |
| 20 | 0.05 | 0.26 | 0.59 | 0.94 | 1.29 | 1.71 | 2.29 | 2.99 | 3.51 | 3.93 | 4.30 | 5.15 | 6.43 | 7.45 | 8.23 | 8.98 | 9.58 | 9.80 | 9.95 | |
| 21 | 0.05 | 0.30 | 0.66 | 1.02 | 1.39 | 1.77 | 2.34 | 2.97 | 3.43 | 3.83 | 4.21 | 5.09 | 6.35 | 7.33 | 8.08 | 8.87 | 9.49 | 9.77 | 9.96 | |
| 22 | 0.04 | 0.23 | 0.54 | 0.90 | 1.28 | 1.66 | 2.20 | 2.82 | 3.29 | 3.69 | 4.09 | 5.04 | 6.35 | 7.35 | 8.10 | 8.84 | 9.42 | 9.73 | 9.95 | |
| 23 | 0.05 | 0.27 | 0.58 | 0.91 | 1.26 | 1.64 | 2.19 | 2.78 | 3.21 | 3.69 | 4.19 | 5.16 | 6.47 | 7.46 | 8.20 | 8.87 | 9.43 | 9.75 | 9.95 | |
| 24 | 0.05 | 0.24 | 0.52 | 0.86 | 1.25 | 1.63 | 2.15 | 2.71 | 3.09 | 3.52 | 4.01 | 5.03 | 6.36 | 7.38 | 8.15 | 8.81 | 9.39 | 9.74 | 9.96 | |
| 25 | 0.05 | 0.23 | 0.47 | 0.77 | 1.15 | 1.56 | 2.05 | 2.53 | 2.84 | 3.21 | 3.74 | 4.88 | 6.26 | 7.24 | 8.00 | 8.73 | 9.35 | 9.75 | 9.98 | |
| 26 | 0.05 | 0.20 | 0.45 | 0.78 | 1.17 | 1.58 | 2.08 | 2.63 | 2.99 | 3.40 | 3.95 | 5.02 | 6.39 | 7.44 | 8.21 | 8.89 | 9.48 | 9.80 | 9.96 | |
| 27 | 0.05 | 0.22 | 0.48 | 0.78 | 1.15 | 1.56 | 2.03 | 2.56 | 2.91 | 3.29 | 3.86 | 4.93 | 6.33 | 7.38 | 8.14 | 8.91 | 9.56 | 9.85 | 9.98 | |
| 28 | 0.04 | 0.17 | 0.40 | 0.74 | 1.16 | 1.52 | 1.88 | 2.39 | 2.76 | 3.04 | 3.46 | 4.54 | 5.99 | 7.07 | 7.90 | 8.75 | 9.45 | 9.80 | 9.98 | |
| 29 | 0.03 | 0.18 | 0.47 | 0.80 | 1.19 | 1.51 | 1.79 | 2.27 | 2.68 | 3.01 | 3.41 | 4.35 | 5.68 | 6.76 | 7.66 | 8.62 | 9.38 | 9.75 | 9.96 | |
| 30 | 0.01 | 0.13 | 0.45 | 0.82 | 1.19 | 1.45 | 1.69 | 2.23 | 2.75 | 3.13 | 3.50 | 4.35 | 5.61 | 6.66 | 7.54 | 8.47 | 9.27 | 9.67 | 9.91 | |
| Years | | | | | | | | | | | | | | | | | | | | |

Figure 1 The Multiple Sclerosis Severity Score – adapted according to (Roxburgh et al., 2005)

### *1.6. Epidemiology of multiple sclerosis*

Research performed so far has indicated that there are two main determinants of MS distribution: 1) familial clustering, defined by genetic burden of the individual; and 2) geographic variation in prevalence, in which probably both genetic factors and environmental risk factors play a role (Dean et al., 2008; Dyment et al., 2006).

The most recent update of Atlas of MS, which incorporates data on MS prevalence from 84% of the countries in the world, estimates that there are 2.9 million PwMS worldwide, which amounts to a prevalence of 36/100,000 persons (Multiple Sclerosis International Federation, 2020). This is an increase from the 2.3 million reported in 2013, and besides the growth of the human population, which has reached 8 billion on November 15, 2022 according to the United Nations; the other factors contributing to this increase in the number of PwMS are better diagnostics, improved reporting, and prolonged life span for PwMS (Multiple Sclerosis International Federation, 2020). The highest prevalence of MS has been reported in Europe WHO region (133/100,000), followed by the Americas (112/100,000), Eastern Mediterranean (30/100,000), South-East Asia (9/100,000), and finally Africa and the Western Pacific WHO regions (both with a prevalence of 5/100,000) (Multiple Sclerosis International Federation, 2020). The five countries with highest prevalence of MS in the world are San Marino (337/100,000 persons), Germany (303/100,000), USA (288/100,000), Denmark (282/100,000), and Canada (250/100,000) (Multiple Sclerosis International Federation, 2020).

According to data obtained from the population-based Belgrade MS Registry, on December 31, 2023, prevalence of MS in Belgrade was 140/100,000, 100/100,000 for males, and 200/100,000 for females (unpublished data).

The incidence data of the Atlas of MS is less reliable, stemming from only 65% of the 84% countries that responded with prevalence data. The estimate from data from available 75 countries is that 300 persons are diagnosed each day, for a 107,000 persons diagnosed with MS annually, for an annual incidence of 2.1/100,000 (Multiple Sclerosis International Federation, 2020). This number is an underestimate of the true distribution, bearing in mind that data for many countries is missing.

### 1.6.1. Demographic characteristics of persons with multiple sclerosis

Demographic characteristics are an important asset when studying MS occurrence, because of their influence on MS risk. The possibility of acquiring MS varies based on age, sex, and socio-economic status of the individual.

As previously stated, MS is primarily a disease of young adults, with mean age of 32 years at MS diagnosis (Multiple Sclerosis International Federation, 2020). However, the mean age at diagnoses varies based on MS course, with RRMS having a mean age of onset of 30 years, and PPMS patients having on average onset 10 years after, at age 40 (Compston and Coles, 2008). Birth order has been studied as a potential factor in MS occurrence, with large studies refuting the existence of difference in birth order between PwMS and their healthy siblings (Ahlgren and Andersen, 2005; Sadovnick et al., 2005). Although it is much more frequent in adults, MS can occur in children too, with an estimated of 30,000 cases of paediatric-onset MS worldwide (Multiple Sclerosis International Federation, 2020).

Women are affected by RRMS more frequently than men, with a female to male ratio of 2-3:1, while the sex distribution in PPMS is basically identical (Compston and Coles, 2008). There has been observed an increase of this ratio, which is attributed to increase of incidence of MS in women (Voskuhl, 2020). On the other hand, males with RRMS have been found to have an increased risk of progressing to SPMS compared with females (Koch et al., 2010). The mechanisms behind these differences are still unclear, with the differences in sex chromosomes and in hormonal status between sexes being proposed as possible solutions (Voskuhl, 2020).

Education level is another demographic factor that has been associated with MS risk, with PwMS having lower levels of education compared to their siblings, as well as PwMS with higher levels of education having lower rates of relapse compared to PwMS with lower levels of education (Bjørnevik et al., 2017; D'hooghe et al., 2016). Also, PwMS with higher levels of education were less prone to developing cognitive defects compared with PwMS with lower education levels (Martins Da Silva et al., 2015). A relationship has also been found between higher maternal education levels at age 16 of the PwMS and slower disease progression in these patients, as well as the shorter delay between symptom onset and diagnosis, while no such relationship was found for paternal education levels (Flemmen et al., 2021).

### 1.6.2. Etiology of multiple sclerosis

The etiology of MS remains unknown, although significant breakthroughs have been made in the past few years on the path to understanding this disease. It is apparent that an interaction between several factors lies at the root of MS: a) infectious agents, namely viruses; b) genetic background of susceptible individual, with *HLA-DRB1*15:01* and *HLA-A*02* haplotypes being the key genetic determinants; c) hormonal factors, such as vitamin D deficiency; and d) lifestyle factors such as smoking and passive smoking. Studies currently being undertaken might further elucidate the exact roles of individual environmental and genetic risk factors and the mechanisms of their interactions.

#### *1.6.2.1. Environmental risk factors for multiple sclerosis*

Studies of environmental risk factors in MS have encompassed many factors such as infectious agents, smoking, vitamin D, latitude, obesity, breastfeeding, etc, with diverse levels of evidence being obtained for different factors in various studies. Among these, the factors that have so far been shown to be most strongly associated with MS development are Epstein-Barr virus (EBV) infection, smoking, low levels of vitamin D, and obesity in adolescence.

The role of the EBV, the infectious agent that causes infectious mononucleosis (IM), in MS risk and occurrence has been much investigated during the years. It has been discovered decades ago, and confirmed multiple times, that the risk of MS increases in persons that have had IM, with the increased risk still being maintained even 30 years after infection (Ascherio et al., 2001; Handel et al., 2010a; Nielsen et al., 2007). Recent studies have elucidated the magnitude of the role that EBV in MS occurrence, with the most convincing results being obtained from a cohort study performed in the USA, including more than 10 million veterans of the US military (Bjornevik et al., 2022). The authors observed conversion rates from a EBV seronegative subset of the cohort that was also free of MS at baseline, and compared the seroconversion rates in individuals who developed MS and in those who didn't (Bjornevik et al., 2022). Seroconversion was observed in 97% of individuals that developed MS during follow-up, and only in 57% of those that remained healthy, displaying a hazard ratio of 32.4 (Bjornevik et al., 2022). The authors have argued that there isn't a possibility that a factor that has enough magnitude of effect exists in order to confound these results (Bjornevik et al., 2022). These findings indicate that EBV infection has a seminal role in MS development, and open the doors for potential prevention measures (Bjornevik et al., 2022; Bjornevik et al., 2023; VanderWeele and Ding, 2017). The patophysiology of EBV influence on MS risk is unclear, although antiviral immunity and autoimmunity have been suggested (Aloisi, Giovannoni and Salvetti, 2023). A recent study proposed that *HLA-DRB1*15:01* has a role as a coreceptor for EBV, possibly providing the pathophysiological mechanism of the EBV- *HLA-DRB1*15:01* interaction (Menegatti et al., 2021).

Besides EBV, other viruses, such as human herpesvirus 6 (HHV-6), varicella zoster virus (VZV), human immunodeficiency virus (HIV), cytomegalovirus (CMV), measles, coronaviruses, endogenous retroviruses, and others have been explored as potential risk factors for MS, with results being inconclusive because of the studies being underpowered (Mentis et al., 2017).

Vitamin D is a fat-soluble hormone that is synthesized under the influence of ultraviolet radiation B from direct sunlight (Ellison and Moran, 2021). Diet can also be a source of vitamin D, but in magnitude that is inferior to exposure to sunlight, with oily fish being a particularly rich source of vitamin D (Gombash et al., 2022). The main functions of vitamin D is the homeostasis of calcium, but it also has immunomodulatory properties (Carmeliet, Dermauw and Bouillon, 2015; Maretzke et al., 2020). Deficiency of vitamin D has been linked to MS risk, as well as relapse occurrence in large prospective cohort studies and case-control studies (Munger et al., 2004; Munger et al., 2006; Simpson et al., 2010). Another finding that can be explained by the link between MS and vitamin D deficiency is the observed increasing trend of MS with the increase of latitude, seeing how there is a variance of amount of direct sunlight at different latitudes (Simpson et al., 2019). It is still not definitely clear at which point during a person's lifetime will vitamin D deficiency influence increased MS risk, with studies showing an increased risk of MS in offspring of mothers that had low vitamin D in pregnancy; in individuals born in the spring; in persons that had low vitamin D as newborns, and in those that had lower levels of sunlight exposure at ages 6-20 (Dobson, Giovannoni, and Ramagopalan, 2013b; Kampman, Wilsgaard, and Mellgren, 2007; Munger et al., 2016; Van der Mei et al., 2003). The findings that further strengthen the link between MS and vitamin D deficiency are that at higher latitudes MS risk is decreased in populations consuming oily fish rich in vitamin D, as well as that there is a reduced risk of MS in women using vitamin D supplements compared to those that do not (Munger et al., 2004; Swank et al., 1952).

The key lifestyle aspect related with MS is smoking, having a population attributable fraction of 13% for MS, and an odds ratio of 1.46, indicating a modest effect of smoking on MS occurrence risk, with a dose-response relationship present and the risk increasing with cigarette pack-years increase (Manouchehrinia et al., 2022; Poorolajal et al., 2017). Studies have demonstrated that smoking also increases the risk of debilitating relapses and generally accelerates disease progression in PwMS (O'Gorman et al., 2014a; O'Gorman et al., 2014b; Ramagopalan et al., 2013). The explanations regarding the pathophysiological mechanism by which smoking influences MS risk are still indefinite, however, multiple explanations have been suggested, including damage of the blood-brain barrier, production of metabolites in the organism that lead to demyelination, and pro-inflammatory effects of smoking (Arneth, 2020). On the other hand, some authors suggest that smoking facilitates the already present genetic susceptibility in *HLA-DRB1*15:01+* and *HLA-A*02-* individuals (Hedström et al., 2011; Sawcer and Hellenthal, 2011a). There are still some conflicts regarding the role of interaction of EBV and smoking with the genetic burden of the individual (Simon et al., 2010; Sundqvist et al., 2012). Besides active smoking, passive smoking has also been related with increased MS risk (Zhang et al., 2016).

Breastfeeding is very beneficial for both the infant, and the mother, with children that have been breastfed having lower incidence of respiratory infections, diarrheal disease, as well as mortality, and mothers that have breastfed having lower risk of type II diabetes, ovarian and breast cancer (Victora et al., 2016). Studies investigating the effect on breastfeeding on MS occurrence risk have employed different designs, and obtained conflicting results, although a meta-analysis published recently has shown that there exists a protective effect on MS occurrence in persons that were breastfed, with an odds ratio (OR) of 0.82 (Baldin et al., 2021; Hedström et al., 2020a; Holz et al., 2022).

Another risk factor for MS explored in observational studies is adolescence obesity, with multiple studies corroborating this association (Alfredsson and Olsson, 2019; Munger, 2013; Schreiner and Genes, 2021). Genetic profile of persons has been shown to modify the effects of obesity on MS risk, with persons that are *HLA-DRB1*15:01* positive and *HLA-A*02* negative and obese having 16 times higher odds of MS than persons that are *HLA-DRB1*15:01* positive, *HLA-A*02* negative and not obese (Hedström et al., 2014). Also, obesity has been shown to interact with EBV infection, with an OR of 15 when comparing obese persons with previous IM to non-obese individuals without past history of IM (Hedström et al., 2015).

#### *1.6.2.2. Genetic risk factors for multiple sclerosis*

Complex models are needed to pinpoint the exact contribution of genetics in MS development, seeing how data on twin concordance and recurrence rates suggest an absence of Mendelian inheritance (Hollenbach and Oksenberg, 2015). Concordances rates have been estimated at 3-5% for dizygotic twins, while as high as 25-30% concordance rates have been observed in monozygotic twins (Hansen et al., 2005). When observing risks in non-twin relatives of PwMS, first-degree relatives have been estimated at 3-5% lifetime risk for MS, 15-25 times higher than the lifetime MS risk of 0.2% in general population (Sadovnick, Dircks and Ebers, 1999).

The information obtained so far indicates that a polygenic model, with a combination of a single moderate-effect allele, and a large number of small-effect alleles, is the most likely one to explain the participation of genetic variants in MS occurrence risk (O'Gorman et al., 2013; Sadovnick, Dyment, and Ebers, 1997). More than 200 genes, loci and SNPs have been associated with MS risk in GWAS, however, the degree in which each of these factors participates in MS occurrence is likely low (International Multiple Sclerosis Genetics Consortium, 2019). The human leukocyte antigen (*HLA*) system, which has a key role in immune response regulation, but also in autoimmunity, has been shown to influence MS risk decades ago (Choo, 2007; Jersild, Svejgaard and Fog, 1972). However, a differentiation between Class I and II of the *HLA* has been made, where

particular variants of Class I are shown to have a protective effect on MS development as opposed to Class II variants which have a detrimental effect (Goodin et al., 2021; Moutsianas et al., 2015). The two haplotypes that have been shown to have the highest magnitude of association with MS are *HLA-DRB1*15:01*, which has been shown to increase the risk of MS; and *HLA-A*02*, the presence of which reduces MS risk (Brynedal et al., 2007; Sawcer et al., 2011b).

A recent study performing genomic mapping in almost 50,000 PwMS and 70,000 controls based on data from 15 GWAS has found over 30 susceptibility gene variants within the major histocompatibility complex (*MHC*), and that some of them have multiple independent effects, such as the *HLA-DRB1* having six independent effects on MS susceptibility (International Multiple Sclerosis Genetics Consortium, 2019). This study also detected some interactions within the extended MHC class II alleles, and overall found a model explaining 48% of heritability for MS susceptibility (International Multiple Sclerosis Genetics Consortium, 2019). Up to date, 236 gene variants affecting MS risk have been found, most of which are common, implying that all individuals have some degree of MS susceptibility (Goris et al., 2022).

Studies have attempted to discern the connection between the carriage of *HLA-DRB1*15:01* allele and disability progression in PwMS, with differing results (Brownlee et al., 2023; Jackson et al., 2020; Jokubaitis et al., 2023a). In a prospective cohort study, 107 patients categorized by *HLA-DRB1*15:01* status were followed for 15 years (Brownlee et al., 2023). The results of this study indicate that *HLA-DRB1*15:01* carriers have a slightly faster disease progression (about 1 difference in EDSS progression after 15 years), and more MRI detected lesions compared to *HLA-DRB1*15:01* negative PwMS (Brownlee et al., 2023). The authors hypothesize that *HLA-DRB1*15:01* allele carriers could be better responders to disease-modifying therapy (Brownlee et al., 2023). This is possibly why previous studies failed to find the association between *HLA-DRB1*15:01* carrier status and disease prognosis, seeing how these studies have been performed in mostly treated patients, which could have confounded the effect of *HLA-DRB1*15:01* on longitudinal outcomes (Jokubaitis et al., 2023b).

Examination of rare genetic variants is an emerging field in exploring the etiology of MS, with genetic variants of genes *CYP27B1* and *TYK2* being found as moderately contributing to MS risk in two studies performed at the beginning of the last decade (Dyment et al., 2012; Ramagopalan et al., 2011). A newer study has identified variants in *NLRP1* gene to be related with MS occurrence risk, with authors arguing that environmental factors stimulating *NRLP1* pathway in individuals with pathological rare gene variants could disrupt the normal functioning of the blood-brain barrier, facilitating pathological immune response in the CNS (Maver et al., 2017).

### 1.6.3. Familial multiple sclerosis

There are multiple definitions of fMS, the most commonly used being: a) fMS is a case of MS with a first-degree relative with MS; and b) fMS is a case of MS with a first-, second-, or third-degree relative with MS (Andrijauskis et al., 2019; Hader and Yee, 2014; O'Gorman et al., 2011).

Meta-analyses on the prevalence of fMS in MS population performed in 2018 and 2021 have found the global prevalence of fMS to be 12.6% and 11.8%, respectively (Ehtesham, Rafie and Mosallaei, 2021; Harirchian et al., 2018). However, the estimates of fMS prevalence vary significantly based on population and geographical location, with estimates ranging from 2.2% in Hungary to 32.7% in Saskatchewan, Canada (Fricska-Nagy et al., 2007; Hader and Yee, 2014). When observing the differences between sexes, the most recent meta-analysis found no significance between the prevalence of fMS in females (15.4%) and males (13.7%) (Ehtesham, Rafie and Mosallaei, 2021).

An important issue for PwMS is the magnitude of risk of developing MS in their blood relatives. Several studies have attempted to tackle this issue, with data from Australian population

demonstrating the highest risk for sisters, mothers, daughters, and brothers of PwMS (2.88%, 1.09%, 0.94%, and 0.94% respectively), while an UK-based study obtained results that are slightly different, with risk being highest in sisters, and then declining in brothers, mothers, fathers and daughters (3.74%, 2.65%, 2.08%, 1.96%, and 1.01% respectively) (O'Gorman et al., 2011; Robertson et al., 1996). A meta-analysis on the topic has found a higher risk in siblings than offspring and parents, and in half-sibs compared with aunts/uncles and nieces/nephews, which the authors have explained with a environmental factor that acts in a family at a given time, initiating the process of MS development (O'Gorman et al., 2013).

Studies investigating the clinical and radiological specificities of fMS have been performed, tackling this complex issue. Studies comparing disease phenotypes between fMS and sMS have provided conflicting results, with a registry-based study performed in Denmark found evidence towards the existence of difference in disease phenotypes in fMS compared to sMS, with fMS cases more often having RRMS phenotype; while no difference was found between the fMS and sMS in studies of smaller sample sizes (Andrijauskis et al., 2019; Peterlin et al., 2006; Steenhof et al., 2019a). Additionally, a difference in disease onset has been found, with slower onset in familial cases, and a difference in symptoms with fMS cases more often having pyramidal, and brainstem-related disorders, cortical lesions and headaches (Andrijauskis et al., 2019). Also, a decrease in the delay between symptoms onset and diagnosis between the first family member with MS and subsequent family members diagnosed has been observed, however, this may be contributed to raised awareness of the risk of MS in the family both by the physicians and the family members themselves (Steenhof et al., 2019b).

Anticipation phenomenon manifests in some neurological diseases, and it signifies the onset of the disease at earlier ages and more severe clinical features of the disease in younger generations of the family, the classic examples being Huntington’s disease and similar repeat expansion diseases (Paulson, 2018; Teisberg, 1995). So far, the evidence regarding anticipation phenomenon in MS has been inconclusive. The difficulty in assessing this phenomenon arises from the nature of the disease, with uncertainty arising regarding the exact moment of onset of the disease, and the divergence between the time at diagnosis of the disease and age at disease onset. The difference in the obtained results occur due to differently defining the baseline moment (age at onset vs. age at diagnosis), and due to difference in tackling the necessary adjustments for difference in intergenerational length of follow-up (Alonso-Magdalena et al., 2010; Romero-Pinel et al., 2010; Steenhof et al., 2019a). The previously mentioned gap in delay in diagnosis between the first and subsequent generations provides another challenge in investigating this complex phenomenon (Steenhof et al., 2019b).

## 2. OBJECTIVES

The objectives of this doctoral dissertation were:

1. To estimate the prevalence of fMS in the population of Belgrade;
2. To assess the risk factors for developing fMS in comparison with sMS and controls;
3. To determine the predictive factors associated with disease outcome in persons with fMS and sMS.
4. To characterize the rare gene variants in patients with fMS by using whole genome sequencing

# 3. MATERIAL AND METHOD

## *3.1. Study design*

In order to realize the aims set in the dissertation, several observational study designs were utilized: prevalence (cross-sectional), case-control, and retrospective cohort. The studies were performed at the Clinic of Neurology, University Clinical Center of Serbia, and the Clinical Institute for Medical Genetics, University Medical Centre Ljubljana, Ljubljana, Slovenia between 2021 and 2023.

## *3.2. Selection of the participants*

*a) Cross-sectional study assessing the prevalence of fMS in Belgrade region*

The cross-sectional study investigating the prevalence of fMS in Belgrade MS population was performed on the basis of the Belgrade population MS Registry. The registry has been regularly updated since its inception in 1996, and it is being maintained at the Clinic of Neurology, University Clinical Center of Serbia which is the national referral center for MS patients in the Republic of Serbia. The registry comprises information on all living PwMS diagnosed with MS in Belgrade region, with all the diagnoses made based on McDonald Criteria. Data included in the registry are: demographic characteristics of PwMS, such as sex, age, address and municipality of residence, as well as the data on clinical characteristics of patients including the family history of MS, the relationship with the family member with MS, the phenotype of MS, EDSS score, and age at diagnosis.

The definition used for fMS in this study was: a case of MS with a first-, second-, or third-degree relative with MS. Patients with registered family members with MS were contacted, and interviewed in order to obtain pedigrees for each proband of fMS families. Proband was defined as the first person in family to be diagnosed with MS. In cases where the proband was deceased or unavailable, the pedigree was obtained from the living family member with MS. If multiple persons from the same family were available, they were all interviewed, however, only the proband's pedigree was included in the analysis in the cross-sectional study. Patients with MS that had a relative with MS that was a more distant relation than third-degree were excluded from the study.

*b) Case-control study investigating the risk factors for developing fMS*

In order to assess the risk factors for fMS development, a case-control study was conducted at the Clinic of Neurology, University Clinical Center of Serbia in Belgrade, between March 2021 and January 2023. For the purpose of the analysis, three groups were formed:

I. The case group (the fMS group)

All PwMS with a positive family history of MS from the Belgrade population MS Registry, as well as the PwMS with positive family history of MS that have residence in other municipalities, were considered for inclusion in the case-control study.

The inclusion criteria for the fMS group were: presence of a first-, second-, or third-degree relative with MS, and age ≥18 years.

The exclusion criteria for the fMS group were: Adopted relatives and half-sibling status in the family, refusal of participation, family history of MS in more distant degree of relation than third-degree.

II. Control group 1 (the sMS group)

In order to assess whether differences exist in the risk factor profile between PwMS that have burden of MS in the family, and those that do not, a group of sMS cases was included in the study. A PwMS was considered a case of sMS if they had no family relations of any degree with MS. These participants were matched with fMS cases by sex, year of birth (±2 years) and disease course.

III. Control group 2 (the spousal control group)

Second control group, the healthy control (HC) group, comprised spouses of persons with fMS and sMS in order to enable a comparison with persons that do not have MS. In cases a person had no spouse, or the spouse was deceased, a close non-blood related friend or neighbor of the proband was included in the control group 2.

*c) Retrospective cohort study investigating the predictive factors associated with disease outcomes in fMS and sMS*

Patient histories of the patients recruited at the Clinic of Neurology, University Clinical Center of Serbia in Belgrade were used as the data source for the retrospective cohort study assessing predictive factors associated with disease outcomes in fMS and sMS. All participants were evaluated in terms of their MS course and level of physical disability (EDSS and MSSS) from the time of MS symptoms onset until the end of the follow-up. Clinical data (age at onset, phenotype, duration of MS, EDSS) were collected from medical records.

The unexposed group consisted of patients with sMS with no family relations of any degree with MS. These participants were individually matched with the fMS cases by sex, year of birth (±2 years), and disease course.

*d) Case-control study characterizing rare and uncommon gene variants in patients with fMS*

In order to investigate the rare and uncommon gene variants in persons with fMS in the Serbian population, a case-control study design was utilized. The group of cases comprised fMS patients with a first-degree relative with MS, while the control group consisted of patients with neurological diseases other than MS. Both groups were recruited at the Clinic of Neurology, University Clinical Centre of Serbia, and were exclusively comprised of persons of Serbian ethnicity. Matching in this case-control study was performed solely for ethnicity.

### *3.3. Measurements*

*a) Cross-sectional study assessing the prevalence of fMS in Belgrade region*

In the cross-sectional study, the prevalence of fMS was calculated as the proportion of cases with fMS among all cases of MS in the Belgrade population MS Registry. The prevalence of MS among family members of probands with fMS was calculated as the proportion of relatives with MS and the total number of relatives in that category. A case of MS was considered as pediatric MS onset if MS was diagnosed prior to age 18.

We also explored the vertical transmission of MS in families where MS occurred in different generations. We proceeded by categorizing family members into an older generation and a younger generation, with the goal of identifying any differences in clinical and demographic features, as well as investigating the possibility of anticipation phenomenon. The older generation included parents, grandparents, aunts, and uncles of persons with fMS, while the younger generation consisted of children, grandchildren, nieces, and nephews of persons with fMS. We used the progression index, calculated as the current EDSS score divided by the years since disease onset, to measure disease

progression. In order to avoid inadequate follow-up bias, we employed a strategy of limiting our analysis to groups with equal observation durations. Specifically, we conducted a secondary analysis that included only those patients who experienced symptom onset before the age of 39 - the 75th percentile for onset age - and those who were 39 years or older (Alonso-Magdalena et al., 2010).

*b) Case-control study investigating the risk factors for developing fMS*

In order to obtain data on risk factors from participants, a specific questionnaire was utilized in the case-control study. This questionnaire was previously translated, validated and culturally adapted for use in the Serbian population (Pugliatti et al., 2012). The first part of the questionnaire contains questions regarding the demographic data of the participant, including sex, year of birth, the respondent's and parents' highest degree of education, number of siblings, the siblings' sex and year of birth. The second part of the questionnaire contains information on sun exposure, the participant's phototype (skin colour, skin reaction to first sun exposure, hair colour and eye colour), frequency of outdoor activities in the summer and in the winter, occupational sun light exposure, frequency of sun exposure during holidays and frequency of use of sun blocks and sun beds. The third section contains questions on the dietary habits of the participants, food intake in the teenage years and in different seasons. Items include foods rich in vitamin D and dietary supplements. This part also contains questions regarding breastfeeding habits in infanthood. The fourth part includes information of medical history of the study subjects and their families. Participants were asked whether they suffer from chronic, predominantly autoimmune diseases and about the age of onset of the comorbid condition. Cigarette smoking and other lifestyle factors - details on the type of exposure, age at start, duration and amount of the exposure, are also requested in the questionnaire. Participants' body shape at 5-year intervals from birth to the age 30 or MS onset and during the last 3 years was rated with the use of a figure rating scale, by choosing an image corresponding to their figure on a scale from 1 (thinnest) to 9 (largest). Current height and weight were collected to estimate the body mass index (BMI). The last section contains questions regarding hormonal factors, which only women filled in. This section aims at investigating reproductive factors (age at menarche, timing and outcome of each pregnancy, such as live birth, stillbirth, miscarriage, abortion, number of living children), use of sex hormones (oral contraceptives, hormonal treatments for infertility).

*c) Retrospective cohort study investigating the predictive factors associated with disease outcomes in fMS and sMS*

Patient histories of the patients recruited at the Clinic of Neurology, University Clinical Center of Serbia in Belgrade were used as a source of data for the retrospective cohort study assessing predictive factors associated with disease outcomes in fMS and sMS. All participants were evaluated in terms of their MS course and level of physical disability. Clinical data (age at onset, age at diagnosis, phenotype, duration of MS, EDSS, and DMT therapy) were collected from medical records.

*d) Case-control study characterizing rare and uncommon gene variants in patients with fMS*

Whole exome sequencing (WES) was conducted using DNA extracted from whole blood samples of the participants on Illumina HiSeq-2000 platform with an average 30x coverage. Sequence reads were aligned with the hg38 reference genome using Burrows-Wheeler Aligner in line with genome analysis toolkit best practices (McKenna et al., 2010). Agilent-All-Exon 2/5/6 and Illumina Nextera-Exome exome capture kits were used for next generation sequencing library preparation. For quality control, variants were filtered by read depth ($DP \geq 10$), call quality ($GQ \geq 20$) and those that passed quality control by the Genome Analysis Toolkit predictor (GATK). The gnomADe database was used as a source of gene minor allele frequency (MAF) data (Chen et al., 2022).

The virtual gene panel was selected based on previously published GWAS study (International Multiple Sclerosis Genetics Consortium, 2019). From this study, MS-associated autosomal protein-coding genes with detected exonic, intronic, downstream, upstream or untranslated region (UTR) variants were selected. A total of 111 genes were included - 99 genes outside of the extended *MHC* region, and 12 *MHC* genes. Variants containing synonymous genetic changes were excluded from the original data. Only rare variants with allele frequency < 5% were included.

### *3.4. Statistical analysis*

*a) Cross-sectional study assessing the prevalence of fMS in Belgrade region*

Comparisons of demographic and clinical characteristics between patients with fMS and sMS in the registry were made using chi-square test in case of categorical variables, t-test in case of continuous variables with normal distribution, and Mann-Whitney U test in case of non-normally distributed variables. The same approach was utilized to compare family members with MS across generations. In order to assess the differences in MS sex distribution of the probands' family members, odds ratios (OR) with 95% confidence intervals (95% CI) were computed comparing female with male sex in the same category of relation with proband.

*b) Case-control study investigating the risk factors for developing fMS*

To evaluate the risk factors for fMS in comparison with sMS subjects and spousal controls in the case-control study, logistic regression analysis with ORs and corresponding 95% CIs as effect measure were used. Conditional logistic regression was used when comparing matched groups (fMS vs. sMS), while unconditional logistic regression was utilized when comparing non-matched groups (fMS vs. HC, sMS vs. HC). In order to control for confounding factors, each of the variables found statistically significant in the univariate model were reassessed separately while controlling for known MS confounders (history of infectious mononucleosis, history of smoking, educational level; as well as sex in comparisons including HC). All analyses were performed in Stata statistical environment (Stata, version 18.0; StataCorp).

*c) Retrospective cohort study investigating the predictive factors associated with disease outcomes in fMS and sMS*

Calculation of MSSS was based on the most recent EDSS score and the duration of the disease (Roxburgh et al., 2005). Delay to diagnosis was calculated as the difference between the age at diagnosis and the age at symptom onset. Progression index was calculated by dividing the most recent EDSS score with the duration of the disease. Mann-Whitney U and chi-square test were utilized.

*d) Case-control study characterizing rare and uncommon gene variants in patients with fMS*

A burden analysis of rare variants was conducted with the generalised linear model (GLM) using the CMGgenomics package in the R statistical environment (Juvan, Maver, and Majnik, 2023; R Core Team, 2023). The burden analysis was conducted with comparing the fMS cohort with the group of patients with other neurological diseases. Rare variants that fit the study criteria were aggregated by genes, and compared between the fMS cohort and controls using the GLM. The burden of rare variants was calculated for the fMS cohort. Similarly, the burden analysis was also conducted for all genes included in the present study's panel. The threshold for significance was set as $p = 0.05$ after false discovery rate (FDR) correction.

### *3.5. Ethical approval*

Informed consent was obtained from all participants. The study was approved by the Ethics Committee of the Faculty of Medicine, University of Belgrade, Serbia (approval number [1322/XII-10]).

# 4. RESULTS

## *4.1. Cross-sectional study assessing the prevalence of fMS in Belgrade region*

On the prevalence day of the cross-sectional study, June 30th 2022, there were 2765 PwMS in the Belgrade population MS Registry, 1927 of which were female (69.7%), and 838 male (30.3%). The mean age of PwMS in the registry was 56.3±14.4 years. When observing the distribution of PwMS in regards to MS phenotype, 1689 (63.2%) of PwMS in the registry have RRMS, 638 (23.8%) have SPMS, and 347 (13.0%) have PPMS phenotype.

Among all 2765 PwMS in the registry, there were 178 registered cases of fMS (prevalence of 6.4%). The prevalence of fMS was similar between sexes: 6.5% in females, and 6.2% in males. There were 8 cases of pediatric onset MS in patients with fMS (prevalence of pediatric onset fMS of 4.5%). The comparison of patients with fMS and sMS in the registry are presented in Table 1. In comparison with sMS cases, fMS cases were on average younger (48.4±13.9 vs. 56.9±14.2 years), with significantly earlier age at onset (30.4±9.5 vs. 32.3±10.1 years), and shorter duration of the disease (18.3±11.9 vs. 24.6±12.3 years). Also, the median EDSS score was lower in fMS cases (2.5, range 1.0-6.0) in comparison with the sMS cases (4.0, range 2.0-6.5).

Table 1 Demographic and clinical characteristics of familial and sporadic MS cases in the Belgrade population

| | fMS n=178 | sMS n=2587 | p value |
|---|---|---|---|
| Sex | | | |
| Male | 52 (29.2) | 786 (30.4) | 0.800 |
| Female | 126 (70.8) | 1801 (69.6) | |
| Age* | 48.4±13.9 | 56.9±14.2 | **<0.001** |
| Age at MS onset* | 30.4±9.5 | 32.3±10.1 | **0.022** |
| Disease duration* | 18.3±11.9 | 24.6±12.3 | **<0.001** |
| MS phenotype | | | |
| RMS | 150 (87.7) | 2277 (87.0) | 0.779 |
| PPMS | 21 (12.3) | 326 (13.0) | |
| EDSS** | 2.5(1.0-6.0) | 4.0 (2.0-6.5) | **0.001** |

Abbreviation: MS - multiple sclerosis; fMS – familial multiple sclerosis; sMS – sporadic multiple sclerosis; RMS - relapsing multiple sclerosis; PPMS - primary progressive multiple sclerosis; EDSS - expanded disability status scale; results in table are presented as frequency (%); *mean±sd; **median (IQR); bold values denote statistical significance

An interview was conducted with 96 fMS probands in order to obtain their full pedigrees. The prevalence of MS among relations of persons with fMS is presented in Table 2. The highest prevalence found was in offspring (18.6%), parents (16.7%), and siblings (16.2%), and the lowest was reported in cousins (1.8%). After stratification of family members by sex, the highest prevalence of MS observed was in sisters (27.1%), mothers (22.9%), and daughters (21.7%) of fMS probands, while the lowest prevalence was found in grandfathers (1.0%). The prevalence of fMS was higher in female relatives of all categories, when compared with their male counterparts, with odds ratios ranging from 9.3 (sisters/brothers) to 1.6 (aunts/uncles).

Table 2 The prevalence of MS among family members of the fMS probands

| | No. cases | No. relatives | Prevalence % (95% CI) | Odds ratio F/M (95% CI) |
|---|---|---|---|---|
| Parent | 32 | 192 | 16.7 (11.7-22.7) | 2.6 (1.1-5.7) |
| Father | 10 | 96 | 10.4 (5.1-18.3) | |
| Mother | 22 | 96 | 22.9 (14.9-32.6) | |
| Offspring | 19 | 102 | 18.6 (11.6-27.6) | 1.7 (0.6-4.8) |
| Son | 6 | 42 | 14.3 (5.4-28.5) | |
| Daughter | 13 | 60 | 21.7 (12.1-34.2) | |
| Sibling | 18 | 111 | 16.2 (9.9-24.4) | 9.3 (2.0-42.8) |
| Brother | 2 | 52 | 3.8 (0.4-1.3) | |
| Sister | 16 | 59 | 27.1 (16.4-40.3) | |
| Nephew/Niece | 5 | 132 | 3.8 (1.2-8.6) | 5.6 (0.6-51.4) |
| Nephew | 1 | 75 | 1.3 (0.1-7.2) | |
| Niece | 4 | 57 | 7.0 (1.9-17.0) | |
| Uncle/Aunt | 15 | 459 | 3.3 (1.8-5.3) | 1.6 (0.6-4.9) |
| Uncle | 5 | 206 | 2.4 (0.8-5.6) | |
| Aunt | 10 | 253 | 4.0 (1.9-7.1) | |
| Grandparent | 8 | 384 | 2.1 (0.9-4.1) | 3.1 (0.6-15.5) |
| Grandfather | 2 | 192 | 1.0 (0.1-3.7) | |
| Grandmother | 6 | 192 | 3.1 (1.2-6.7) | |
| Cousin | 15 | 825 | 1.8 (1.0-3.0) | N/A |

Abbreviation: MS- multiple sclerosis; fMS – familial multiple sclerosis; F-female; M-male; 95% CI – 95% confidence intervals

The comparison of fMS cases across generation showed no statistically significant disparities in sex distribution, progression index, or MS phenotype between the older and younger generations (Table 3). However, the younger generation reported significantly earlier onset of symptoms, with an average age of 25.8±7.2 years, compared to 35.7±11.6 years in the older generation ($p<0.001$). After adjustment for different length of follow-up, the difference in the ages of onset remained statistically significant, albeit with smaller difference (onset at 30.0±7.9 years for the younger vs. 36.4±11.9 years for the older generation, $p=0.04$).

Table 3 Demographic, clinical features and anticipation phenomenon in different generations of familial MS

| | Older generation (N=64) | Younger generation (N=66) | p value |
|---|---|---|---|
| Sex | | | |
| Male | 20 (31.2) | 20 (30.3) | 0.907 |
| Female | 44 (68.8) | 46 (69.7) | |
| Age at MS onset* | 35.7±11.6 | 25.8±7.2 | **<0.001** |
| Adjusted age at MS onset* | 36.4±11.9 | 30.0±7.9 | **0.016** |
| Progression index* | 0.28±0.35 | 0.41±0.81 | 0.482 |
| MS phenotype | | | |
| RMS | 56 (87.5) | 61 (92.4) | 0.349 |
| PPMS | 8 (12.5) | 5 (7.6) | |

Abbreviations: SD – standard deviation; MS - multiple sclerosis; RMS - relapsing multiple sclerosis; PPMS - primary progressive multiple sclerosis; results in table are presented as frequency (%); *mean±sd; bold values denote statistical significance

## *4.2. Case-control study investigating the risk factors for developing fMS*

### 4.2.1. Descriptive statistics

A total of 393 participants filled in the questionnaire (131 fMS, 131 sMS, and 131 HC). Demographic characteristics of the participants in the case-control study are presented in Table 4.

Table 4 Demographic characteristics of the participants included in the case-control study

| | fMS n=131 | sMS n=131 | HC n=131 |
|---|---|---|---|
| Sex | | | |
| Male | 37 (28.2) | 37 (28.2) | 84 (64.1) |
| Female | 94 (71.8) | 94 (71.8) | 47 (35.9) |
| Age (years)* | 41.47±12.22 | 41.37±11.96 | 41.72±12.81 |
| Education | | | |
| Secondary or lower | 69 (52.6) | 75 (57.3) | 68 (51.9) |
| College | 15 (11.5) | 11 (8.4) | 8 (6.1) |
| Bachelor | 30 (22.9) | 37 (28.2) | 34 (26.0) |
| Masters/PhD | 17 (13.0) | 8 (6.1) | 21 (16.0) |
| Years of education* | 14.25±2.88 | 13.93±2.50 | 14.44±2.71 |
| Maternal education | | | |
| Secondary or lower | 90 (68.6) | 89 (67.9) | 88 (67.2) |
| College | 17 (13.0) | 13 (9.9) | 18 (13.7) |
| Bachelor | 20 (15.3) | 24 (18.4) | 21 (16.0) |
| Masters/PhD | 4 (3.1) | 5 (3.8) | 4 (3.1) |
| Paternal education | | | |
| Secondary or lower | 77 (59.2) | 86 (66.1) | 76 (58.5) |
| College | 21 (16.2) | 14 (10.8) | 18 (13.8) |
| Bachelor | 27 (20.8) | 27 (20.8) | 28 (21.5) |
| Masters/PhD | 5 (3.8) | 3 (2.3) | 8 (6.2) |
| Employment | | | |
| Employed | 84 (64.1) | 74 (56.5) | 94 (71.8) |
| Unemployed | 25 (19.1) | 37 (28.2) | 30 (22.9) |
| Retired | 22 (16.8) | 20 (15.3) | 7 (5.3) |
| Marital status | | | |
| Married | 86 (65.6) | 81 (61.8) | 106 (80.9) |
| Single | 41 (31.3) | 46 (35.1) | 24 (18.3) |
| Divorced | 2 (1.5) | 2 (1.5) | 0 (0.0) |
| Widowed | 2 (1.5) | 2 (1.5) | 1 (0.8) |
| Number of siblings** | 1 (0-4) | 1 (0-5) | 1 (0-6) |
| Birth order*** | 1 (1-2) | 2 (1-2) | 1.5 (1-2) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; ; results in table are presented as frequency (%); *mean±standard deviation; **median (range); ***median (interquartile range)

The characteristics of participants in regard to skin color, tanning reaction to first sun exposure, and natural hair and eye color are presented in Table 5.

Table 5 The distribution of the participants according to skin phototype

| | fMS n=131 | sMS n=131 | HC n=131 |
|---|---|---|---|
| Skin colour* | 3.9±1.6 | 3.7±1.5 | 4.1±1.7 |
| Tanning reaction to first sun | | | |
| Always burns, never tans | 10 (7.6) | 11 (8.4) | 8 (6.1) |
| Usually burns/sometimes tans | 28 (21.4) | 38 (29.0) | 28 (21.4) |
| Tan average/sometimes mild burn | 49 (37.4) | 56 (42.7) | 55 (42.0) |
| Rarely burns/more than average tan | 44 (33.6) | 26 (19.8) | 40 (30.5) |
| Natural hair colour | | | |
| Black | 21 (16.0) | 15 (11.5) | 20 (15.3) |
| Dark brown | 64 (48.9) | 78 (59.5) | 60 (45.8) |

| | | | |
|---|---|---|---|
| Light brown | 42 (32.1) | 34 (26.0) | 42 (32.1) |
| Blonde or red | 4 (3.1) | 4 (3.1) | 9 (6.8) |
| Natural eye colour | | | |
| Black | 11 (8.4) | 4 (3.1) | 6 (4.6) |
| Brown or hazel | 70 (53.4) | 78 (60.0) | 74 (56.5) |
| Green | 34 (26.0) | 33 (25.4) | 24 (18.3) |
| Blue | 16 (12.2) | 15 (11.5) | 27 (20.6) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; results in table are presented as frequency (%);*mean±standard deviation

A high proportion of participants in all groups have spent most of their time during summer performing outdoor activities in the first 10 years of their life, while these proportions are lower in older age categories (Table 6).

Table 6 The distribution of the participants regarding the frequency of outdoor activities during summer at ages 0-30 years

| | fMS n=131 | sMS n=131 | HC n=131 |
|---|---|---|---|
| 0–5 years of age | | | |
| Not that often | 2 (1.5) | 1 (0.8) | 2 (1.5) |
| Reasonably often | 10 (7.6) | 8 (6.1) | 5 (3.8) |
| Quite often | 19 (14.5) | 22 (16.8) | 35 (26.7) |
| Virtually all the time | 100 (76.3) | 100 (76.3) | 89 (67.9) |
| 6–10 years of age | | | |
| Not that often | 1 (0.8) | 2 (1.5) | 1 (0.8) |
| Reasonably often | 6 (4.6) | 5 (3.8) | 6 (4.6) |
| Quite often | 24 (18.3) | 23 (17.6) | 33 (25.2) |
| Virtually all the time | 100 (76.3) | 101 (77.1) | 91 (69.5) |
| 11–15 years of age | | | |
| Not that often | 2 (1.5) | 2 (1.5) | 2 (1.5) |
| Reasonably often | 9 (6.9) | 10 (7.6) | 10 (7.6) |
| Quite often | 38 (29.0) | 26 (19.8) | 34 (26.0) |
| Virtually all the time | 82 (62.6) | 93 (71.0) | 85 (64.9) |
| 16–20 years of age | | | |
| Not that often | 7 (5.3) | 5 (3.8) | 6 (4.6) |
| Reasonably often | 25 (19.1) | 23 (17.6) | 25 (19.1) |
| Quite often | 45 (34.4) | 38 (29.0) | 46 (35.1) |
| Virtually all the time | 54 (41.2) | 65 (49.6) | 54 (41.2) |
| 21–25 years of age | | | |
| Not that often | 15 (11.8) | 11 (8.5) | 12 (9.4) |
| Reasonably often | 45 (35.4) | 39 (30.2) | 37 (28.9) |
| Quite often | 41 (32.3) | 33 (25.6) | 46 (35.9) |
| Virtually all the time | 26 (20.5) | 46 (35.7) | 33 (25.8) |
| 26–30 years of age | | | |
| Not that often | 21 (18.3) | 19 (16.1) | 16 (13.5) |
| Reasonably often | 40 (34.8) | 34 (28.8) | 39 (33.1) |
| Quite often | 29 (25.2) | 31 (26.3) | 36 (30.5) |
| Virtually all the time | 25 (21.7) | 34 (28.8) | 27 (22.9) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; results in table are presented as frequency (%)

A lower proportion of participants in all three groups reported performing outdoor activities during winter compared to summer, however, still the highest proportion in all three groups reported that they spend “virtually all their time” outdoors when aged 15 and below (Table 7).

Table 7 The distribution of the participants regarding the frequency of outdoor activities during winter at ages 0-30 years

| | fMS n=131 | sMS n=131 | HC n=131 |
|---|---|---|---|
| 0–5 years of age | | | |
| Not that often | 10 (7.6) | 5 (3.8) | 5 (3.8) |
| Reasonably often | 18 (13.7) | 15 (11.5) | 22 (16.8) |
| Quite often | 27 (20.7) | 25 (19.1) | 37 (28.2) |
| Virtually all the time | 76 (58.0) | 86 (65.6) | 67 (51.2) |
| 6–10 years of age | | | |
| Not that often | 6 (4.6) | 5 (3.8) | 3 (2.3) |
| Reasonably often | 19 (14.5) | 13 (9.9) | 13 (9.9) |
| Quite often | 29 (22.1) | 27 (20.6) | 43 (32.8) |
| Virtually all the time | 77 (58.8) | 86 (65.7) | 72 (55.0) |
| 11–15 years of age | | | |
| Not that often | 6 (4.6) | 6 (4.6) | 4 (3.1) |
| Reasonably often | 26 (19.8) | 20 (15.3) | 18 (13.7) |
| Quite often | 33 (25.2) | 27 (20.6) | 38 (29.0) |
| Virtually all the time | 66 (50.4) | 78 (59.5) | 71 (54.2) |
| 16–20 years of age | | | |
| Not that often | 14 (10.7) | 8 (6.1) | 9 (6.9) |
| Reasonably often | 47 (35.8) | 42 (32.1) | 35 (26.7) |
| Quite often | 31 (23.7) | 29 (22.1) | 42 (32.1) |
| Virtually all the time | 39 (29.8) | 52 (39.7) | 45 (34.3) |
| 21–25 years of age | | | |
| Not that often | 31 (24.4) | 17 (13.2) | 19 (15.0) |
| Reasonably often | 50 (39.4) | 52 (40.3) | 45 (35.4) |
| Quite often | 26 (20.5) | 26 (20.1) | 33 (26.0) |
| Virtually all the time | 20 (15.7) | 34 (26.4) | 30 (23.6) |
| 26–30 years of age | | | |
| Not that often | 38 (33.0) | 24 (20.2) | 25 (21.2) |
| Reasonably often | 40 (34.8) | 46 (38.6) | 41 (34.7) |
| Quite often | 21 (18.3) | 19 (16.0) | 27 (22.9) |
| Virtually all the time | 16 (13.9) | 30 (25.2) | 25 (21.2) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; results in table are presented as frequency (%)

The majority of the participants in all three groups have worked or studied indoors from age 16 until age 30 (Table 8).

Table 8 The distribution of the participants regarding the frequency of outdoor professional activities at ages 16-30 years

| | fMS n=131 | sMS n=131 | HC n=131 |
|---|---|---|---|
| 16–20 years of age | | | |
| Mostly indoors | 116 (88.5) | 113 (86.3) | 99 (75.6) |
| Mostly outdoors | 8 (6.1) | 5 (3.8) | 10 (7.6) |
| Equally indoors and outdoors | 7 (5.4) | 13 (9.9) | 22 (16.8) |
| 21–25 years of age | | | |
| Mostly indoors | 112 (88.2) | 107 (82.9) | 88 (68.8) |
| Mostly outdoors | 9 (7.1) | 9 (7.0) | 15 (11.7) |
| Equally indoors and outdoors | 6 (4.7) | 13 (10.1) | 25 (19.5) |
| 26–30 years of age | | | |
| Mostly indoors | 101 (87.8) | 94 (79.0) | 78 (66.1) |
| Mostly outdoors | 6 (5.2) | 8 (6.7) | 15 (12.7) |
| Equally indoors and outdoors | 8 (7.0) | 17 (14.3) | 25 (21.2) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; results in table are presented as frequency (%)

The majority of participants reported spending more than 4 hours per day on weekends exposed to sun ages 6-15, while this proportion was lower when patients reached later age (Table 9).

Table 9 The distribution of the participants regarding the amount of weekend sun exposure at ages 6-30 years

| | fMS n=131 | sMS n=131 | HC n=131 |
|---|---|---|---|
| 6–10 years of age | | | |
| <1h/day | 1 (0.8) | 3 (2.3) | 4 (3.1) |
| 1-2h/day | 10 (7.6) | 5 (3.8) | 8 (6.1) |
| 2-3h/day | 14 (10.7) | 10 (7.6) | 12 (9.2) |
| 3-4h/day | 14 (10.7) | 14 (10.7) | 17 (13.0) |
| >4h/day | 92 (70.2) | 99 (75.6) | 90 (68.6) |
| 11–15 years of age | | | |
| <1h/day | 2 (1.5) | 2 (1.5) | 3 (2.3) |
| 1-2h/day | 11 (8.4) | 6 (4.6) | 6 (4.6) |
| 2-3h/day | 13 (9.9) | 12 (9.2) | 13 (9.9) |
| 3-4h/day | 25 (19.1) | 27 (20.6) | 22 (16.8) |
| >4h/day | 80 (61.1) | 84 (64.1) | 87 (66.4) |
| 16–20 years of age | | | |
| <1h/day | 5 (3.8) | 2 (1.5) | 5 (3.8) |
| 1-2h/day | 20 (15.3) | 10 (7.6) | 10 (7.6) |
| 2-3h/day | 29 (22.1) | 28 (21.4) | 26 (19.9) |
| 3-4h/day | 26 (19.7) | 27 (20.6) | 26 (19.9) |
| >4h/day | 51 (38.9) | 64 (48.9) | 64 (48.8) |
| 21–25 years of age | | | |
| <1h/day | 10 (7.9) | 10 (7.7) | 8 (6.3) |
| 1-2h/day | 27 (21.3) | 17 (13.3) | 14 (10.9) |
| 2-3h/day | 30 (23.6) | 28 (21.9) | 36 (28.1) |
| 3-4h/day | 20 (15.7) | 28 (21.9) | 26 (20.3) |
| >4h/day 26– | 40 (31.5) | 45 (35.2) | 44 (34.4) |
| 30 years of age | | | |
| <1h/day | 21 (18.2) | 13 (10.9) | 11 (9.3) |
| 1-2h/day | 22 (19.1) | 21 (17.6) | 14 (11.9) |
| 2-3h/day | 23 (20.0) | 28 (23.6) | 30 (25.4) |
| 3-4h/day | 13 (11.3) | 21 (17.6) | 25 (21.2) |
| >4h/day | 36 (31.4) | 36 (30.3) | 38 (32.2) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; results in table are presented as frequency (%)

Most participants in all three groups reported never being exposed to UV lamps in their life (Table 10).

Table 10 The distribution of the participants regarding the frequency of UV lamp exposure at ages 16-30

| | fMS n=131 | sMS n=131 | HC n=131 |
|---|---|---|---|
| 16–20 years of age | | | |
| Never | 120 (91.6) | 115 (87.8) | 121 (92.3) |
| <1/year | 8 (6.1) | 5 (3.8) | 4 (3.1) |
| <1/month | 1 (0.8) | 6 (4.6) | 4 (3.1) |
| ≥1/month | 2 (1.5) | 5 (3.8) | 2 (1.5) |
| 21–25 years of age | | | |
| Never | 112 (88.2) | 109 (84.5) | 113 (88.3) |
| <1/year | 8 (6.3) | 11 (8.5) | 4 (3.1) |
| <1/month | 3 (2.4) | 5 (3.9) | 8 (6.3) |
| ≥1/month | 4 (3.1) | 4 (3.1) | 3 (2.3) |
| 26–30 years of age | | | |
| Never | 107 (90.7) | 106 (88.3) | 108 (90.7) |

| | | | |
|---|---|---|---|
| <1/year | 6 (5.1) | 7 (5.8) | 4 (3.4) |
| <1/month | 4 (3.4) | 5 (4.2) | 4 (3.4) |
| ≥1/month | 1 (0.8) | 2 (1.7) | 3 (2.5) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; results in table are presented as frequency (%)

Most participants reported seldom or never using sunscreen at ages 0-15 in all three groups (Table 11).

Table 11 The distribution of the participants regarding the frequency of sun protection use at ages 0-30

| | fMS n=131 | sMS n=131 | HC n=131 |
|---|---|---|---|
| 0–5 years of age | | | |
| Seldom/never | 79 (60.3) | 72 (55.0) | 71 (54.2) |
| Sometimes | 11 (8.4) | 21 (16.0) | 17 (13.0) |
| Quite often | 18 (13.7) | 7 (5.3) | 13 (9.9) |
| Almost always | 23 (17.6) | 31 (23.7) | 30 (22.9) |
| 6–10 years of age | | | |
| Seldom/never | 78 (59.5) | 71 (54.2) | 70 (53.5) |
| Sometimes | 12 (9.2) | 21 (16.0) | 19 (14.5) |
| Quite often | 17 (13.0) | 9 (6.9) | 13 (9.9) |
| Almost always | 24 (18.3) | 30 (22.9) | 29 (22.1) |
| 11–15 years of age | | | |
| Seldom/never | 76 (58.0) | 71 (54.2) | 66 (50.4) |
| Sometimes | 13 (9.9) | 20 (15.2) | 22 (16.8) |
| Quite often | 18 (13.7) | 9 (6.9) | 16 (12.2) |
| Almost always | 24 (18.3) | 31 (23.7) | 27 (20.6) |
| 16–20 years of age | | | |
| Seldom/never | 71 (54.2) | 61 (46.6) | 60 (45.8) |
| Sometimes | 17 (13.0) | 24 (18.3) | 27 (20.6) |
| Quite often | 16 (12.2) | 13 (9.9) | 14 (10.7) |
| Almost always | 27 (20.6) | 33 (25.2) | 30 (22.9) |
| 21–25 years of age | | | |
| Seldom/never | 56 (44.1) | 42 (32.6) | 54 (42.2) |
| Sometimes | 26 (20.5) | 29 (22.5) | 25 (19.5) |
| Quite often | 16 (12.6) | 15 (11.6) | 18 (14.1) |
| Almost always | 29 (22.8) | 43 (33.3) | 31 (24.2) |
| 26–30 years of age | | | |
| Seldom/never | 37 (32.2) | 36 (30.3) | 45 (38.1) |
| Sometimes | 26 (22.6) | 25 (21.0) | 28 (23.7) |
| Quite often | 16 (13.9) | 13 (10.9) | 18 (15.3) |
| Almost always | 36 (31.3) | 45 (37.8) | 27 (22.9) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; results in table are presented as frequency (%)

In the age 13-19 years, the majority of participants in all three groups consummated milk and dairy products throughout the year (Table 12).

Table 12 The distribution of the participants regarding the consumption of milk and dairy products in different seasons in the age 13-19 years

| | fMS n=131 | sMS n=131 | HC n=131 |
|---|---|---|---|
| Milk usage | | | |
| Never | 38 (29.0) | 39 (29.8) | 30 (22.9) |
| Ever | 93 (71.0) | 92 (70.2) | 101 (77.1) |
| Winter | 93 (71.0) | 92 (70.2) | 101 (77.1) |
| Spring | 91 (69.5) | 92 (70.2) | 99 (75.6) |
| Summer | 91 (69.5) | 92 (70.2) | 98 (74.8) |
| Fall | 91 (69.5) | 92 (70.2) | 99 (75.6) |

| | | | | |
|---|---|---|---|---|
| Yogurt usage | | | | |
| Never | | 22 (16.8) | 26 (19.8) | 16 (12.2) |
| Ever | | 109 (83.2) | 105 (80.2) | 115 (87.8) |
| | Winter | 108 (82.4) | 104 (79.4) | 115 (87.8) |
| | Spring | 109 (83.2) | 105 (80.2) | 114 (87.1) |
| | Summer | 109 (83.2) | 105 (80.2) | 114 (87.1) |
| | Fall | 108 (82.4) | 105 (80.2) | 114 (87.1) |
| Cheese usage | | | | |
| Never | | 15 (11.5) | 21 (16.0) | 13 (9.9) |
| Ever | | 116 (88.5) | 110 (84.0) | 118 (90.1) |
| | Winter | 115 (87.8) | 110 (84.0) | 117 (89.3) |
| | Spring | 114 (87.1) | 107 (81.7) | 113 (86.3) |
| | Summer | 114 (87.1) | 107 (81.7) | 112 (85.5) |
| | Fall | 115 (87.8) | 107 (81.7) | 116 (88.5) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; results in table are presented as frequency (%)

Similarly, most participants in all three groups reported consuming meat and meat products throughout the year in the age 13-19 years (Table 13).

Table 13 The distribution of the participants regarding the consumption of meat and meat products in different seasons in the age 13-19 years

| | | fMS n=131 | sMS n=131 | HC n=131 |
|---|---|---|---|---|
| Meat usage | | | | |
| Never | | 7 (5.3) | 8 (6.1) | 3 (2.3) |
| Ever | | 124 (94.7) | 123 (93.9) | 128 (97.7) |
| | Winter | 124 (94.7) | 123 (93.9) | 128 (97.7) |
| | Spring | 124 (94.7) | 123 (93.9) | 127 (96.9) |
| | Summer | 124 (94.7) | 123 (93.9) | 124 (94.7) |
| | Fall | 124 (94.7) | 123 (93.9) | 126 (96.2) |
| Meat products usage | | | | |
| Never | | 32 (24.4) | 27 (20.6) | 17 (13.0) |
| Ever | | 99 (75.6) | 104 (89.4) | 114 (87.0) |
| | Winter | 99 (75.6) | 103 (78.6) | 112 (85.5) |
| | Spring | 94 (71.8) | 101 (77.1) | 108 (82.4) |
| | Summer | 93 (71.0) | 101 (77.1) | 105 (80.2) |
| | Fall | 95 (72.5) | 103 (78.6) | 108 (82.4) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; results in table are presented as frequency (%)

About half of the participants in all three groups reported using fish in the age 13-19 years, while only few reported using seafood in the same age (Table 14).

Table 14 The distribution of the participants regarding the consumption of fish and seafood in different seasons in the age 13-19 years

| | | fMS n=131 | sMS n=131 | HC n=131 |
|---|---|---|---|---|
| Fresh fish usage | | | | |
| Never | | 67 (51.1) | 55 (42.0) | 48 (36.6) |
| Ever | | 64 (48.9) | 76 (58.0) | 83 (63.4) |
| | Winter | 47 (35.9) | 61 (46.1) | 67 (51.1) |
| | Spring | 46 (35.1) | 65 (49.6) | 66 (50.4) |
| | Summer | 59 (45.0) | 72 (55.0) | 74 (56.5) |
| | Fall | 45 (34.4) | 65 (49.6) | 63 (48.1) |

| | | | | |
|---|---|---|---|---|
| Frozen fish usage | | | | |
| Never | | 64 (48.9) | 59 (45.0) | 64 (48.9) |
| Ever | | 67 (51.1) | 72 (55.0) | 67 (51.1) |
| | Winter | 65 (49.6) | 68 (51.9) | 61 (46.6) |
| | Spring | 56 (42.7) | 66 (50.4) | 57 (43.5) |
| | Summer | 51 (38.9) | 64 (48.9) | 52 (39.7) |
| | Fall | 53 (40.5) | 66 (50.4) | 52 (39.7) |
| Canned fish usage | | | | |
| Never | | 72 (55.0) | 62 (47.3) | 62 (47.3) |
| Ever | | 59 (45.0) | 69 (52.7) | 69 (52.7) |
| | Winter | 58 (44.3) | 66 (50.4) | 56 (42.7) |
| | Spring | 56 (42.7) | 65 (49.6) | 58 (44.3) |
| | Summer | 55 (42.0) | 66 (50.4) | 55 (42.0) |
| | Fall | 53 (40.5) | 65 (49.6) | 51 (38.9) |
| Seafood usage | | | | |
| Never | | 113 (86.3) | 99 (75.6) | 104 (79.4) |
| Ever | | 18 (13.7) | 32 (24.4) | 27 (20.6) |
| | Winter | 9 (6.9) | 19 (14.5) | 15 (11.5) |
| | Spring | 8 (6.1) | 19 (14.5) | 16 (12.2) |
| | Summer | 17 (13.0) | 31 (23.7) | 25 (19.1) |
| | Fall | 8 (6.1) | 20 (15.3) | 14 (10.7) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; results in table are presented as frequency (%)

When observing distribution regarding consumption of different kinds of fish in the age 13-19 years, the most commonly consumed fish in all three groups were European hake, trout, and different kinds of canned fish (Table 15).

Table 15 The distribution of the participants regarding the frequency of fish consumption in the age 13-19 years

| | fMS n=131 | sMS n=131 | HC n=131 |
|---|---|---|---|
| Fresh tuna | | | |
| Never | 118 (90.1) | 121 (92.4) | 120 (91.6) |
| <1/month | 9 (6.9) | 8 (6.1) | 6 (4.6) |
| 1-3/month | 2 (1.5) | 0 (0.0) | 3 (2.3) |
| 1/week | 2 (1.5) | 2 (1.5) | 2 (1.5) |
| European hake | | | |
| Never | 47 (35.9) | 38 (29.0) | 39 (29.8) |
| <1/month | 44 (33.6) | 45 (34.4) | 52 (39.7) |
| 1-3/month | 27 (20.6) | 36 (27.5) | 30 (22.9) |
| 1/week | 11 (8.4) | 10 (7.6) | 8 (6.1) |
| 2-3/week | 2 (1.5) | 2 (1.5) | 2 (1.5) |
| Sardine | | | |
| Never | 94 (71.7) | 106 (80.9) | 98 (74.8) |
| <1/month | 23 (17.6) | 12 (9.2) | 18 (13.7) |
| 1-3/month | 9 (6.9) | 11 (8.4) | 12 (9.2) |
| 1/week | 2 (1.5) | 2 (1.5) | 3 (2.3) |
| 2-3/week | 3 (2.3) | 0 (0.0) | 0 (0.0) |
| Salmon | | | |
| Never | 100 (76.3) | 108 (82.4) | 108 (82.4) |
| <1/month | 24 (18.3) | 14 (10.7) | 16 (12.2) |
| 1-3/month | 5 (3.8) | 5 (3.8) | 6 (4.6) |
| 1/week | 1 (0.8) | 3 (2.3) | 1 (0.8) |
| 2-3/week | 1 (0.8) | 1 (0.8) | 0 (0.0) |
| Trout | | | |
| Never | 57 (43.5) | 60 (45.8) | 40 (30.5) |
| <1/month | 42 (32.0) | 37 (28.2) | 50 (38.2) |
| 1-3/month | 23 (17.6) | 26 (19.8) | 31 (23.7) |
| 1/week | 7 (5.3) | 7 (5.3) | 8 (6.1) |
| 2-3/week | 1 (0.8) | 1 (0.8) | 2 (1.5) |

| | | | |
|---|---|---|---|
| >3/week | 1 (0.8) | 0 (0.0) | 0 (0.0) |
| Whitefish | | | |
| Never | 105 (80.1) | 103 (78.6) | 100 (76.3) |
| <1/month | 17 (13.0) | 19 (14.5) | 19 (14.5) |
| 1-3/month | 8 (6.1) | 6 (4.6) | 11 (8.4) |
| 1/week | 1 (0.8) | 3 (2.3) | 1 (0.8) |
| Mackerel | | | |
| Never | 78 (59.5) | 71 (54.2) | 71 (54.2) |
| <1/month | 34 (25.9) | 35 (26.7) | 30 (22.9) |
| 1-3/month | 15 (11.5) | 22 (16.8) | 22 (16.8) |
| 1/week | 3 (2.3) | 2 (1.5) | 8 (6.1) |
| 2-3/week | 0 (0.0) | 1 (0.8) | 0 (0.0) |
| >3/week | 1 (0.8) | 0 (0.0) | 0 (0.0) |
| Smoked fish | | | |
| Never | 92 (70.2) | 104 (79.3) | 91 (69.4) |
| <1/month | 30 (22.9) | 22 (16.8) | 28 (21.4) |
| 1-3/month | 7 (5.3) | 4 (3.1) | 11 (8.4) |
| 1/week | 1 (0.8) | 1 (0.8) | 1 (0.8) |
| 2-3/week | 1 (0.8) | 0 (0.0) | 0 (0.0) |
| >3/week | | | |
| Canned fish | | | |
| Never | 43 (32.7) | 48 (36.6) | 38 (29.0) |
| <1/month | 46 (35.1) | 31 (23.7) | 51 (38.9) |
| 1-3/month | 28 (21.4) | 29 (22.1) | 23 (17.6) |
| 1/week | 9 (6.9) | 21 (16.0) | 14 (10.7) |
| 2-3/week | 4 (3.1) | 1 (0.8) | 4 (3.1) |
| >3/week | 1 (0.8) | 1 (0.8) | 1 (0.8) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; results in table are presented as frequency (%)

Most of the participants in all three groups reported using tap water for drinking and cooking in the age 13-19 years (Table 16).

Table 16 The distribution of the participants regarding the type of water used in the age 13-19 years

| | fMS n=131 | sMS n=131 | HC n=131 |
|---|---|---|---|
| Well water, spring water | | | |
| Rarely/never used | 108 (82.4) | 112 (85.5) | 98 (74.8) |
| Frequently used | 23 (17.6) | 19 (14.5) | 33 (25.2) |
| For drinking | 23 (17.6) | 19 (14.5) | 32 (24.4) |
| For cooking | 17 (13.0) | 17 (13.0) | 29 (22.1) |
| Tap water | | | |
| Rarely/never used | 12 (9.2) | 12 (9.2) | 19 (14.5) |
| Frequently used | 119 (90.8) | 119 (90.8) | 112 (85.5) |
| For drinking | 115 (87.8) | 115 (87.8) | 108 (82.4) |
| For cooking | 117 (89.3) | 118 (90.1) | 110 (84.0) |
| Bottled water | | | |
| Rarely/never used | 110 (84.0) | 114 (87.0) | 125 (95.4) |
| Frequently used | 21 (16.0) | 17 (13.0) | 6 (4.6) |
| For drinking | 21 (16.0) | 17 (13.0) | 6 (4.6) |
| For cooking | 3 (2.3) | 2 (1.5) | 2 (1.5) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; results in table are presented as frequency (%)

Butter, margarine, oil, pork fat, and mayonnaise were all commonly used in all groups in the age 13-19 years, while very few participants reported ever using sardine past and tuna sauce (Table 17).

Table 17 The distribution of the participants regarding the frequency of consumption of sauces in the age 13-19 years

| | fMS n=131 | sMS n=131 | HC n=131 |
|---|---|---|---|
| Butter | | | |
| Never | 48 (36.6) | 53 (40.4) | 48 (36.6) |
| <1x/month | 24 (18.3) | 24 (18.3) | 27 (20.6) |
| 1-3x/month | 14 (10.7) | 14 (10.7) | 12 (9.2) |
| 1x/week | 9 (6.9) | 12 (9.2) | 8 (6.1) |
| 2-3x/week | 25 (19.1) | 17 (13.0) | 20 (15.3) |
| 4-5x/week | 2 (1.5) | 4 (3.1) | 6 (4.6) |
| >5x/week | 9 (6.9) | 7 (5.3) | 10 (7.6) |
| Margarine | | | |
| Never | 38 (29.0) | 37 (28.2) | 30 (22.9) |
| <1x/month | 22 (16.8) | 23 (17.6) | 27 (20.6) |
| 1-3x/month | 14 (10.7) | 22 (16.8) | 25 (19.1) |
| 1x/week | 29 (14.5) | 12 (9.2) | 14 (10.7) |
| 2-3x/week | 30 (22.9) | 25 (19.1) | 19 (14.5) |
| 4-5x/week | 2 (1.5) | 5 (3.8) | 11 (8.4) |
| >5x/week | 6 (4.6) | 7 (5.3) | 5 (3.8) |
| Oil | | | |
| Never | 3 (2.3) | 3 (2.3) | 6 (4.6) |
| <1x/month | 1 (0.8) | 4 (3.1) | 6 (4.6) |
| 1-3x/month | 4 (3.1) | 1 (0.8) | 6 (4.6) |
| 1x/week | 9 (6.9) | 7 (5.3) | 9 (6.9) |
| 2-3x/week | 25 (19.0) | 25 (19.0) | 32 (24.4) |
| 4-5x/week | 12 (9.2) | 13 (9.9) | 15 (11.4) |
| >5x/week | 77 (58.7) | 78 (59.6) | 57 (43.5) |
| Pork fat | | | |
| Never | 30 (22.9) | 39 (29.8) | 20 (15.3) |
| <1x/month | 14 (10.7) | 18 (13.7) | 13 (9.9) |
| 1-3x/month | 10 (7.6) | 7 (5.3) | 9 (6.9) |
| 1x/week | 5 (3.8) | 10 (7.6) | 15 (11.5) |
| 2-3x/week | 16 (12.2) | 14 (10.7) | 22 (16.8) |
| 4-5x/week | 7 (5.3) | 2 (1.5) | 10 (7.6) |
| >5x/week | 49 (37.5) | 41 (31.4) | 42 (32.1) |
| Mayonnaise | | | |
| Never | 40 (30.5) | 58 (44.3) | 45 (34.4) |
| <1x/month | 16 (12.2) | 20 (15.3) | 16 (12.2) |
| 1-3x/month | 19 (14.5) | 11 (8.4) | 20 (15.3) |
| 1x/week | 9 (6.9) | 11 (8.4) | 8 (6.1) |
| 2-3x/week | 26 (19.8) | 19 (14.5) | 26 (19.8) |
| 4-5x/week | 4 (3.1) | 2 (1.5) | 8 (6.1) |
| >5x/week | 17 (13.0) | 10 (7.6) | 8 (6.1) |
| Sardine paste | | | |
| Never | 128 (97.7) | 121 (92.3) | 127 (96.9) |
| <1/month | 2 (1.5) | 6 (4.6) | 3 (2.3) |
| 1-3/month | 0 (0.0) | 3 (2.3) | 1 (0.8) |
| 1/week | 1 (0.8) | 1 (0.8) | 0 (0.0) |
| Tuna sauce | | | |
| Never | 128 (97.7) | 122 (93.1) | 124 (94.6) |
| <1/month | 2 (1.5) | 5 (3.8) | 6 (4.6) |
| 1-3/month | 1 (0.8) | 2 (1.5) | 1 (0.8) |
| 1/week | 0 (0.0) | 1 (0.8) | 0 (0.0) |
| 2-3/week | 0 (0.0) | 1 (0.8) | 0 (0.0) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; results in table are presented as frequency (%)

The majority of the participants in all three groups reported not consuming either cod oil supplements or multivitamins in age 13-19 years (Table 18).

Table 18 The distribution of the participants regarding the consumption of supplements in the age 13-19 years

| | fMS n=131 | sMS n=131 | HC n=131 |
|---|---|---|---|
| Cod liver oil | | | |
| Yes | 12 (9.2) | 5 (3.8) | 16 (12.2) |
| No | 119 (90.8) | 126 (96.2) | 115 (87.8) |
| Cod liver oil pearls | | | |
| Yes | 15 (11.5) | 15 (11.5) | 17 (13.0) |
| No | 116 (88.5) | 116 (88.5) | 114 (87.0) |
| Cod liver oil capsules | | | |
| Yes | 9 (6.9) | 11 (8.4) | 14 (10.7) |
| No | 122 (93.1) | 120 (91.6) | 117 (89.3) |
| Multivitamins | | | |
| Yes | 27 (20.6) | 28 (21.4) | 33 (25.2) |
| No | 104 (79.4) | 103 (78.6) | 98 (74.8) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; results in table are presented as frequency (%)

Most participants in all three groups reported being breastfeed for the first 6 months of life, while this distribution differed between groups in the later age (Table 19).

Table 19 The distribution of the participants regarding the consumption of breast milk and other types of milk after birth

| | fMS n=131 | sMS n=131 | HC n=131 |
|---|---|---|---|
| 1-3 months | | | |
| Breastfeeding without other types of milk | 100 (76.3) | 100 (76.3) | 103 (78.6) |
| Breastfeeding and other types of milk | 9 (6.9) | 11 (8.4) | 11 (8.4) |
| Infant formula (without breast milk) | 14 (10.7) | 16 (12.2) | 11 (8.4) |
| Other types of milk (without breast milk) | 3 (2.3) | 1 (0.8) | 3 (2.3) |
| Don’t know | 5 (3.8) | 3 (2.3) | 3 (2.3) |
| 4-6 months | | | |
| Breastfeeding without other types of milk | 74 (56.5) | 86 (65.6) | 94 (71.7) |
| Breastfeeding and other types of milk | 18 (13.7) | 16 (12.2) | 15 (11.5) |
| Infant formula (without breast milk) | 28 (21.4) | 22 (16.8) | 14 (10.7) |
| Other types of milk (without breast milk) | 6 (4.6) | 4 (3.1) | 5 (3.8) |
| Don’t know | 5 (3.8) | 3 (2.3) | 3 (2.3) |
| 7-9 months | | | |
| Breastfeeding without other types of milk | 52 (39.7) | 75 (57.2) | 73 (55.7) |
| Breastfeeding and other types of milk | 30 (22.9) | 24 (18.3) | 24 (18.3) |
| Infant formula (without breast milk) | 36 (27.5) | 25 (19.1) | 19 (14.5) |
| Other types of milk (without breast milk) | 8 (6.1) | 4 (3.1) | 12 (9.2) |
| Don’t know | 5 (3.8) | 3 (2.3) | 3 (2.3) |
| 10+ months | | | |
| Breastfeeding without other types of milk | 48 (36.6) | 71 (54.2) | 66 (50.4) |
| Breastfeeding and other types of milk | 33 (25.2) | 25 (19.1) | 28 (21.4) |
| Infant formula (without breast milk) | 37 (28.3) | 25 (19.1) | 20 (15.2) |
| Other types of milk (without breast milk) | 8 (6.1) | 7 (5.3) | 14 (10.7) |
| Don’t know | 5 (3.8) | 3 (2.3) | 3 (2.3) |
| Artificial food | | | |
| Never used | 48 (38.1) | 71 (55.5) | 66 (51.6) |
| Ever used | 78 (61.9) | 57 (44.5) | 62 (48.4) |
| Infant formula | 41 (32.5) | 27 (21.1) | 28 (21.9) |
| Cow milk | 47 (37.3) | 31 (24.2) | 44 (34.4) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; results in table are presented as frequency (%)

The majority of the participants in all three groups reported having chickenpox, followed by measles and pneumonia prior to the age 30 (Table 20). More than 10% in both fMS and sMS group reported having mononucleosis, while less than 5% in the HC group reported the same (Table 20).

Table 20 The distribution of the participants regarding history of infections

| | fMS n=131 | sMS n=131 | HC n=131 |
|---|---|---|---|
| Measles | 52 (39.7) | 43 (32.8) | 45 (34.4) |
| Mumps | 23 (17.6) | 32 (24.4) | 31 (23.7) |
| German Measles | 28 (21.4) | 24 (18.3) | 26 (19.8) |
| Chickenpox | 110 (84.0) | 108 (82.4) | 104 (79.4) |
| Tonsillectomy | 26 (19.8) | 30 (22.9) | 30 (22.9) |
| Pneumonia | 31 (23.7) | 20 (15.3) | 30 (22.9) |
| Mononucleosis | 15 (11.5) | 17 (13.0) | 6 (4.6) |
| Mononucleosis confirmed by blood tests | 13 (9.9) | 13 (9.9) | 4 (3.1) |
| Spring (Mononucleosis season of infection) | 6 (4.6) | 1 (0.8) | 4 (3.1) |
| Summer (Mononucleosis season of infection) | 2 (1.5) | 2 (1.5) | 2 (1.5) |
| Fall (Mononucleosis season of infection) | 2 (1.5) | 6 (4.6) | 0 (0.0) |
| Winter (Mononucleosis season of infection) | 5 (3.8) | 5 (3.8) | 0 (0.0) |
| Parasite infection | 9 (6.9) | 9 (6.9) | 16 (12.2) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; results in table are presented as frequency (%)

In Table 21 is presented the distribution of participants regarding history of allergies, autoimmune diseases, cancer, and kidney disease, while in Table 22 is presented the distribution of participants regarding positive family history of these conditions.

Table 21 The distribution of the participants regarding history of allergies, autoimmune diseases, cancer, and kidney disease

| | fMS n=131 | sMS n=131 | HC n=131 |
|---|---|---|---|
| Allergy | | | |
| Pollens | 20 (15.3) | 16 (12.2) | 23 (17.6) |
| House dust | 11 (8.4) | 17 (13.0) | 22 (16.8) |
| Animal dander | 8 (6.1) | 7 (5.3) | 9 (6.9) |
| Foods | 4 (3.1) | 4 (3.1) | 8 (6.1) |
| Drugs | 18 (13.7) | 14 (10.7) | 16 (12.2) |
| Other | 4 (3.1) | 1 (0.8) | 5 (3.8) |
| Autoimmune disease | | | |
| Systemic Lupus Erythematosus | 0 (0.0) | 1 (0.8) | 0 (0.0) |
| Rheumatoid Arthritis | 2 (1.5) | 4 (3.1) | 0 (0.0) |
| Hypothyroidism | 10 (7.6) | 14 (10.7) | 7 (5.3) |
| Hyperthyroidism | 4 (3.1) | 2 (1.5) | 0 (0.0) |
| Neuromyelitis optica | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Inflammatory bowel disease | 2 (1.5) | 0 (0.0) | 1 (0.8) |
| Coeliac disease | 0 (0.0) | 1 (0.8) | 0 (0.0) |
| Type 1 diabetes | 0 (0.0) | 3 (2.3) | 0 (0.0) |
| Psoriasis | 3 (2.3) | 4 (3.1) | 0 (0.0) |
| Cancer | | | |
| Leukemia | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Hodgkin's lymphoma | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Non-Hodgkin's lymphoma | 0 (0.0) | 0 (0.0) | 1 (0.8) |
| Melanoma | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Non-melanoma skin cancer | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Kidney disease | 2 (1.5) | 1 (0.8) | 2 (1.5) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; results in table are presented as frequency (%)

Table 22 The distribution of the participants regarding family history of autoimmune diseases, cancer, and kidney disease

| | fMS n=131 | sMS n=131 | HC n=131 |
|---|---|---|---|
| Autoimmune disease | | | |
| Systemic Lupus Erythematosus | 1 (0.8) | 0 (0.0) | 3 (2.3) |
| Rheumatoid Arthritis | 6 (4.6) | 9 (6.9) | 11 (8.4) |
| Hypothyroidism | 26 (19.8) | 20 (15.3) | 17 (13.0) |
| Hyperthyroidism | 6 (4.6) | 9 (6.9) | 8 (6.1) |
| Neuromyelitis optica | 2 (1.5) | 0 (0.0) | 0 (0.0) |
| Inflammatory bowel disease | 1 (0.8) | 4 (3.1) | 1 (0.8) |
| Coeliac disease | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Type 1 diabetes | 2 (1.5) | 2 (1.5) | 1 (0.8) |
| Psoriasis | 12 (9.2) | 6 (4.6) | 4 (3.1) |
| Cancer | | | |
| Leukemia | 0 (0.0) | 2 (1.5) | 1 (0.8) |
| Hodgkin's lymphoma | 2 (1.5) | 1 (0.8) | 2 (1.5) |
| Non-Hodgkin's lymphoma | 1 (0.8) | 2 (1.5) | 2 (1.5) |
| Melanoma | 4 (3.1) | 0 (0.0) | 3 (2.3) |
| Non-melanoma skin cancer | 4 (3.1) | 2 (1.5) | 2 (1.5) |
| Kidney disease | 7 (5.3) | 4 (3.1) | 7 (5.3) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; results in table are presented as frequency (%)

The smoking habits of participants, smoking habits of mother during pregnancy and exposure to second-hand smoking are presented in Table 23.

Table 23 The distribution of the participants regarding smoking habits, mother's smoking habits during pregnancy, and exposure to second-hand smoking

| | fMS n=131 | sMS n=131 | HC n=131 |
|---|---|---|---|
| Smoker* | 73 (55.7) | 75 (57.3) | 71 (54.2) |
| Age at smoking onset** | 18.5±5.1 | 18.7±3.8 | 19.9±6.7 |
| Age at smoking cessation** | 31.7±10.6 | 33.6±11.6 | 35.7±10.4 |
| Smoking length (years)** | 19.0±12.0 | 20.5±12.3 | 20.5±11.5 |
| How many cigarettes?** | | | |
| 11-15 | 10.2±7.2 | 11.2±13.1 | 4.9±3.2 |
| 16-20 | 13.0±6.7 | 13.6±7.6 | 14.5±8.8 |
| 21-25 | 15.6±9.1 | 17.1±9.0 | 18.4±10.8 |
| 26-30 | 18.6±10.4 | 19.1±11.1 | 22.1±10.6 |
| Pack-years** | 5.8±5.4 | 6.2±6.0 | 6.2±5.6 |
| Mother smoked in pregnancy* | 21 (16.0) | 24 (18.3) | 24 (18.3) |
| How many cigarettes?** | 14.6±9.6 | 15.4±8.5 | 12.1±7.0 |
| Father smoked in the apartment* | 59 (45.0) | 68 (51.9) | 63 (48.1) |
| How many cigarettes?** | 18.4±12.8 | 17.0±12.7 | 15.9±11.4 |
| Mother smoked in the apartment* | 44 (33.6) | 40 (30.5) | 37 (28.2) |
| How many cigarettes?** | 15.9±12.3 | 16.5±12.3 | 15.8±9.5 |
| 18-25 lived with a smoker* | 54 (41.2) | 65 (49.6) | 48 (36.6) |
| How many cigarettes?** | 22.5±14.9 | 19.4±15.3 | 21.6±16.7 |
| 26-30 lived with a smoker* | 46 (35.1) | 49 (37.4) | 42 (32.1) |
| How many cigarettes?** | 21.0±14.3 | 17.7±13.1 | 20.4±16.3 |
| Colleagues smoked in the workplace* | 60 (45.8) | 55 (42.0) | 65 (49.6) |
| How many cigarettes?** | 19.9±19.3 | 27.6±30.9 | 36.6±34.3 |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; *frequency (%); **mean±standard deviation; pack years were calculated up to age 30

Participants' self-assessement of body figure, their height, weight and waist size, as well as physical activity intensity aged 13-19 are presented in Table 24.

Table 24 The distribution of the participants regarding weight and physical activity

| | fMS n=131 | sMS n=131 | HC n=131 |
|---|---|---|---|
| Figure aged 5* | 2 (1-4) | 2 (1-3) | 2 (1-3) |
| Figure aged 10* | 2 (2-4) | 2 (2-4) | 2.5 (2-4) |
| Figure aged 15* | 3 (2-4) | 3 (2-4) | 3 (2-4) |
| Figure aged 20* | 3 (2-4) | 3 (2-4) | 3 (2-5) |
| Figure aged 25* | 3 (2-4) | 3 (2-5) | 4 (3-5) |
| Figure aged 30* | 3 (2-5) | 3 (3-5) | 4 (3-5) |
| Figure now* | 4 (3-5) | 4 (3-5) | 5 (4-6) |
| Height** | | | |
| Males | 181.4±7.4 | 184.9±8.1 | 183.0±6.8 |
| Females | 167.6±6.8 | 168.0±7.4 | 167.3±6.4 |
| Weight** | | | |
| Males | 78.8±13.6 | 88.2±20.8 | 91.6±15.1 |
| Females | 64.0±13.0 | 66.4±13.7 | 71.9+±22.1 |
| Waist size** | | | |
| Males | 91.3±13.4 | 93.9±11.9 | 99.0±12.4 |
| Females | 81.0±12.5 | 79.2±11.1 | 86.2±19.6 |
| Physically active*** | 103 (78.6) | 95 (72.5) | 108 (82.4) |
| 13-19 mild physical activity*** | | | |
| None | 5 (3.8) | 4 (3.1) | 4 (3.1) |
| <1/week | 3 (2.3) | 2 (1.5) | 4 (3.1) |
| 1-2/week | 4 (3.1) | 8 (6.1) | 8 (6.2) |
| >2/week | 119 (90.8) | 117 (89.3) | 113 (87.6) |
| 13-19 vigorous physical activity*** | | | |
| None | 35 (26.7) | 39 (29.8) | 26 (20.3) |
| <1/week | 5 (3.8) | 8 (6.1) | 9 (7.0) |
| 1-2/week | 5 (3.8) | 11 (8.4) | 18 (14.1) |
| >2/week | 86 (65.6) | 73 (55.7) | 75 (58.6) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; *median (IQR); **mean±standard deviation; ***frequency (%)

The distribution of the female participants in regards to reproductive health factors is presented in Table 25.

Table 25 The distribution of the female participants regarding reproductive health factors

| | fMS n=94 | sMS n=94 | HC n=46 |
|---|---|---|---|
| Age at first period (years)* | 13.0±1.5 | 12.8±1.5 | 12.8±1.4 |
| Number of pregnancies** | | | |
| 0 | 29 (30.9) | 26 (27.7) | 20 (43.5) |
| 1 | 16 (17.0) | 19 (20.1) | 10 (21.7) |
| 2 | 30 (31.8) | 29 (30.9) | 8 (17.4) |
| 3 | 9 (9.6) | 10 (10.6) | 5 (10.9) |
| 4 | 9 (9.6) | 6 (6.4) | 3 (6.5) |
| 5 | 1 (1.1) | 4 (4.3) | 0 (0.0) |
| Age at first pregnancy* | 24.8±4.1 | 26.2±5.5 | 26.5±3.7 |
| Used contraceptive pills** | 25 (26.6) | 19 (20.9) | 12 (26.1) |
| Age at first use* | 24.7±8.8 | 24.4±6.6 | 24.7±6.6 |
| Length (years)* | 3.0±3.1 | 2.8±3.1 | 3.8±5.1 |
| Ever used transdermal contraceptives** | 1 (1.1) | 0 (0.0) | 0 (0.0) |
| Ever used a diaphragm** | 2 (2.2) | 1 (1.1) | 2 (4.3) |

| | | | |
|---|---|---|---|
| Hirsutism** | 14 (14.9) | 13 (13.8) | 3 (6.5) |
| Used hormonal therapy** | 3 (21.4) | 4 (30.8) | 1 (33.3) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; *mean±standard deviation; **frequency (%)

### 4.2.2. Logistic regression analysis

Skin phototype characteristics are assessed as risk factors for fMS compared to sMS and HC, as well as for sMS compared to HC in Table 26. No factors related to skin phototype have been associated with fMS or sMS (Table 26).

Table 26 Logistic regression analysis of skin phototype across fMS, sMS and HC

| | fMS vs. sMS OR (95% CI)* | fMS vs. HC OR (95% CI)** | sMS vs. HC OR (95% CI)** |
|---|---|---|---|
| Skin colour | 1.11 (0.95-1.29) | 0.93 (0.80-1.08) | 0.85 (0.73-1.00) |
| Tanning reaction to first sun | | | |
| Never tans/sometimes tans | Reference | Reference | Reference |
| Tan average/more than average tan | 1.42 (0.86-2.35) | 0.92 (0.54-1.59) | 0.63 (0.38-1.07) |
| Natural hair colour | | | |
| Black/ Dark brown | 0.73 (0.42-1.27) | 1.06 (0.63-1.80) | 1.44 (0.84-2.47) |
| Light brown | Reference | Reference | Reference |
| Blonde/red | 0.79 (0.19-3.37) | 0.44 (0.13-1.56) | 0.55 (0.16-1.94) |
| Natural eye colour | | | |
| Dark (Black/Brown/Hazel) | 0.97 (0.59-1.59) | 0.97 (0.59-1.59) | 1.10 (0.67-1.82) |
| Light (Green/Blue) | Reference | Reference | Reference |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; OR-odds ratio; 95% CI-95% confidence interval;*conditional logistic regression analysis; **unconditional logistic regression

Frequency of outdoor activities during summer was shown not to be associated with the risk of developing fMS or sMS (Table 27).

Table 27 Logistic regression analysis of frequency of outdoor activities during summer at ages 0-30 years across fMS, sMS and HC

| | fMS vs. sMS OR (95% CI)* | fMS vs. HC OR (95% CI)** | sMS vs. HC OR (95% CI)** |
|---|---|---|---|
| 0–5 years of age | | | |
| Not that often/Reasonably often | Reference | Reference | Reference |
| Quite often/Virtually all the time | 0.73 (0.29-1.81) | 0.56 (0.21-1.47) | 0.77 (0.28-2.12) |
| 6–10 years of age | | | |
| Not that often/Reasonably often | Reference | Reference | Reference |
| Quite often/Virtually all the time | 1.00 (0.32-3.10) | 1.00 (0.34-2.94) | 1.00 (0.34-2.94) |
| 11–15 years of age | | | |
| Not that often/Reasonably often | Reference | Reference | Reference |
| Quite often/Virtually all the time | 1.10 (0.47-2.59) | 1.10 (0.47-2.59) | 1.00 (0.43-2.32) |
| 16–20 years of age | | | |
| Not that often/Reasonably often | Reference | Reference | Reference |
| Quite often/Virtually all the time | 0.82 (0.44-1.53) | 0.96 (0.54-1.69) | 1.14 (0.64-2.04) |
| 21–25 years of age | | | |
| Not that often/Reasonably often | Reference | Reference | Reference |
| Quite often/Virtually all the time | 0.68 (0.41-1.12) | 0.69 (0.42-1.14) | 0.98 (0.59-1.62) |
| 26–30 years of age | | | |
| Not that often/Reasonably often | Reference | Reference | Reference |
| Quite often/Virtually all the time | 0.71 (0.43-1.16) | 0.77 (0.46-1.29) | 1.07 (0.64-1.79) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; OR-odds ratio; 95% CI-95% confidence interval;*conditional logistic regression analysis; **unconditional logistic regression

More frequent outdoor activities during winter in ages 16-20 and 21-25 were shown to be a protective factor for fMS when compared to HC in the univariate analysis (OR 0.58, 95% CI 0.35-0.96, and OR 0.59, 95% CI 0.36-0.96, respectively) (Table 28).

Table 28 Logistic regression analysis of frequency of outdoor activities during winter at ages 0-30 years across fMS, sMS and HC

| | fMS vs. sMS OR (95% CI)* | fMS vs. HC OR (95% CI)** | sMS vs. HC OR (95% CI)** |
|---|---|---|---|
| 0–5 years of age | | | |
| Not that often/Reasonably often | Reference | Reference | Reference |
| Quite often/Virtually all the time | 0.69 (0.38-1.26) | 0.96 (0.53-1.73) | 1.44 (0.76-2.72) |
| 6–10 years of age | | | |
| Not that often/Reasonably often | Reference | Reference | Reference |
| Quite often/Virtually all the time | 0.70 (0.38-1.32) | 0.59 (0.30-1.17) | 0.87 (0.42-1.80) |
| 11–15 years of age | | | |
| Not that often/Reasonably often | Reference | Reference | Reference |
| Quite often/Virtually all the time | 0.75 (0.41-1.38) | 0.62 (0.34-1.15) | 0.82 (0.44-1.53) |
| 16–20 years of age | | | |
| Not that often/Reasonably often | Reference | Reference | Reference |
| Quite often/Virtually all the time | 0.71 (0.43-1.16) | **0.58 (0.35-0.96)** | 0.82 (0.49-1.36) |
| 21–25 years of age | | | |
| Not that often/Reasonably often | Reference | Reference | Reference |
| Quite often/Virtually all the time | 0.65 (0.38-1.11) | **0.59 (0.36-0.96)** | 0.86 (0.53-1.39) |
| 26–30 years of age | | | |
| Not that often/Reasonably often | Reference | Reference | Reference |
| Quite often/Virtually all the time | 0.77 (0.47-1.27) | 0.69 (0.42-1.12) | 0.88 (0.54-1.44) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; OR-odds ratio; 95% CI-95% confidence interval;*conditional logistic regression analysis; **unconditional logistic regression; bold values denote statistical significance

Performing professional activities outdoors or equally outdoors and indoors was shown to be a protective factor for developing both fMS compared to HC, and sMS compared to HC across all life periods 16-30 years in the univariate analysis (Table 29).

Table 29 Logistic regression analysis of frequency of outdoor professional activities at ages 16-30 years across fMS, sMS and HC

| | fMS vs. sMS OR (95% CI)* | fMS vs. HC OR (95% CI)** | sMS vs. HC OR (95% CI)** |
|---|---|---|---|
| 16–20 years of age | | | |
| Mostly indoors | Reference | Reference | Reference |
| Mostly outdoors/Equally | 0.81 (0.39-1.69) | **0.40 (0.20-0.78)** | **0.49 (0.26-0.93)** |
| 21–25 years of age | | | |
| Mostly indoors | Reference | Reference | Reference |
| Mostly outdoors/Equally | 0.67 (0.32-1.38) | **0.29 (0.15-0.57)** | **0.45 (0.25-0.82)** |
| 26–30 years of age | | | |
| Mostly indoors | Reference | Reference | Reference |
| Mostly outdoors/Equally | 0.55 (0.27-1.10) | **0.27 (0.14-0.53)** | **0.51 (0.29-0.93)** |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; OR-odds ratio; 95% CI-95% confidence interval;*conditional logistic regression analysis; **unconditional logistic regression; bold values denote statistical significance

The results of the logistic regression analyses indicate that spending more than 4 hours per day on weekends exposed to sun does not influence the risk of developing fMS or sMS (Table 30).

Table 30 Logistic regression analysis of amount of weekend sun exposure at ages 6-30 years across fMS, sMS and HC

| | fMS vs. sMS OR (95% CI)* | fMS vs. HC OR (95% CI)** | sMS vs. HC OR (95% CI)** |
|---|---|---|---|
| 6–10 years of age | | | |
| <=4h/day | Reference | Reference | Reference |
| >4h/day 11–15 years of age | 0.77 (0.45-1.32) | 1.07 (0.64-1.82) | 1.41 (0.82-2.43) |
| <=4h/day | Reference | Reference | Reference |
| >4h/day 16–20 years of age | 0.87 (0.52-1.46) | 0.79 (0.48-1.31) | 0.90 (0.54-1.50) |
| <=4h/day | Reference | Reference | Reference |
| >4h/day 21–25 years of age | 0.67 (0.41-1.10) | 0.67 (0.41-1.10) | 1.00 (0.62-1.62) |
| <=4h/day | Reference | Reference | Reference |
| >4h/day 26–30 years of age | 0.84 (0.50-1.41) | 0.87 (0.52-1.48) | 1.04 (0.62-1.73) |
| <=4h/day | Reference | Reference | Reference |
| >4h/day | 1.04 (0.59-1.82) | 0.96 (0.55-1.67) | 0.91 (0.53-1.58) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; OR-odds ratio; 95% CI-95% confidence interval;*conditional logistic regression analysis; **unconditional logistic regression

Additionally, exposure to UV lamps at the ages 16-30 was not shown to be associated with fMS or sMS risk (Table 31).

Table 31 Logistic regression analysis of frequency of UV lamp exposure at ages 16-30 years across fMS, sMS and HC

| | fMS vs. sMS OR (95% CI)* | fMS vs. HC OR (95% CI)** | sMS vs. HC OR (95% CI)** |
|---|---|---|---|
| 16–20 years of age | | | |
| Never | Reference | Reference | Reference |
| Ever | 0.58 (0.23-1.48) | 1.11 (0.45-2.71) | 1.68 (0.73-3.86) |
| 21–25 years of age | | | |
| Never | Reference | Reference | Reference |
| Ever | 0.67 (0.27-1.63) | 1.01 (0.47-2.16) | 1.38 (0.67-2.84) |
| 26–30 years of age | | | |
| Never | Reference | Reference | Reference |
| Ever | 0.77 (0.34-1.75) | 1.01 (0.42-2.43) | 1.30 (0.56-2.99) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; OR-odds ratio; 95% CI-95% confidence interval;*conditional logistic regression analysis; **unconditional logistic regression

The results of the logistic regression analyses indicate that the risk for developing fMS or sMS does not differ between those that have used sun protection more frequently and those that have never used it, or used it less frequently (Table 32).

Table 32 Logistic regression analysis of frequency of sun protection use at ages 0-30 years across fMS, sMS and HC

| | fMS vs. sMS OR (95% CI)* | fMS vs. HC OR (95% CI)** | sMS vs. HC OR (95% CI)** |
|---|---|---|---|
| 0–5 years of age | | | |
| Seldom/never/Sometimes | Reference | Reference | Reference |
| Quite often/Almost always | 1.13 (0.64-2.02) | 0.93 (0.55-1.57) | 0.84 (0.49-1.41) |
| 6–10 years of age | | | |
| Seldom/never/Sometimes | Reference | Reference | Reference |
| Quite often/Almost always | 1.09 (0.62-1.91) | 0.97 (0.57-1.62) | 0.90 (0.53-1.52) |
| 11–15 years of age | | | |
| Seldom/never/Sometimes | Reference | Reference | Reference |
| Quite often/Almost always | 1.09 (0.62-1.91) | 0.97 (0.58-1.62) | 0.90 (0.53-1.51) |

| | | | |
|---|---|---|---|
| 16–20 years of age | | | |
| Seldom/never/Sometimes | Reference | Reference | Reference |
| Quite often/Almost always | 0.90 (0.54-1.51) | 0.97 (0.58-1.62) | 1.07 (0.64-1.78) |
| 21–25 years of age | | | |
| Seldom/never/Sometimes | Reference | Reference | Reference |
| Quite often/Almost always | 0.65 (0.38-1.11) | 0.88 (0.53-1.47) | 1.32 (0.80-2.17) |
| 26–30 years of age | | | |
| Seldom/never/Sometimes | Reference | Reference | Reference |
| Quite often/Almost always | 0.81 (0.46-1.43) | 1.36 (0.84-2.21) | 1.54 (0.92-2.59) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; OR-odds ratio; 95% CI-95% confidence interval;*conditional logistic regression analysis; **unconditional logistic regression

Usage of milk and dairy products was not associated with the risk of fMS or sMS (Table 33).

Table 33 Logistic regression analysis of milk and dairy products usage, aged 13-19 years across fMS, sMS and HC

| | fMS vs. sMS OR (95% CI)* | fMS vs. HC OR (95% CI)** | sMS vs. HC OR (95% CI)** |
|---|---|---|---|
| Milk usage | | | |
| Never | Reference | Reference | Reference |
| Ever | 1.04 (0.59-1.82) | 0.73 (0.42-1.27) | 0.70 (0.40-1.22) |
| Yogurt usage | | | |
| Never | Reference | Reference | Reference |
| Ever | 1.21 (0.66-2.22) | 0.69 (0.34-1.38) | 0.56 (0.29-1.11) |
| Cheese usage | | | |
| Never | Reference | Reference | Reference |
| Ever | 1.46 (0.72-2.96) | 0.85 (0.39-1.87) | 0.58 (0.28-1.21) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; OR-odds ratio; 95% CI-95% confidence interval;*conditional logistic regression analysis; **unconditional logistic regression

Consumption of meat was not associated with the risk of fMS or sMS, while consumption of meat products was shown to be a protective factor for fMS compared with HC (Table 34).

Table 34 Logistic regression analysis of meat and meat products usage, aged 13-19 years across fMS, sMS and HC

| | fMS vs. sMS OR (95% CI)* | fMS vs. HC OR (95% CI)** | sMS vs. HC OR (95% CI)** |
|---|---|---|---|
| Meat usage | | | |
| Never | Reference | Reference | Reference |
| Ever | 1.14 (0.41-3.15) | 0.42 (0.10-1.64) | 0.36 (0.09-1.39) |
| Meat products usage | | | |
| Never | Reference | Reference | Reference |
| Ever | 0.81 (0.46-1.43) | **0.46 (0.24-0.88)** | 0.57 (0.30-1.11) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; OR-odds ratio; 95% CI-95% confidence interval;*conditional logistic regression analysis; **unconditional logistic regression; bold values denote statistical significance

Consumption of seafood was shown to be a protective factor in fMS when compared with sMS, while fresh fish usage was shown to be a protective factor for fMS when compared with HC (Table 35).

Table 35 Logistic regression analysis of fish and seafood usage, aged 13-19 years across fMS, sMS and HC

| | fMS vs. sMS OR (95% CI)* | fMS vs. HC OR (95% CI)** | sMS vs. HC OR (95% CI)** |
|---|---|---|---|
| Fresh fish usage | | | |
| Never | Reference | Reference | Reference |
| Ever | 0.69 (0.42-1.13) | **0.55 (0.33-0.91)** | 0.80 (0.49-1.31) |
| Frozen fish usage | | | |
| Never | Reference | Reference | Reference |
| Ever | 0.85 (0.52-1.40) | 1.00 (0.62-1.62) | 1.16 (0.72-1.89) |
| Canned fish usage | | | |
| Never | Reference | Reference | Reference |
| Ever | 0.74 (0.45-1.20) | 0.74 (0.45-1.20) | 1.00 (0.62-1.62) |
| Seafood usage | | | |
| Never | Reference | Reference | Reference |
| Ever | **0.52 (0.28-0.96)** | 0.61 (0.32-1.18) | 1.24 (0.70-2.23) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; OR-odds ratio; 95% CI-95% confidence interval;*conditional logistic regression analysis; **unconditional logistic regression; bold values denote statistical significance

Regarding usage of specific types of fish, trout usage was shown to be a protective factor for both fMS and sMS compared to HC in the univariate analysis (OR 0.57, 95% CI 0.34-0.95, and OR 0.52, 95% CI 0.31-0.86, respectively) (Table 36).

Table 36 Logistic regression analysis of fish type usage, aged 13-19 years across fMS, sMS and HC

| | fMS vs. sMS OR (95% CI)* | fMS vs. HC OR (95% CI)** | sMS vs. HC OR (95% CI)** |
|---|---|---|---|
| Fresh tuna usage | | | |
| Never | Reference | Reference | Reference |
| Ever | 1.33 (0.56-3.16) | 1.20 (0.52-2.79) | 0.90 (0.37-2.20) |
| European hake usage | | | |
| Never | Reference | Reference | Reference |
| Ever | 0.71 (0.41-1.23) | 0.76 (0.45-1.27) | 1.04 (0.61-1.77) |
| Sardine usage | | | |
| Never | Reference | Reference | Reference |
| Ever | 1.71 (0.94-3.10) | 1.17 (0.68-2.02) | 0.70 (0.39-1.26) |
| Salmon usage | | | |
| Never | Reference | Reference | Reference |
| Ever | 1.57 (0.80-3.07) | 1.46 (0.80-2.66) | 1.00 (0.53-1.89) |
| Trout usage | | | |
| Never | Reference | Reference | Reference |
| Ever | 1.10 (0.67-1.78) | **0.57 (0.34-0.95)** | **0.52 (0.31-0.86)** |
| Whitefish usage | | | |
| Never | Reference | Reference | Reference |
| Ever | 0.89 (0.47-1.72) | 0.80 (0.44-1.44) | 0.88 (0.49-1.57) |
| Mackerel usage | | | |
| Never | Reference | Reference | Reference |
| Ever | 0.77 (0.45-1.32) | 0.80 (0.49-1.31) | 1.00 (0.62-1.63) |
| Smoked fish usage | | | |
| Never | Reference | Reference | Reference |
| Ever | 1.75 (0.95-3.23) | 0.96 (0.57-1.63) | 0.59 (0.34-1.04) |
| Canned fish usage | | | |
| Never | Reference | Reference | Reference |
| Ever | 1.17 (0.71-1.92) | 0.84 (0.49-1.41) | 0.71 (0.42-1.19) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; OR-odds ratio; 95% CI-95% confidence interval;*conditional logistic regression analysis; **unconditional logistic regression; bold values denote statistical significance

Usage of well water in teenage years was shown to be a protective factor for SMS compared with HC, while bottle water usage in the same age was shown to be a risk factor for both fMS and sMS compared with HC (Table 37).

Table 37 Logistic regression analysis of water type usage, aged 13-19 years across fMS, sMS and HC

| | fMS vs. sMS OR (95% CI)* | fMS vs. HC OR (95% CI)** | sMS vs. HC OR (95% CI)** |
|---|---|---|---|
| Well water, spring water | 1.25 (0.65-2.41) | 0.63 (0.35-1.15) | **0.50 (0.27-0.94)** |
| Tap water | 1.00 (0.41-2.40) | 1.68 (0.78-3.62) | 1.68 (0.78-3.62) |
| Bottled water | 1.36 (0.62-2.97) | **3.98 (1.55-10.21)** | **3.11 (1.18-8.15)** |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; OR-odds ratio; 95% CI-95% confidence interval;*conditional logistic regression analysis; **unconditional logistic regression; bold values denote statistical significance

Out of the sauces analysed, only pork fat usage singled out as a protective factor in sMS compared with HC (Table 38).

Table 38 Logistic regression analysis of sauce usage, aged 13-19 years across fMS, sMS and HC

| | fMS vs. sMS OR (95% CI)* | fMS vs. HC OR (95% CI)** | sMS vs. HC OR (95% CI)** |
|---|---|---|---|
| Butter | 1.19 (0.71-1.98) | 1.00 (0.60-1.65) | 0.85 (0.52-1.40) |
| Margarine | 0.97 (0.58-1.61) | 0.73 (0.42-1.27) | 0.75 (0.43-1.32) |
| Oil | 1.00 (0.20-4.95) | 2.05 (0.50-8.37) | 2.05 (0.50-8.37) |
| Pork fat | 1.41 (0.82-2.43) | 0.61 (0.32-1.14) | **0.43 (0.23-0.78)** |
| Mayonnaise | 1.75 (1.06-2.89) | 1.19 (0.71-2.00) | 0.66 (0.40-1.08) |
| Sardine paste | 0.30 (0.08-1.09) | 0.74 (0.16-3.39) | 2.62 (0.80-8.59) |
| Tuna sauce | 0.33 (0.09-1.23) | 0.42 (0.10-1.64) | 1.31 (0.47-3.62) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; OR-odds ratio; 95% CI-95% confidence interval;*conditional logistic regression analysis; **unconditional logistic regression; bold values denote statistical significance

Usage of cod liver oil in teenage years was shown to be a protective factor for sMS compared to HC (Table 39).

Table 39 Logistic regression analysis of supplement usage, aged 13-19 years across fMS, sMS and HC

| | fMS vs. sMS OR (95% CI)* | fMS vs. HC OR (95% CI)** | sMS vs. HC OR (95% CI)** |
|---|---|---|---|
| Cod liver oil | 2.75 (0.88-8.63) | 0.72 (0.33-1.60) | **0.29 (0.10-0.80)** |
| Cod liver oil pearls | 1.00 (0.45-2.23) | 0.87 (0.41-1.82) | 0.87 (0.41-1.82) |
| Cod liver oil capsules | 0.80 (0.32-2.03) | 0.62 (0.26-1.48) | 0.77 (0.33-1.76) |
| Multivitamins | 0.96 (0.54-1.70) | 0.77 (0.43-1.38) | 0.81 (0.45-1.43) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; OR-odds ratio; 95% CI-95% confidence interval;*conditional logistic regression analysis; **unconditional logistic regression; bold values denote statistical significance

Breastfeeding longer than 6 months has singled out as a protective factor for fMS when compared with both sMS and HC in the logistic regression analysis (Table 41). Artificial food usage in the first year of life has been associated with increased risk for fMS both when compared with sMS and HC, while infant formula and cow milk usage has been associated with increased risk for fMS when compared with sMS (Table 40).

Table 40 Logistic regression analysis of consumption of breast milk and other types of milk after birth across fMS, sMS and HC

| | fMS vs. sMS OR (95% CI)* | fMS vs. HC OR (95% CI)** | sMS vs. HC OR (95% CI)** |
|---|---|---|---|
| 1-3 months | | | |
| Exclusively breastfed | 1.00 (0.53-1.89) | 0.93 (0.51-1.73) | 0.87 (0.47-1.59) |
| Non-exclusively breastfed | Reference | Reference | Reference |
| 4-6 months | | | |
| Exclusively breastfed | 0.68 (0.42-1.13) | 0.67 (0.40-1.10) | 0.74 (0.43-1.27) |
| Non-exclusively breastfed | Reference | Reference | Reference |
| 7-9 months | | | |
| Exclusively breastfed | **0.48 (0.28-0.80)** | **0.53 (0.32-0.87)** | 1.07 (0.65-1.75) |
| Non-exclusively breastfed | Reference | Reference | Reference |
| 10+ months | | | |
| Exclusively breastfed | **0.49 (0.29-0.81)** | **0.58 (0.35-0.95)** | 1.17 (0.72-1.91) |
| Non-exclusively breastfed | Reference | Reference | Reference |
| Artificial food usage | | | |
| Ever used | **2.05 (1.23-3.41)** | **1.73 (1.05-2.85)** | 0.85 (0.52-1.40) |
| Never used | Reference | Reference | Reference |
| Infant formula | **2.15 (1.12-4.16)** | 1.72 (0.98-3.02) | 0.95 (0.53-1.73) |
| Cow milk | **1.81 (1.06-3.08)** | 1.14 (0.68-1.90) | 0.61 (0.36-1.05) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; OR-odds ratio; 95% CI-95% confidence interval;*conditional logistic regression analysis; **unconditional logistic regression; bold values denote statistical significance

Positive history of mononucleosis has singled out from other infections as a risk factor for both fMS and for sMS when compared with HC (Table 41).

Table 41 Logistic regression analysis of history of infections across fMS, sMS and HC

| | fMS vs. sMS OR (95% CI)* | fMS vs. HC OR (95% CI)** | sMS vs. HC OR (95% CI)** |
|---|---|---|---|
| Measles | 1.47 (0.82-2.64) | 1.26 (0.76-2.08) | 0.93 (0.56-1.56) |
| Mumps | 0.50 (0.22-1.11) | 0.69 (0.38-1.26) | 1.04 (0.59-1.84) |
| German Measles | 1.24 (0.65-2.34) | 1.10 (0.60-2.00) | 0.91 (0.49-1.68) |
| Chickenpox | 1.10 (0.60-2.02) | 1.36 (0.72-2.55) | 1.22 (0.66-2.26) |
| Tonsillectomy | 0.83 (0.46-1.51) | 0.83 (0.46-1.51) | 1.00 (0.56-1.78) |
| Pneumonia | 1.65 (0.88-3.09) | 1.04 (0.59-1.85) | 0.61 (0.32-1.14) |
| Mononucleosis | 0.87 (0.41-1.82) | **2.69 (1.01-7.18)** | **3.11 (1.18-8.15)** |
| Parasite Infection | 1.00 (0.38-2.66) | 0.53 (0.23-1.25) | 0.53 (0.23-1.25) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; OR-odds ratio; 95% CI-95% confidence interval;*conditional logistic regression analysis; **unconditional logistic regression; bold values denote statistical significance

When analyzing allergies, autoimmune diseases, cancer and kidney diseases, no disease singled out as a risk factor for developing fMS or sMS (Table 42).

Table 42 Logistic regression analysis of history of allergies, autoimmune diseases, cancer and kidney diseases across fMS, sMS and HC

| | fMS vs. sMS OR (95% CI)* | fMS vs. HC OR (95% CI)** | sMS vs. HC OR (95% CI)** |
|---|---|---|---|
| Pollens | 1.33 (0.63-2.82) | 0.85 (0.44-1.63) | 0.65 (0.33-1.30) |
| House dust | 0.60 (0.26-1.37) | 0.45 (0.21-0.98) | 0.74 (0.37-1.47) |
| Animal dander | 1.20 (0.37-3.93) | 0.88 (0.33-2.36) | 0.77 (0.28-2.12) |
| Foods | 1.00 (0.25-4.00) | 0.48 (0.14-1.65) | 0.48 (0.14-1.65) |
| Drugs | 1.33 (0.63-2.80) | 1.14 (0.56-2.36) | 0.86 (0.40-1.84) |
| Other | 4.09 (0.45-37.13) | 0.79 (0.21-3.02) | 0.19 (0.02-1.68) |
| Rheumatoid Arthritis | 0.50 (0.09-2.73) | N/A | N/A |
| Hypothyroidism | 0.71 (0.32-1.61) | 1.46 (0.54-3.97) | 2.12 (0.83-5.44) |

| | fMS vs. sMS OR (95% CI)* | fMS vs. HC OR (95% CI)** | sMS vs. HC OR (95% CI)** |
|---|---|---|---|
| Hyperthyroidism | 2.00 (0.37-10.92) | N/A | N/A |
| Inflammatory bowel disease | N/A | 2.02 (0.18-22.50) | N/A |
| Psoriasis | 0.75 (0.17-3.35) | N/A | N/A |
| Kidney disease | 2.00 (0.15-22.06) | 1.00 (0.14-7.21) | 0.50 (0.04-5.54) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; OR-odds ratio; 95% CI-95% confidence interval;*conditional logistic regression analysis; **unconditional logistic regression

Similarly, family history of autoimmune diseases, cancer or kidney diseases was shown to have no association with the risk of developing fMS compared with sMS, but family history of psoriasis was associated with the risk of developing fMS compared with HC in the univariate analysis (Table 43).

Table 43 Logistic regression analysis of family history of allergies, autoimmune diseases, cancer and kidney diseases across fMS, sMS and HC

| | fMS vs. sMS OR (95% CI)* | fMS vs. HC OR (95% CI)** | sMS vs. HC OR (95% CI)** |
|---|---|---|---|
| Systemic Lupus Erythematosus | N/A | 0.33 (0.03-3.20) | N/A |
| Rheumatoid Arthritis | 0.67 (0.24-1.87) | 0.52 (0.19-1.46) | 0.80 (0.32-2.01) |
| Hypothyroidism | 1.37 (0.72-2.62) | 1.66 (0.85-3.23) | 1.21 (0.60-2.43) |
| Hyperthyroidism | 0.67 (0.24-1.87) | 0.74 (0.25-2.19) | 1.13 (0.42-3.03) |
| Inflammatory Bowel Disease | 0.25 (0.03-2.24) | 1.00 (0.06-16.16) | 4.09 (0.45-37.13) |
| Type 1 Diabetes | 1.00 (0.14-7.10) | 2.02 (0.18-22.50) | 2.02 (0.18-22.50) |
| Psoriasis | 2.00 (0.75-5.33) | **3.20 (1.01-10.20)** | 1.52 (0.42-5.53) |
| Leukemia | N/A | N/A | 2.02 (0.18-22.50) |
| Hodgkin's Lymphoma | 2.00 (0.18-22.06) | 1.00 (0.14-7.21) | 0.50 (0.04-5.54) |
| Non-Hodgkin's Lymphoma | 0.50 (0.05-5.51) | 0.50 (0.04-5.54) | 1.00 (0.14-7.21) |
| Melanoma | N/A | 1.34 (0.29-6.13) | N/A |
| Non-melanoma Skin Cancer | 2.00 (0.37-10.92) | 2.03 (0.37-11.29) | 1.00 (0.14-7.21) |
| Kidney Disease | 1.75 (0.51-5.98) | 1.00 (0.34-2.94) | 0.56 (0.16-1.95) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; OR-odds ratio; 95% CI-95% confidence interval;*conditional logistic regression analysis; **unconditional logistic regression; bold values denote statistical significance

The smoking habits of participants were not associated with increased risk of fMS or sMS according to the results of the logistic regression analysis (Table 44). Living with a smoker aged 18-25 has shown to be a risk factor for developing sMS compared with HC (OR 1.66, 95% CI 1.01-2.73).

Table 44 Logistic regression analysis of smoking habits and exposure to second-hand smoke up to age 30 across fMS, sMS and HC

| Variable | fMS vs. sMS OR (95% CI)* | fMS vs. HC OR (95% CI)** | sMS vs. HC OR (95% CI)** |
|---|---|---|---|
| Smoker | 0.93 (0.56-1.55) | 1.06 (0.65-1.73) | 1.13 (0.69-1.84) |
| Pack-years | 1.02 (0.95-1.10) | 0.99 (0.93-1.05) | 1.00 (0.94-1.06) |
| Mother smoked in pregnancy | 0.81 (0.39-1.69) | 0.84 (0.44-1.60) | 0.99 (0.53-1.85) |
| Father smoked in the apartment | 0.81 (0.50-1.31) | 0.90 (0.55-1.46) | 1.15 (0.71-1.87) |
| Mother smoked in the apartment | 1.29 (0.73-2.27) | 1.30 (0.77-2.20) | 1.10 (0.65-1.88) |
| 18-25 lived with a smoker | 0.75 (0.46-1.24) | 1.25 (0.76-2.06) | **1.66 (1.01-2.73)** |
| 26-30 lived with a smoker | 1.00 (0.58-1.72) | 1.36 (0.80-2.31) | 1.28 (0.76-2.14) |
| Colleagues smoked in the workplace | 1.47 (0.79-2.72) | 0.88 (0.54-1.44) | 0.74 (0.45-1.21) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; OR-odds ratio; 95% CI-95% confidence interval;*conditional logistic regression analysis; **unconditional logistic regression; bold values denote statistical significance

Self-assessed higher body weight at age 30 was associated with lower fMS and sMS risk compared with HC (Table 45). Physical activity in teenage years was not associated with increase fMS or sMS risk (Table 45).

Table 45 Logistic regression analysis of self-reported figure ages 5-30 and physical activity levels aged 13-19 across fMS, sMS and HC

| | fMS vs. sMS OR (95% CI)* | fMS vs. HC OR (95% CI)** | sMS vs. HC OR (95% CI)** |
|---|---|---|---|
| Figure aged 5 | 1.05 (0.91-1.21) | 1.02 (0.88-1.18) | 0.97 (0.84-1.14) |
| Figure aged 10 | 1.03 (0.89-1.19) | 0.98 (0.84-1.14) | 0.95 (0.81-1.11) |
| Figure aged 15 | 1.00 (0.86-1.16) | 1.06 (0.89-1.25) | 1.06 (0.90-1.25) |
| Figure aged 20 | 1.05 (0.89-1.25) | 0.93 (0.78-1.11) | 0.91 (0.77-1.08) |
| Figure aged 25 | 1.01 (0.86-1.17) | 0.87 (0.73-1.03) | 0.89 (0.75-1.04) |
| Figure aged 30 | 1.03 (0.88-1.21) | **0.81 (0.68-0.95)** | **0.78 (0.65-0.93)** |
| 13-19 mild physical activity | 0.98 (0.68-1.42) | 1.06 (0.73-1.55) | 1.09 (0.74-1.61) |
| 13-19 vigorous physical activity | 1.11 (0.92-1.33) | 0.98 (0.81-1.19) | 0.88 (0.73-1.07) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; OR-odds ratio; 95% CI-95% confidence interval;*conditional logistic regression analysis; **unconditional logistic regression; bold values denote statistical significance

Having had two or more pregnancies during lifetime as opposed to ≤1 has singled out to be a risk factor for both fMS and sMS when compared with HC in the univariate analysis (Table 46).

Table 46 Logistic regression analysis of female participants' reproductive health factors across fMS, sMS and HC

| | fMS vs. sMS OR (95% CI)* | fMS vs. HC OR (95% CI)** | sMS vs. HC OR (95% CI)** |
|---|---|---|---|
| Age at first period (years) | 1.11 (0.91-1.35) | 1.13 (0.88-1.46) | 1.03 (0.81-1.33) |
| Ever pregnant | 0.89 (0.46-1.74) | 1.81 (0.87-3.77) | 2.01 (0.96-4.21) |
| Age at first pregnancy | 0.92 (0.85-1.01) | 0.90 (0.80-1.01) | 0.99 (0.90-1.08) |
| Number of pregnancies ≥2 | 1.06 (0.55-2.05) | **2.11 (1.02-4.36)** | **2.11 (1.02-4.36)** |
| Used contraceptive pills | 1.43 (0.72-2.83) | 1.03 (0.46-2.29) | 0.72 (0.31-1.64) |
| Hirsutism | 1.10 (0.47-2.59) | 2.51 (0.68-9.21) | 2.30 (0.62-8.51) |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; HC-healthy control; OR-odds ratio; 95% CI-95% confidence interval;*conditional logistic regression analysis; **unconditional logistic regression; bold values denote statistical significance

### 4.2.3. Confounding control

In Table 47 are singled out the results of univariate analysis for factors statistically associated with fMS when compared with sMS, as well as the results of multivariate analysis controlling for potential confounders. All of the variables significant in the univariate model remained significant after correction for potential confounders. Protective factors that singled out are seafood usage aged 13-19, and exclusive breastfeeding 7-9 months, and after the ninth month, while using cow's milk or infant formula in infanthood were shown to be risk factors for fMS compared with sMS (Table 47).

Table 47 Univariate and multivariate logistic regression analysis comparing risk factors for developing fMS compared with sMS

| | Univariate analysis OR (95% CI)* | Multivariate analysis OR (95% CI)** |
|---|---|---|
| Seafood usage | | |
| Never | Reference | Reference |
| Ever | **0.52 (0.28-0.96)** | **0.50 (0.26-0.95)** |
| Aged 7-9 months | | |

| | | |
|---|---|---|
| Non-exclusively breastfed | Reference | Reference |
| Exclusively breastfed | **0.48 (0.28-0.80)** | **0.47 (0.28-0.80)** |
| Aged 10+ months | | |
| Non-exclusively breastfed | Reference | Reference |
| Exclusively breastfed | **0.49 (0.29-0.81)** | **0.48 (0.29-0.81)** |
| Artificial food usage as infant | | |
| Never used | Reference | Reference |
| Ever used | **2.05 (1.23-3.41)** | **2.08 (1.24-3.50)** |
| Infant formula | **2.15 (1.12-4.16)** | **2.07 (1.07-4.02)** |
| Cow milk | **1.81 (1.06-3.08)** | **1.97 (1.13-3.44)** |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; OR-odds ratio; 95% CI-95% confidence interval;*conditional logistic regression analysis, base comparison sMS; **results of conditional logistic regression analysis, base comparison sMS, adjusted for history of mononucleosis, smoking history and educational level; participants were matched for sex, age, and disease phenotype; bold values denote statistical significance

In Table 48 are presented the results of univariate and multivariate logistic regression analysis comparing risk factors for developing fMS compared with HC. Out of factors significant in the univariate model, performing professional activities outdoors aged 21-25 and 26-30, fresh fish usage and trout usage aged 13-19 were shown to be protective factors for fMS when compared with HC, while frequently using bottled water aged 13-19 was associated with increased risk of fMS compared with HC (Table 48).

Table 48 Univariate and multivariate logistic regression analysis comparing risk factors for developing fMS compared with HC

| | Univariate analysis OR (95% CI)* | Multivariate analysis OR (95% CI)** |
|---|---|---|
| Winter sun exposure aged 16–20 years | | |
| Not that often/Reasonably often | Reference | Reference |
| Quite often/Virtually all the time | **0.58 (0.35-0.96)** | 0.90 (0.68-1.20) |
| Winter sun exposure aged 21–25 years | | |
| Not that often/Reasonably often | Reference | Reference |
| Quite often/Virtually all the time | **0.59 (0.36-0.96)** | 0.83 (0.63-1.08) |
| Professional activities aged 16–20 years | | |
| Mostly indoors | Reference | Reference |
| Mostly outdoors/Equally | **0.40 (0.20-0.78)** | 0.53 (0.25-1.11) |
| Professional activities aged 21–25 years | | |
| Mostly indoors | Reference | Reference |
| Mostly outdoors/Equally | **0.29 (0.15-0.57)** | **0.38 (0.18-0.78)** |
| Professional activities aged 26–30 years | | |
| Mostly indoors | Reference | Reference |
| Mostly outdoors/Equally | **0.27 (0.14-0.53)** | **0.33 (0.15-0.69)** |
| Meat products usage aged 13-19 | | |
| Never | Reference | Reference |
| Ever | **0.46 (0.24-0.88)** | 0.65 (0.32-1.31) |
| Fresh fish usage aged 13-19 | | |
| Never | Reference | Reference |
| Ever | **0.55 (0.33-0.91)** | **0.52 (0.30-0.90)** |
| Trout usage aged 13-19 | | |
| Never | Reference | Reference |
| Ever | **0.57 (0.34-0.95)** | **0.57 (0.33-0.95)** |
| Frequently using bottled water aged 13-19 | | |
| No | Reference | Reference |
| Yes | **3.98 (1.55-10.21)** | **4.95 (1.77-13.88)** |
| Aged 7-9 months | | |
| Non-exclusively breastfed | Reference | Reference |
| Exclusively breastfed | **0.53 (0.32-0.87)** | 0.63 (0.37-1.09) |
| Aged 10+ months | | |
| Non-exclusively breastfed | Reference | Reference |
| Exclusively breastfed | **0.58 (0.35-0.95)** | 0.68 (0.39-1.17) |

| | | |
|---|---|---|
| Artificial food usage as infant | | |
| Never used | Reference | Reference |
| Ever used | **1.83 (1.11-3.02)** | 1.47 (0.85-2.54) |
| Family history of psoriasis | | |
| No | Reference | Reference |
| Yes | **3.20 (1.01-10.20)** | 3.41 (0.99-11.72) |
| Figure aged 30 | **0.81 (0.68-0.95)** | 0.92 (0.76-1.11) |
| Number of pregnancies | | |
| ≤1 | Reference | Reference |
| ≥2 | **2.11 (1.02-4.36)** | 1.84 (0.86-3.92) |

Abbreviations: fMS-familial multiple sclerosis; HC-healthy control; OR-odds ratio; 95% CI-95% confidence interval;*unconditional logistic regression analysis, base comparison HC; **results of unconditional logistic regression analysis, base comparison HC, adjusted for sex, history of mononucleosis, smoking history and educational level; bold values denote statistical significance

In Table 49 are presented the results of the univariate analysis for factors statistically associated with sMS when compared with HC, as well as the results of multivariate analysis controlling for potential confounders. Out of the variables significant in the univariate analysis, professional activities outdoors aged 21-25 and 26-30, trout, pork fat, and cod liver oil usage aged 13-19 were shown to be protective factors in the multivariate analysis, while frequently using bottled water was shown to be a risk factor for sMS when compared with HC (Table 49).

Table 49 Univariate and multivariate logistic regression analysis comparing risk factors for developing sMS compared with HC

| | Univariate analysis OR (95% CI)* | Multivariate analysis OR (95% CI)** |
|---|---|---|
| Professional activities aged 16–20 years | | |
| Mostly indoors | Reference | Reference |
| Mostly outdoors/Equally | **0.49 (0.26-0.93)** | 0.59 (0.29-1.19) |
| Professional activities aged 21–25 years | | |
| Mostly indoors | Reference | Reference |
| Mostly outdoors/Equally | **0.45 (0.25-0.82)** | **0.47 (0.24-0.92)** |
| Professional activities aged 26–30 years | | |
| Mostly indoors | Reference | Reference |
| Mostly outdoors/Equally | **0.51 (0.29-0.93)** | **0.51 (0.26-0.99)** |
| Trout usage aged 13-19 | | |
| Never | Reference | Reference |
| Ever | **0.52 (0.31-0.86)** | **0.56 (0.32-0.99)** |
| Frequently using bottled water aged 13-19 | | |
| No | Reference | Reference |
| Yes | **3.11 (1.18-8.15)** | **3.40 (1.12-10.31)** |
| Frequently using well water aged 13-19 | | |
| No | Reference | Reference |
| Yes | **0.50 (0.27-0.94)** | 0.63 (0.32-1.24) |
| Pork fat usage aged 13-19 | | |
| No | Reference | Reference |
| Yes | **0.43 (0.23-0.78)** | **0.87 (0.78-0.98)** |
| Cod liver oil usage aged 13-19 | | |
| No | Reference | Reference |
| Yes | **0.29 (0.10-0.80)** | **0.27 (0.09-0.83)** |
| 18-25 lived with a smoker | | |
| No | Reference | Reference |
| Yes | **1.66 (1.01-2.73)** | 1.21 (0.69-2.10) |
| Figure aged 30 | **0.78 (0.65-0.93)** | 0.98 (0.80-1.20) |
| Number of pregnancies | | |
| ≤1 | Reference | Reference |
| ≥2 | **2.11 (1.02-4.36)** | 1.70 (0.79-3.68) |

Abbreviations: sMS-sporadic multiple sclerosis; HC-healthy control; OR-odds ratio; 95% CI-95% confidence interval;*unconditional logistic regression analysis, base comparison HC; **results of

unconditional logistic regression analysis, base comparison HC, adjusted for sex, history of mononucleosis, smoking history and educational level; bold values denote statistical significance

### *4.3. Retrospective cohort study investigating the predictive factors associated with disease outcomes in fMS and sMS*

A total of 262 participants were included in the matched retrospective cohort study (131 fMS, and 131 sMS). Detailed demographic characteristics of the study cohorts are presented in Table 4 in section 4.2.1. In Table 50 are presented clinical characteristics of the fMS and sMS cohort. There were no statistically significant differences between any of the clinical variables between the fMS and sMS cohort. Although age at symptom onset was lower in fMS cohort, this difference did not reach a statistical significance (Table 50).

Table 50 Clinical characteristics of participants in the matched retrospective cohort study

| | fMS n=131 | sMS n=131 | p-value |
|---|---|---|---|
| MS phenotype<br>RMS<br>PPMS | <br>118 (90.1)<br>13 (9.9) | <br>118 (90.1)<br>13 (9.9) | <br>* |
| DMTs | 82 (62.6) | 77 (58.8) | 0.527 |
| Age at symptom onset | 29.9 ± 9.3 | 31.3 ± 11.0 | 0.548 |
| Age at diagnosis | 33.7 ± 10.2 | 35.1 ± 11.5 | 0.327 |
| Delay to diagnosis | 3.7 ± 5.7 | 3.8 ± 6.0 | 0.721 |
| Disease duration | 10.4 ± 8.9 | 10.5 ± 9.6 | 0.721 |
| EDSS | 2.4 ± 1.8 | 2.7 ± 1.8 | 0.241 |
| MSSS | 3.1 ± 2.7 | 3.5 ± 2.4 | 0.208 |
| Progression index | 0.4 ± 0.5 | 0.5 ± 0.6 | 0.127 |

Abbreviations: fMS-familial multiple sclerosis; sMS-sporadic multiple sclerosis; MS-multiple sclerosis; RMS-relapsing multiple sclerosis; PPMS-primary progressive multiple sclerosis; DMT-disease modifying therapy; EDSS-Expanded Disability Status Scale; Multiple Sclerosis Severity Score; *matched for disease phenotype

### *4.4. Case-control study characterizing rare and uncommon gene variants in patients with fMS*

This study included 29 fMS patients with a first-degree relative diagnosed with MS, and 89 patients without MS in the control group. The WES analysis identified 6 rare and three uncommon variants predicted pathogenic in the fMS group. These variants had MAF<5% in the gnomADe database, along with a Combined Annotation Dependent Depletion (CADD) score exceeding 20. Among these, 7 variants were missense mutations with a moderate predicted impact on gene function, and 2 were stop-gain mutations, estimated to have a high impact on gene function (Table 51).

Table 51 Rare and uncommon gene variants found in cohort of fMS cases

| Gene | Chr:location | Coding change | Amino acid change | Consequence | MAF | gnomADe AF | CADD | p |
|---|---|---|---|---|---|---|---|---|
| *HLA-A* | 6:29943450 | c.526G>T | p.Glu176Ter | stop gain | 0.024690 | 0.01584 | 33 | 0.359 |
| *CLEC16A* | 16:11126134 | c.2629G>A | p.Asp877Asn | missense | 0.005000 | Novel | 25 | 0.488 |
| *ALPK2* | 18:58523972 | c.5592C>A | p.Tyr1864Ter | stop gain | 0.005051 | Novel | 35 | 0.488 |
| *TYK2* | 19:10378250 | c.157G>A | p.Ala53Thr | missense | 0.004386 | 0.007351 | 24.6 | 0.488 |
| *SLC9B1* | 4:102932166 | c.787A>G | p.Ile263Val | missense | 0.005051 | 0.002163 | 23.9 | 0.488 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *WWOX* | 16:78115103 | c.358C>T | p.Arg120Trp | missense | 0.004237 | 0.00749 | 28.4 | 0.577 |
| *GATA3* | 10:8073744 | c.1056C>A | p.Asn352Lys | missense | 0.009524 | Novel | 25.3 | 0.660 |
| *INAVA* | 1:200898444 | c.44T>A | p.Ile15Asn | missense | 0.010530 | 0.007512 | 27.9 | 0.660 |
| *BACH2* | 6:89951235 | c.871C>G | p.Leu291Val | missense | 0.020200 | 0.01971 | 24.4 | 0.990 |

Abbreviations: Chr – chromosome; MAF - minor allele frequency; gnomADe AF - allele frequency of the variant allele in the gnomAD database; CADD - Combined Annotation Dependent Depletion score; p – p-value of burden analysis after false discovery rate correction

The GLM burden analysis indicated no statistically significant enrichment of these variants in the fMS group after FDR adjustment (Table 51). However, five rare variants with a gnomADe allele frequency of less than 1% — *CLEC16A* chr16:11126134_G/A, *ALPK2* chr18:58523972_G/T, *TYK2* chr19:10378250_C/T, *SLC9B1* chr4:102932166_T/C, and *WWOX* chr16:78115103_C/T — were exclusively found in the fMS patients and not in the control group. The distribution of these uncommon and rare variants between the fMS cases and controls is depicted in Figure 2.

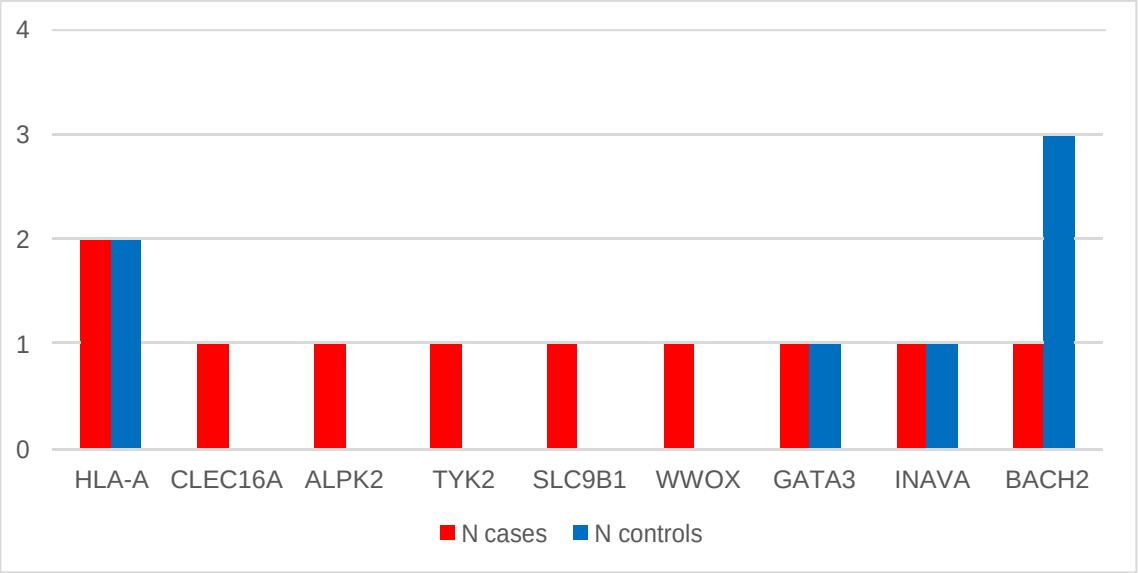


Figure 2 Distribution of uncommon and rare variants in fMS cases and controls

# 5. DISCUSSION

Our study aimed to assess different epidemiological, clinical, and genetic aspects of fMS. We found the prevalence of fMS in the Belgrade region to be 6.4%, and it was similar between males and females. Sisters were the family members of patients with fMS with the highest prevalence of MS, followed by mothers and daughters, while the lowest prevalence was found in grandfathers. The prevalence of fMS was higher in female relatives of all categories, when compared with their male counterparts.

We also aimed to assess the risk factor profile in fMS, and to investigate whether there are any differences when compared with sMS. We found that use of cow's milk and infant formula in infanthood increases the risk for fMS when compared with sMS. On the other hand, exclusive breastfeeding longer than 6 months and usage of seafood were shown to be protective factors in fMS compared to sMS.

When compared with HC, we found that risk for fMS is increased in case of positive history of mononucleosis, and in persons using bottled water as a primary source of water aged 13-19. On the contrary, performing professional activities outdoors aged 21-30, consumption of fresh fish and consummation of trout aged 13-19 were shown to be protective factors for fMS when compared with HC.

In our matched retrospective cohort study we found no statistically significant differences in any of the clinical variables between the fMS and sMS cohort. Although age at symptom onset was lower in fMS cohort compared with sMS, this difference did not reach a statistical significance. On the other hand, using registry-based data, we did find a statistically significant earlier age of onset in the fMS group compared to sMS group.

Using whole exome sequencing, we found 9 rare or uncommon gene variants predicted pathogenic in the fMS group. Two of these were stop-gain mutations predicted to have a high impact on gene function, while 7 variants were missense mutations with a moderate predicted impact on gene function. Although the burden analysis indicated no statistically significant enrichment of these variants in the fMS group after adjusting for the false discovery rate, we found that five rare variants are present exclusively in the fMS patients and not in the control group.

The prevalence of fMS of 6.4% found in our study is aligned with the literature data, where a wide range of fMS prevalence has been reported, ranging from 2.2% in Hungary to almost 33% in Saskatchewan, Canada (Fricska-Nagy et al., 2007; Hader and Yee, 2014). A meta-analysis published in 2021 has obtained the pooled estimate of global fMS prevalence of 11.8% (95% CI: 10.7–13.0%) (Ehtesham, Rafie and Mosallaei, 2021). This systematic review has found similar prevalence among sexes (15.4% in females and 13.7% in males), albeit slightly lower in males, which is in alignment with our findings (6.5% in females 6.2% in males) (Ehtesham, Rafie and Mosallaei, 2021).

Risks of developing MS among relatives of fMS probands couldn't be calculated due to the cross-sectional design of our registry-based study. However, we have used prevalence of MS among the probands' relatives to approximate this risk. We observed the highest prevalence of MS in sisters of probands with fMS (27.1%), followed by mothers (22.9%) and daughters (21.7%). Similar findings were obtained in an Australian study (O'Gorman et al., 2011). The authors of the study observed the highest crude risk for MS in sisters of MS probands (2.88%), followed by mothers (1.09%), daughters (0.94%), and brothers (0.94%) (O'Gorman et al., 2011). Another study performed in the United Kingdom also found that sisters are the family members of probands with

the highest crude MS risk (3.74%), followed by brothers (2.65%), mothers (2.08%), fathers (1.96%), and daughters (1.01%) (Robertson et al., 1996).

Our research highlights that individuals with fMS generally exhibit symptoms of MS at an earlier age (30.4±9.5 years) than those with sMS (32.3±10.1 years). This observation aligns closely with results from an Argentinian study which reported similar ages at symptom onset as found in our study for both sMS (32.4±9.4 years) and fMS (29.4±5.1 years) groups (Rojas et al., 2016). Similarly, a Danish study which utilized age at diagnosis as a proxy for symptom onset, also found that fMS cases are usually earlier diagnosed compared to sMS cases (Steenhof et al., 2019b). We believe that our approach of focusing on the age at symptom onset rather than age at diagnosis, has some merit. This is because previous studies have indicated that subsequent family members diagnosed with MS in fMS families experience shorter delays to diagnosis compared to the initial family member, likely influenced by an awareness of familial disease history (Steenhof et al., 2019b). This reduced diagnostic delay for subsequent family members with MS could skew comparisons based on age at diagnosis. By concentrating on symptom onset, we believe to have neutralized this potential confounding factor. It is noteworthy that our findings show consistency with the results of the Danish study, despite the stricter definition of fMS used in the Danish study (Steenhof et al., 2019b).

When comparing fMS cases across generations, a significantly lower mean age at MS symptom onset was observed in the younger generation (25.8±7.2 years) compared to the older generation (35.7±11.6 years). This observation aligns with the findings of the previously mentioned Argentinean study, where a younger mean age at onset was also noted in the younger fMS generation (24.1±3.7 years) versus the older generation (30.3±5.7 years) (Rojas et al., 2016). Inadequate follow-up time bias is a well documented bias when comparing ages of onset across generations (Alonso-Magdalena et al., 2010; Rabinowitz and Yang, 1999; Romero-Pinel et al., 2010). We took measures to control for this by including in our secondary analysis only those individuals who were diagnosed before the age of 39 - the 75th percentile in our dataset (Alonso-Magdalena et al., 2010). Even with the adjustments, the age at onset difference remained statistically significant (30.0±7.9 years in the younger generation versus 36.4±11.9 years in the older generation, p=0.04), which supports the existence of the anticipation phenomenon within our study cohort (Jovanovic et al., 2024a).

To the best of our knowledge, no studies have been performed aimed at evaluating the risk factor profile of fMS other than analyzing it as a part of the broader MS patient cohort. Therefore we evaluated the potential risk factors for fMS by making comparisons with sMS and HC. This study had several sub-segments: we investigated different factors related to sun exposure and consumption of food in youth, which are factors related with vitamin D levels; explored the patients history of communicable and non-communicable diseases, as well as the family history of autoimmune diseases and cancer; assessed the patients' smoking habits, alongside their exposure to second-hand smoking; investigated their physical activity levels and body weight at different time periods; and finally, assessed different reproductive factors in female participants. The aim of this comprehensive overview was to evaluate in the context of fMS the most notable risk factors for MS such as EBV infection, smoking, low levels of vitamin D, and obesity in adolescence. Several notable observations have stemmed from our approach.

Vitamin D plays a crucial role in mineral balance, regulating cellular proliferation, differentiation, apoptosis, and immunity (Carmeliet, Dermauw and Bouillon, 2015; Maretzke et al., 2020). Humans obtain vitamin D in two primary ways: through sun exposure and from dietary sources (Chen et al., 2007). When skin is exposed to sunlight, specifically ultraviolet B (UVB) rays, it synthesizes vitamin D. The efficiency of this process depends on the amount of UVB radiation and the level of skin pigmentation. The key factors influencing the amount of UVB radiation reaching the skin is the solar zenith angle, which has been shown to vary with changes in latitude,

seasons, and time of day (Bener, 1969; Chen, 1998; Webb, Kline and Holick, 1988). Skin pigmentation also plays a role in the efficiency of the vitamin D synthesis process, with darker skin requiring longer exposure to synthesize the same amount of vitamin D as lighter skin (Chen et al., 2007).

Several studies have associated the deficiency of vitamin D with increased MS occurrence and relapse risk (Munger et al., 2004; Munger et al., 2006; Simpson et al., 2010). Additionally, it has been shown that higher amounts of sunlight exposure have protective effect on MS risk, which is traditionally linked to higher vitamin D levels in persons that are more exposed to sunlight (Alonso et al., 2011; van der Mei et al., 2001). However, findings of several studies, including a randomized controlled trial indicate that sun exposure is a risk factor for CNS demyelination independent of vitamin D levels (Hedström et al., 2020b; Lucas et al., 2011)

This can be explained by independent suppression of Th-1 immune response, stimulation of IL-10 secretion, and reduction of IL-17 secretion by vitamin D and UV radiation (Daniel et al., 2008; Ponsonby, Lucas and van der Mei, 2005). Sun exposure appears to affect MS risk both directly, and indirectly, by affecting vitamin D synthesis (Hedström et al., 2020b).

In our case-control study involving 131 fMS cases, 131 sMS cases and 131 HC, we investigated the factors related to skin phototype and sun exposure up to age 30 that could influence the cutaneous synthesis of vitamin D. These were: skin, eye and hair colour, tanning reaction, the amount of outdoor activities during winter, summer and weekends, the setting of professional activities (indoors vs. outdoors), UV lamp exposure, and the frequency of sun screen use. We found no association between any of these factors and fMS risk when compared to sMS. On the other hand, out of these factors, performing professional activities outdoors or equally indoors and outdoors was associated with decreased MS risk when compared with performing professional activities mostly indoors. This was true both when compared fMS with HC, and sMS with HC. Our findings can be viewed in the context that Serbia stands in a relatively small geographical area, with the majority of the study participants spending the longest period of life in the country. Therefore, the established effect of latitude on vitamin D synthesis and subsequent MS risk should not be so pronounced in this setting (Simpson et al., 2019).

Notably, we did find a protective effect of spending occupational activities partly or fully outdoors on MS risk. This finding is in line with the association between higher sun exposure leading to higher vitamin D levels, which have a protective effect on MS risk. The occupations most frequently reported by these persons were drivers, handymen, working as waiters. The association between occupational sun exposure and MS risk has been scarcely researched in other studies. Interestingly, the authors of a study performed in Denmark that did investigate this relationship have found that exposure to outdoor work actually increases MS risk (OR 1.94, 95% CI 1.06-3.55), although this finding has been based on only 12 MS cases with this exposure, and the authors have dismissed the finding as being etiologically negligible (Magyari et al., 2013). The lack of other associations regarding sun exposure and MS risk highlight the importance of dietary habits as the main driver of differences between vitamin D levels across a relatively small geographical area such as Serbia.

Fish liver, offal, and egg yolks have the highest concentration of vitamin D, while meat and dairy products are generally low in vitamin D, with butter being an exception (Schmid and Walther, 2013). Studies report that vitamin D deficiency is an increasing problem worldwide, and recommend supplementation due to the difficulty of achieving adequate intake through diet alone (Kučan et al., 2018; Benedik, 2021).

When analyzing food with low content of vitamin D in our study, we found no association between intake of different dairy products in teenage years and fMS and sMS risk. Similar findings were obtained in a recent study, albeit yogurt consumption was found to have a small protective

effect on MS risk (Dieu et al., 2022). The possible beneficial effects of yogurt consumption on MS risk could be explained by the probiotics present in yogurt potentially affecting the gut microbiota favorably (Chen et al., 2016). Similarly, after adjusting for potential confounders, we found no association between meat consumption and fMS or sMS risk. A study found non-processed red meat to reduce MS risk, even when controlling for vitamin D serum concentrations (Black et al., 2019a). There have been studies associating meat consumption with decreased MS risk (Black et al., 2019a; Black et al., 2019b). The fact that the questionnaire we utilized doesn't differentiate between different types of meat could be the drive behind the lack of association of MS risk with meat consumption found in our study.

Regarding food richer in vitamin D contents, we found that seafood consumption in teenage years was associated with decreased fMS risk compared with sMS (adjusted OR 0.50, 0.26-0.95), while fresh fish usage was shown to be a protective factor for fMS when compared with HC, and trout consumption was associated with decreased fMS and sMS risk when compared with HC. Cod liver oil usage aged 13-19 was found to be a protective factor for sMS when compared with HC but not for fMS. The finding of fresh fish, seafood, and cod liver oil associated with decrease of MS risk has been observed in several studies (Abdollahpour et al., 2021; Bäärnhielm, Olsson and Alfredsson, 2014; Black et al., 2020; Cortese et al., 2015; Hoare et al., 2016; Langer-Gould et al., 2020). Our finding of seafood usage in teenage years contributing to reduced fMS risk when compared with sMS, while intriguing, should be interpreted with caution, as the number of participants who have used seafood in any of the groups was low, seeing how seafood consumption is uncommon in Serbia, it being a continental country.

Interestingly, frequently using bottled water aged 13-19 has been associated with both increased fMS and sMS risk when compared with HC. To the best of our knowledge, there have been no studies reporting this association thus far. Regarding other neurological diseases, aluminum which can be found in bottled water has been reported to be associated with increased risk of dementia (Rondeau et al., 2008). This association is an intriguing finding, warranting further investigation in other study populations.

Perhaps the most notable finding of our study was that exclusive breastfeeding longer than 6 months in infanthood lowers risk for fMS in adulthood compared with sMS. Leading authorities such as the World Health Organization and UNICEF recommend infants to be exclusively breastfed for the first 6 months (World Health Organization, 2021). The implication of our finding is that it could be beneficial for mothers of infants with burden of MS in the family to prolong this period, seeing how we found that exclusive breastfeeding 7-9 months and longer than 10 months are both protective factors for fMS when compared with sMS.

Breastfeeding has been shown to reduce the risk of different autoimmune diseases (Diamanti, Capriati and Bizzarri, 2016; Patelarou et al., 2012; Vieira Borba, Sharif and Shoenfeld, 2017). This is also true for MS (Alkhawajah et al., 2021; Brenton et al., 2017; Conradi et al., 2013; Hedström et al., 2020a; Holz et al., 2022). However, our study was the first to investigate this association in fMS as an entity separate from sporadic cases (Jovanovic et al., 2024b). Authors of a multicentric study utilizing the same questionnaire have observed that breasfeeding for 4 months and longer has a protective effect on MS risk (Ragnedda et al., 2015). Notably, this effect seems to be population-specific, as well as sex-specific, with the beneficial effect noted among participants from Italy, but not from Norway, as well as the effect being present only in male population (Ragnedda et al., 2015). The findings of our study segregating fMS from sMS have also found the association only in the male population (Jovanovic et al., 2024b). Several mechanisms of the protective effect of breastfeeding on MS development have been proposed. They include its components interleukin 10, immunoglobulins, human milk oligosaccharides, which have immunomodulatory properties and a beneficial effect on the gut microbiota (Atarashi and Honda, 2011; Fernandez et al., 2013; Prioult, Pecquet, and Fliss, 2004).

Another interesting finding in the part of our study exploring factors related to breastfeeding is the increased risk of fMS compared with sMS in infants that have been fed using infant formula or cow's milk. Research indicates that the use of formula instead of breastfeeding may promote a Th1 cytokine response in infants due to the increased occurrence of acute infections. This effect likely arises because formula-fed infants do not receive secretory IgA antibodies, which are abundant in mother's milk (Goldman, 1993; Winkler et al., 2015). Furthermore, a structural similarity exists between bovine butyrophilin in cow's milk and the human myelin oligodendrocyte glycoprotein, a potential autoantigen implicated inMS. This similarity suggests immunological cross-reactivity between these proteins (Bronge et al., 2019). The distinct impact of these artificial foods in infanthood on fMS compared to sMS could possibly be related to genetic variants that predispose fMS to a more pronounced immune response when exposed to certain proteins found in cow's milk or formula. Further research is warranted to corroborate these findings.

In our case-control study, we analyzed the participant's history of different infections, allergies, autoimmune diseases, cancers, and kidney disease, as well as their close family members' history of autoimmune diseases, cancers and kidney disease in order to assess whether any of these characteristics have an implication in developing fMS or sMS. In our study, only history of mononucleosis proved to be a risk factor for both fMS (OR 2.69, 95% CI 1.01-7.18), and sMS (OR 3.11, 95% CI 1.18-8.15), when compared with HC, and family history of psoriasis was also a risk factor in the univariate analysis (OR 3.69, 95% CI 1.01-7.18) for fMS compared with HC. However, the association between family history of psoriasis and fMS risk was lost in the multivariate analysis (adjusted OR 3.41, 95% CI 0.99-11.72). On the other hand, seeing how mononucleosis is a well-known factor for development of MS, we didn't assess it in the multivariate analysis, but instead incorporated it in the multivariate logistic regression models when assessing other variables that were shown significant for confounders. Interestingly, a most recent Mendelian randomization study has shown that psoriasis reduces the risk of MS, and vice versa (Zhou et al., 2024). Findings of a recent meta-analysis indicated higher prevalence and incidence of psoriasis among PwMS (Liu et al., 2019). Whether our finding of increased burden of psoriasis among family members of fMS is incidental, remains to be elucidated in further studies.

Finding of a study performed in Kuwait indicate that presence of chronic conditions leads to increased MS risk (Al-Shammri et al., 2015). The most frequent comordbidities found in the Kuwait study among persons with MS were migraine, osteoporosis, hypothyroidism, and asthma, which were not present in the control population (Al-Shammri et al., 2015). On the other hand, similar findings to our own were found in a more recent study performed also in Kuwait, where the authors of a matched case-control study observed no difference between MS cases and controls regarding history of chicken pox, mumps and measles infections, history of inflammatory bowel disease, systemic lupus erythematosus, rheumatoid arthritis, and Graves' disease (El-Muzaini, Akhtar and Alroughani, 2020). Our previous analysis of the Belgrade population MS Registry has shown that the prevalence of autoimmune disorders among PwMS in the Belgrade region is 6.1%, while the prevalence of malignant diseases was 2.5% (Maric et al., 2021). Notably, the results of a large Swedish registry-based study show that the risk of MS is increased in persons with family members affected by other autoimmune diseases (Hemminki et al., 2009). Another study performed in the United States also showed that family history of autoimmune conditions is a modest risk factor for MS, while the same study showed no association with history of allergies (Alonso, Hernán and Ascherio, 2008).

The factor that singled out in our analysis as a risk factor for both fMS and sMS when compared with HC is a history of infectious mononucleosis. The importance of EBV infection, the causative agent of infectious mononucleosis in MS risk is well known, with recent studies further elucidating the magnitude of its role (Bjornevik et al., 2022). Findings of a most recent study indicate that ineffective control of EBNA-1-mediated autoimmune response is at the basis of MS pathogenesis (Vietzen et al., 2023). As the significance of EBV infection in MS get further

explored, the possibilities for prevention of MS via EBV vaccination indicate a promising future for reducing the incidence of MS (Zhong et al., 2022).

As part of the case-control studies we also explored smoking habits of the participants, and exposure to second-hand smoke. We found no association between any of the examined variables regarding smoking and the risk for fMS and sMS, except for living with a smoker aged 18-25 which was a risk factor for sMS compared with HC (OR 1.66, 95% CI 1.01-2.73). However, this association was lost after adjusting for confounders (adjusted OR 1.21, 95% CI 0.69-2.10). While this finding is unusual, given the well established link between smoking and MS risk, it could be explained by the unusually high prevalence of smokers in our HC group, which was 54.2%, which is significantly higher than the national smoking prevalence of 39% in Serbia (Global Burden of Disease Collaborative Network, 2021).

Data from epidemiological studies show that smoking is related both with increased MS risk and faster disease progression (Manouchehrinia et al., 2022; van der Mei et al., 2011). On the other hand, a prospective study showed that smoking during pregnancy was not associated with an increased risk of early-onset MS among offspring (Montgomery et al., 2008). It was also demonstrated that there was no association between smoking during pregnancy and the onset of MS in adulthood (Handel et al., 2010b). Smoking during pregnancy is closely linked to passive smoking exposure in childhood because it is assumed that mothers who smoked during pregnancy are likely to continue this habit during their children's childhood (Handel et al., 2010b). Our study showed that mothers were mostly non-smokers during pregnancy in all three groups, and there was no significant difference in passive smoking exposure by parents in the household during childhood between the groups. A population-based case-control study conducted in France examined the possible connection between MS and passive smoking, specifically whether passive exposure to tobacco smoke in childhood increased the risk of developing MS before the age of 16 (Mikaeloff et al., 2007). Researchers concluded that the risk of MS was higher in children of parents who smoked at home compared to children whose parents did not smoke. This study was the first to provide evidence of an increased risk of childhood MS due to passive exposure to parental smoking at home (Mikaeloff et al., 2007).

Our study also explored the relationship between the figure of the participants at ages 5-30 and fMS and sMS risk, as well as physical activity levels in teenage years with the fMS and sMS risk. After adjustment in the multivariate analysis, no factor investigated in this segment of the study was a statistically significant predictor of fMS or sMS risk. Using self-assessment of patients based on sketches of different body figures as an indirect way to assess BMI has limitations related to the participants' self-perception. This apparent during the data collection process where persons of similar waist sizes and BMIs had widely different self-perception of present body figure. This difference in subjective perception of present body image could have easily translated into differences in past body images. This is not unlikely, seeing how a study investigating the relationship between body size and MS risk performed in Italy and Norway using the same questionnaire has provided conflicting evidence (Wesnes et al., 2015). In the study, a positive association between larger body size, especially at age 25, and MS risk found in Norway, but not in Italy (Wesnes et al., 2015).

Obesity in adolescence is an established risk factor for MS (Alfredsson and Olsson, 2019; Munger, 2013; Schreiner and Genes, 2021). Authors of a recently conducted Mendelian randomization study investigating the relationship between BMI and MS risk have found that interleukin-6 signaling has an important role, and concluded that interleukin-6 and lower vitamin D levels explain about half of the association between obesity and MS (Vandebergh et al., 2022). A multicentric case-control study performed in Norway, Sweden, and Italy utilizing the same questionnaire as ours has found that vigorous physical activity in teen age reduces MS risk, while light physical activity has no influence on MS risk (Wesnes et al., 2018). Results of another recent

Mendelian randomization study with a large sample size have implicated that there is a causal link between moderate physical activity and decrease of MS risk (Li et al., 2022). This link could be due to immunomodulatory properties of physical activity and its effect on enhancing the release of interleukin-6 from muscles, but this link still remains to be elucidated (Barry et al., 2016; Krüger, Mooren and Pilat, 2016; Sharif et al., 2018).

The final segment of our case-control study investigating the risk factors for development of fMS was related to reproductive factors in women. We found a positive association between having two or more pregnancies and fMS and sMS risk compared with HC in the univariate analysis (OR 2.11, 95% CI 1.02-4.36), however this association was lost in the multivariate analysis both for fMS (OR 1.84, 95% CI 0.86-3.92) and for sMS (OR 1.70, 95% CI 0.79-3.68) when compared with HC. There was no association between other reproductive factors such as age at first period, age at first pregnancy, use of contraceptive pills, and hirsutism for either fMS or sMS risk in our study. Older age at menarche, older maternal age at first childbirth, and higher number of pregnancies were found to be protective factors for MS development, while oral contraceptive pills use was associated with a higher risk of MS (Mohammadbeigi, Kazemitabaee and Etemadifar, 2016; Salehi et al., 2018). Parenthood was found to be generally a protective factor in MS development, but there have been concerns of it being due to reverse causality (Nielsen et al., 2011). Further studies with larger power are necessary in order to demystify this link both in general MS, and in fMS subcohort.

Although we did find an earlier age of onset among fMS compared with sMS in the matched retrospective cohort study (29.9±9.3 vs. 31.3±11.0), this association was without statistical significance (p=0.548). This is likely only due to inadequate power of our matched cohort study, keeping in mind that findings of our population-based registry do indicate an earlier age of onset in the group of fMS compared with sMS. The participants in the retrospective cohort study were matched by MS phenotype, with 90% having relapsing MS and 10% having PPMS. Age at diagnosis (33.7±10.2 in fMS vs. 35.1 ± 11.5 years in sMS) was also earlier in fMS, but without statistical significance, while delay to diagnosis (3.7±5.7in fMS vs. 3.8±6.0 years in sMS) and disease duration were almost identical in the two cohorts (10.4±8.9 in fMS vs.10.5 ± 9.6 years in sMS). There was also no significant difference in disease progression between fMS and sMS cohorts measured either by MSSS or by progression index. Although matching by disease phenotype has its advantages in term of eliminating the effect of this factor on the prognosis, the downside is that it is not possible to test the effect of difference in familial and sporadic occurrence of MS on occurrence of disease phenotype. A larger study performed only in families with first degree relatives with MS has shown that familial occurrence of MS increases the risk of progressive disease course (Hensiek et al., 2007). However, no difference in prognosis was found in this study (Hensiek et al., 2007). Similarly, another study has found increased progression rate in fMS compared with sMS, and a slightly earlier age at disease onset, which was statistically significant due to the size of the sample (29.01 in fMS vs. 29.44 years in sMS, $p = 0.049$) (Wellek et al., 2011). On the other hand, a study performed in Spain has found no difference in between age at onset between fMS and sMS (Regal et al., 2018). Due to the nature of MS, establishing the exact age of onset can be difficult at times, since not all patients will seek medical help after the first attack of the disease, depending on its severity and simptomatology. Since awareness of familial risk in second and subsequent cases of MS in families leads to earlier diagnosis, using age at diagnosis is not an adequate solution. More sophisticated efforts are necessary to further discern the specific clinical profile of fMS.

The contribution of a person's genetic background to the MS risk cannot be overstated. Recent GWAS have identified 32 genetic variants in the *HLA* region and an additional 201 variants outside the *HLA* region to be linked with MS (International Multiple Sclerosis Genetics Consortium, 2019). These findings account for approximately 48% of the genetic predisposition towards MS, suggesting that a significant portion of genetic susceptibility is due to uncommon and

rare variants (MAF between 1-5% and less than 1%, respectively). Notably, a greater burden of rare risk variants has been observed in fMS patients as compared to sMS (Everest et al., 2022). Nine rare or uncommon gene variants predicted pathogenic with a CADD score exceeding 20 were found in our study. Out of these nine, we found exclusively in fMS five rare variants (all with a gnomADe allele frequency <1%): *CLEC16A* chr16:11126134_G/A, *ALPK2* chr18:58523972_G/T, *TYK2* chr19:10378250_C/T, *SLC9B1* chr4:102932166_T/C, and *WWOX* chr16:78115103_C/T. Some of these variants were previously implicated in MS, while some are a novel finding in our study.

*CLEC16A*, known to participate in neurodegeneration and autoimmunity, encodes a critical autophagy protein essential for the autolysosome, impacting Purkinje cell viability and suggesting a neuroprotective role (Pandey, Bakay and Hakonarson, 2023; Redmann et al., 2016). In MS, *CLEC16A* has been shown to influence antigen presentation and B-cell receptor-mediated antigen uptake, however its role in T-cell selection and reactivity still require further clarification (Eriksson et al., 2021; Rijvers et al., 2020).

*WWOX* is a regulator of cellular lipid homeostasis, and is localized mainly in the Golgi region, hinting at a role in myelogenesis (Iatan et al., 2014). Its expression is notably increased in microglia following inflammation in murine models, linking it to the inflammatory processes within the central nervous system (Aldaz and Hussain, 2020). Loss of function in *WWOX* has been connected to demyelination in rodents and to cortical thinning in MS patients (Aldaz and Hussain, 2020; Matsushita et al., 2015; Tochigi et al., 2019).

*TYK2*, a member of the Janus kinase family, is integral to cytokine signaling, impacting immune responses and hematopoiesis. It interacts with the IFNAR type I interferon receptor, influences interferon-β, interleukin-6, and interleukin-10 pathways, and affects T-cell polarization (Couturier et al., 2011). Observations suggest that reduced *TYK2* activity could promote a Th2 immune response, beneficial in MS where Th1 and Th17 responses are detrimental (Couturier et al., 2011). Moreover, interferon-β treatments, which are known to decrease MS activity, also reduce *TYK2* expression in CD8+ T cells, further underscoring its role in MS (Oliver-Martos et al., 2011).

On the other hand, *ALPK2* and *SLC9B1* have not been studied extensively in MS. *ALPK2* has been found to be highly transcribed in Th1 and Th17 cells in experimental autoimmune encephalitis, the murine disease model (Qian et al., 2021). Its variants are also prevalent in humans with multiple autoimmune syndrome (Arcos-Burgos et al., 2014). Although less is known about *SLC9B1*'s role in MS, variants have been identified in individuals with type 1 diabetes, migraine, and schizophrenia, suggesting broader implications (Haris et al., 2021).

The exclusive identification of rare genetic variants in fMS patients supports the notion of a specific genetic footprint integral to MS pathogenesis. This signature might vary by ethnicity, highlighting the importance of personalized medical strategies in MS research and treatment.

# 6. CONCLUSIONS

1. Among all 2765 PwMS in the registry, there were 178 registered cases of fMS (prevalence of 6.4%). The prevalence of fMS was similar between sexes: 6.5% in females, and 6.2% in males.

2. In comparison with sMS cases, fMS cases in the registry were on average younger (48.4±13.9 vs. 56.9±14.2 years), with significantly earlier age at onset (30.4±9.5 vs. 32.3±10.1 years), and shorter duration of the disease (18.3±11.9 vs. 24.6±12.3 years). Also, the median EDSS score was lower in fMS cases (2.5, range 1.0-6.0) in comparison with the sMS cases (4.0, range 2.0-6.5).

3. The highest prevalence among relations of persons with fMS found was in offspring (18.6%), parents (16.7%), and siblings (16.2%), and the lowest was reported in cousins (1.8%). After stratification of family members by sex, the highest prevalence of MS observed was in sisters (27.1%), mothers (22.9%), and daughters (21.7%) of fMS probands, while the lowest prevalence was found in grandfathers (1.0%).

4. The prevalence of fMS was higher in female relatives of all categories when compared with their male counterparts, with odds ratios ranging from 9.3 (sisters/brothers) to 1.6 (aunts/uncles).

5. After exploring vertical transmission of MS, we found an earlier age of symptom onset in the younger generation of fMS cases (35.7±11.6 in fMS vs. 25.8±7.2 years in sMS, $p<0.001$). This association remained significant after adjustment for the different follow-up length.

6. In the matched case-control study exploring risk factors for fMS, we found no factors regarding sun exposure in childhood and adolescence, associated with fMS risk when compared with sMS.

7. When compared to HC, more frequent outdoor activities during winter aged 16-25 were shown to be a protective factor for fMS, however, this association was lost after adjusting for possible confounding factors.

8. On the other hand, performing professional activities outdoors or equally outdoors and indoors aged 16-30 years was shown to be a protective factor for developing both fMS compared to HC, and sMS compared to HC, with ages 21-25 and 26-30 remaining statistically significant after adjustment for potential confounders in both comparisons (fMS vs. HC – adjusted OR 0.38, 95% CI 0.18-0.78 for aged 21-25, adjusted OR 0.33, 95% CI 0.15-0.69 for aged 26-30; sMS vs. HC – adjusted OR 0.47, 95% CI 0.24-0.92 for aged 21-25, adjusted OR 0.51, 95% CI 0.26-0.99 for aged 26-30).

9. Of the factors related to the consumption of food aged 13-19, seafood usage was found to be a protective factor for fMS when compared with sMS (adjusted OR 0.50, 95% CI 0.26-0.95), while fresh fish usage (adjusted OR 0.52, 95% CI 0.30-0.90) and trout usage (adjusted OR 0.57, 95% CI 0.33-0.95) were found to be protective factors for fMS when compared with HC. Trout was similarly found to be a protective factor for sMS when compared with HC (adjusted OR 0.56, 95% CI 0.32-0.99).

10. Frequently using bottled water aged 13-19 was shown to be a risk factor for both fMS (adjusted OR 4.95, 95% CI 1.77-13.88) and sMS (adjusted OR 3.40, 95% CI 1.12-10.31) when compared with HC.

11. Regarding breastfeeding practices, we found exclusive breastfeeding for 7-9 months (adjusted OR 0.47, 95% CI 0.28-0.80) and 10+ months (adjusted OR 0.48, 95% CI 0.29-0.81) to be protective factors for fMS when compared with sMS. On the other hand, use of cow's milk (adjusted OR 1.97, 95% CI 1.13-3.44) and infant formula (adjusted OR 2.07, 95% CI 1.07-4.02) in infanthood increased the risk of fMS when compared with sMS.

12. When compared with HC, we found that risk for both fMS (OR 2.69, 95% CI 1.01-7.18) and sMS (OR 3.11, 95% CI 1.18-8.15) is increased in case of positive history of mononucleosis. None of the other medical history data have shown to be associated with either fMS or sMS risk. Family history of psoriasis was associated with increased fMS risk when compared with HC, but this association was lost after adjustment for possible confounding variables.

13. Larger self-estimated figure aged 30 and number of pregnancies ≥2 were shown to be associated with increased risk of both fMS and sMS when compared to HC, however, these associations were lost after adjusting for potential confounders.

14. In the matched retrospective cohort study including 262 patients (131 fMS, and 131 sMS) we found no statistically significant differences in any of the clinical variables between the fMS and sMS cohort.

15. Although age at symptom onset was lower in fMS cohort compared with sMS, this difference did not reach statistical significance (29.9 ± 9.3 vs 31.3 ± 11.0 years, p= 0.548.)

16. In the case-control study investigating rare and uncommon gene variants in fMS, we identified 6 rare and three uncommon variants predicted pathogenic in the fMS group. Among these, 7 variants were missense mutations with a moderate predicted impact on gene function, and 2 were stop-gain mutations, estimated to have a high impact on gene function.

17. No statistically significant enrichment of these variants was found in the fMS group after FDR adjustment. However, five rare variants: *CLEC16A* chr16:11126134_G/A, *ALPK2* chr18:58523972_G/T, *TYK2* chr19:10378250_C/T, *SLC9B1* chr4:102932166_T/C, and *WWOX* chr16:78115103_C/T were exclusively found in the fMS patients.

18. Our overall findings suggest that there are certain differences between fMS and sMS; however, confirmatory studies are necessary to accurately determine their implications for prevention, treatment, and research of MS.

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***List of abbreviations:***

**95% CI** - 95% confidence intervals

**CADD** - Combined Annotation Dependent Depletion

**CIS** - Clinically isolated syndrome

**CMV** - cytomegalovirus

**CNS** - central nervous system

**CSF** - Cerebrospinal fluid

**DMT** - Disease modifying therapy

**DP** - read depth

**EBV** - Epstein-Barr virus

**EDSS** - Expanded Disability Status Scale

**FDR** - false discovery rate

**fMS** - familial multiple sclerosis

**GATK** - Genome Analysis Toolkit predictor

**GLM** - generalised linear model

**GQ** - call quality

**GWAS** - genome-wide association studies

**HC** - healthy control

**HHV-6** - human herpesvirus 6

**HIV** - human immunodeficiency virus

**HLA** - human leukocyte antigen

**IM** - infectious mononucleosis

**MAF** - minor allele frequency

**MHC** - major histocompatibility complex

**MRI** - magnetic resonance imaging

**MS** - Multiple sclerosis

**MSSS** - Multiple Sclerosis Severity Score

**OR** – odds ratio

**PPMS** - primary progressive multiple sclerosis

**PwMS** - persons with MS

**RIS** - radiologically isolated syndrome

**RRMS** - relapsing-remitting multiple sclerosis

**sMS** - multiple sclerosis

**SNP** - single nucleotide polymorphism

**SPMS** - secondary progressive multiple sclerosis

**UTR** - untranslated region

**UVB** - ultraviolet B

**VZV** - varicella zoster virus

**WES** - Whole exome sequencing

**Biography**

Dr. Aleksa Jovanović was born in 1992 in Belgrade. He graduated from the Faculty of Medicine at the University of Belgrade in 2017 with a grade point average of 9.66. He passed the board exam for medical doctors in 2018 and began his residency in epidemiology in 2021. He is employed at the Institute of Epidemiology, Faculty of Medicine, University of Belgrade as a teaching assistant in the field of epidemiology.

Dr. Jovanović is the project lead of "One Health or Many Health? A Multidisciplinary, Multicultural Approach to Global Health - VersaHealth," funded by Circle U. European University Alliance. He is also a member of the project of institutional funding of science at the Faculty of Medicine, University of Belgrade. He was a member of two Horizon 2020 projects: “Study of Monitoring Immunization Effects Against COVID-19 Diseases in Serbia” within the VACCELERATE clinical research network, and “TAXINOMISIS - A Multidisciplinary Approach for the Stratification of Patients with Carotid Artery Disease.” He was also a member of projects funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, the Ministry of Health of the Republic of Serbia, the Health Secretariat of the City of Belgrade, and AmCham Serbia.

He has completed several international training programs, including those by the Johns Hopkins Center for Communication Programs, UNICEF, MediPIET, FENS, TEMPUS program, and Circle U. European University Alliance.

Dr. Jovanović has conducted several seminars and workshops, including a workshop at the 7th Global Student’s Conference of Biomedical Sciences in 2021, a workshop at the 61st Congress of Biomedical Science Students of Serbia, and the VersaHealth workshop, which he also organized. He has mentored eight student papers presented at the Congress of Biomedical Science Students of Serbia with international participation, two of which were awarded the best paper in session (in 2022 and 2023). He was also a mentor in the IFMSA scientific exchange programs from 2019 to 2021.

He is the author and co-author of 19 papers published in journals on the JCR list, four of which he is the first author, as well as four papers in non-JCR journals and a chapter in the book "Adrenal Gland Surgery." He has presented his work at several international and national congresses, one of which has received the award for the best poster presentation at the XIII/XIX Congress of Neurologists of Serbia with international participation in 2023. Dr. Jovanović is a reviewer for eight international journals, seven of which are indexed on the JCR list. He is a member of the editorial board of the journal Medical Youth and a member of the Serbian Association of Neuroimmunologists.

**Biografija**

Dr Aleksa Jovanović rođen je 1992. godine u Beogradu. Diplomirao je na Medicinskom fakultetu Univerziteta u Beogradu 2017. godine sa prosečnom ocenom 9,66. Stručni ispit za doktora medicine položio je 2018. godine, a specijalizaciju iz epidemiologije upisao 2021. godine. Zaposlen je na Institutu za epidemiologiju Medicinskog fakulteta Univerziteta u Beogradu u zvanju asistenta za užu naučnu oblast epidemiologija.

Rukovodilac je projekta „*One Health or Many Health? A Multidisciplinary, Multicultural Approach to Global Health - VersaHealth*“ finansiranog u okviru *Circle U. European University Alliance*. Član je projekta institucionalnog finansiranja nauke na Medicinskom fakultetu Univerziteta u Beogradu. Bio je član dva *Horizon 2020* projekta: “*Study of monitoring immunization effects against COVID-19 diseases in Serbia*” u okviru kliničke istraživačke mreže *VACCELERATE* i “*TAXINOMISIS - A multidisciplinary approach for the stratification of patients with carotid artery disease*”, kao i projekata finansiranih od strane Ministarstva prosvete, nauke i tehnološkog razvoja Republike Srbije, Ministarstva zdravlja Republike Srbije, Sekretarijata za zdravstvo Grada Beograda i *AmCham Serbia*.

Završio je više međunarodnih edukacija, uključujući programe *Johns Hopkins Center for Communication Programs, UNICEF*-a, *MediPIET*-a, *FENS*-a, *TEMPUS* programa i *Circle U. European University Alliance*.

Dr Jovanović je održao više seminara i radionica, uključujući radionicu na *7th Global Student's Conference of Biomedical Sciences 2021*, radionicu na 61. Kongresu studenata biomedicinskih nauka Srbije i *VersaHealth* radionicu, koju je i organizovao. Bio je mentor 8 studentskih radova koji su prezentovani na Kongresima studenata biomedicinskih nauka Srbije sa međunarodnim učešćem, od kojih su dva nagrađena za najbolji rad na sesiji (2022. i 2023. godine). Takođe, bio je mentor na *IFMSA* programima naučne razmene u periodu 2019-2021.

Autor je i koautor 19 radova publikovanih u časopisima sa JCR liste, od kojih u četiri prvi autor, kao i četiri rada u časopisima van JCR liste i poglavlja u knjizi „Hirurgija nadbubrežne žlezde“. Svoje radove je prezentovao na više međunarodnih i nacionalnih kongresa, od kojih je jedan dobio nagradu za najbolju poster prezentaciju na XIII/XIX Kongresu neurologa Srbije sa međunarodnim učešćem 2023. godine. Recenzent je u 8 međunarodnih časopisa, od kojih je 7 indeksirano na SCI listi. Član je uredničkog odbora časopisa „Medicinski Podmladak“ i član udruženja Neuroimunologa Srbije.

**Publications resulting from the thesis**


1. Jovanovic, A., Pekmezovic, T., Mesaros, S., Novakovic, I., Peterlin, B., Veselinovic, N., Tamas, O., Ivanovic, J., Maric, G., Andabaka, M., Momcilovic, N., & Drulovic, J. (2024).Exclusive breastfeeding may be a protective factor in individuals with familial multiple sclerosis. A population registry-based case-control study. *Multiple sclerosis and related disorders*, *82*, 105392. https://doi.org/10.1016/j.msard.2023.105392

2. Jovanovic, A., Pekmezovic, T., Mesaros, S., Novakovic, I., Peterlin, B., Veselinovic, N., Tamas, O., Ivanovic, J., Maric, G., Andabaka, M., Momcilovic, N., & Drulovic, J. (2024).
   Earlier age of symptom onset in younger generation of familial cases of multiple sclerosis. *Neurological sciences: official journal of the Italian Neurological Society and ofthe Italian Society of Clinical Neurophysiology*, 10.1007/s10072-024-07512-w. Advance online publication. https://doi.org/10.1007/s10072-024-07512-w

3. Jovanovic, A., Pekmezovic, T., Mesaros, S., Novakovic, I. (2025). Significance of interactions between genetic and environmental factors in etiology of multiple sclerosis.*Medicinski podmladak*, 76, 3. DOI: 10.5937/mp76-46939

# Изјава о ауторству

Име и презиме аутора: Алекса Јовановић

Број индекса: 2017/5098

**Изјављујем**

да је докторска дисертација под насловом

**Investigation of the prevalence, epidemiological and clinical characteristics of familial occurrence of multiple sclerosis in the population of Belgrade**

- резултат сопственог истраживачког рада;
- да дисертација у целини ни у деловима није била предложена за стицање друге дипломе према студијским програмима других високошколских установа;
- да су резултати коректно наведени и
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**Потпис аутора**

У Београду, 30.04.2024.

А. Јовановић

**Изјава о истоветности штампане и електронске верзије докторског рада**

Име и презиме аутора: Алекса Јовановић

Број индекса: 2017/5098

Студијски програм: Епидемиологија

Наслов рада: Investigation of the prevalence, epidemiological and clinical characteristics of familial occurrence of multiple sclerosis in the population of Belgrade

Ментор: проф. др Татјана Пекмезовић

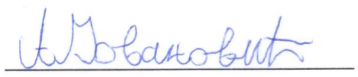
Изјављујем да је штампана верзија мог докторског рада истоветна електронској верзији коју сам предао/ла ради похрањивања у **Дигиталном репозиторијуму Универзитета у Београду.**

Дозвољавам да се објаве моји лични подаци везани за добијање академског назива доктора наука, као што су име и презиме, година и место рођења и датум одбране рада.

Ови лични подаци могу се објавити на мрежним страницама дигиталне библиотеке, у електронском каталогу и у публикацијама Универзитета у Београду.

**Потпис аутора**

У Београду, 30.04.2024.



# Изјава о коришћењу

Овлашћујем Универзитетску библиотеку „Светозар Марковић“ да у Дигитални репозиторијум Универзитета у Београду унесе моју докторску дисертацију под насловом:

**Investigation of the prevalence, epidemiological and clinical characteristics of familial occurrence of multiple sclerosis in the population of Belgrade**

која је моје ауторско дело.

Дисертацију са свим прилозима предао/ла сам у електронском формату погодном за трајно архивирање.

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