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THE ASSOCIATION BETWEEN NEFL GENETIC POLYMORPHISMS AMONG MULTIPLE SCLEROSIS PATIENTS

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
BIOLOGY

BY

ALYAA ADEEB SALLAMA SALLAMA

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THE ASSOCIATION BETWEEN NEFL GENETIC POLYMORPHISMS AMONG MULTIPLE SCLEROSIS PATIENTS

By Alyaa Adeeb Sallama SALLAMA

December 2023

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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Alyaa Adeeb Sallama SALLAMA

ABSTRACT

THE ASSOCIATION BETWEEN NEFL GENETIC POLYMORPHISMS AMONG MULTIPLE SCLEROSIS PATIENTS

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Master of Science in Biology

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December 2023

Multiple Sclerosis (MS) is a chronic and progressive neurodegenerative disease characterized by the demyelination of axons within the central nervous system (CNS), leading to a spectrum of neurological impairments. Predominantly affecting young adults, MS poses a significant burden on both patients and healthcare systems worldwide. The CNS, comprising the brain, cerebellum, brain stem, and spinal cord, serves as the epicenter of MS pathology, with multifocal lesions manifesting throughout these regions. The term "multiple" in MS reflects its propensity to affect various areas of the CNS, while "sclerosis" denotes the characteristic hardening of tissues in areas of damage. Understanding the genetic underpinnings of MS is crucial for elucidating its etiology and identifying potential therapeutic targets. In this context, this study aimed to investigate the relationship between genetic polymorphisms of neurofilament light (NEFL) and MS. Analysis of Apa I and Fok I polymorphisms yielded nonsignificant results, contrasting with prior findings. However, a significant difference was observed in Taq I polymorphism (p:0.025) between MS patients and control groups, highlighting a potential genetic susceptibility marker for MS development. This study serves as a preliminary exploration, paving the way for future investigations encompassing larger cohorts and additional parameters. Incorporating variables such as serum calcitriol levels, disease subtypes differentiation, bone densitometry, and body mass index correlations will enhance our understanding of the complex interplay between genetic predisposition, environmental factors, and MS pathogenesis. By expanding our knowledge base, these endeavors will facilitate the development of more targeted and

effective therapeutic strategies for individuals afflicted by this debilitating neurological condition.

2023, 53 pages

Keywords: NEFL, Genetic polymorphisms, SNPs, Autoimmune disease, PCR

ÖZET

MULTİPL SKLEROZ HASTALARINDA NEFL GENETİK POLİMORFİZMLERİ ARASINDAKİ İLİŞKİ

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Multipl Skleroz (MS), merkezi sinir sistemi (CNS) içindeki aksonların demiyelinizasyonu ile karakterize edilen ve çeşitli nörolojik bozukluklara yol açan kronik ve ilerleyici bir nörodejeneratif hastalıktır. Ağırlıklı olarak genç yetişkinleri etkileyen MS, dünya çapında hem hastalar hem de sağlık sistemleri üzerinde önemli bir yük oluşturmaktadır. Beyin, beyincik, beyin sapı ve omurilikten oluşan CNS, MS patolojisinin merkez üssü olarak hizmet eder ve bu bölgelerde çok odaklı lezyonlar ortaya çıkar. MS'teki "multipl" terimi MSS'nin çeşitli bölgelerini etkileme eğilimini yansıtırken, "skleroz" hasar alanlarındaki dokuların karakteristik sertleşmesini ifade eder. MS'in genetik temellerini anlamak, etiyolojisini aydınlatmak ve potansiyel tedavi hedeflerini belirlemek açısından çok önemlidir. Bu bağlamda bu çalışma, nörofilament ışığın (NEFL) genetik polimorfizmleri ile MS arasındaki ilişkiyi araştırmayı amaçlamıştır. Apa I ve Fok I polimorfizmlerinin analizi, önceki bulgularla çelişen, anlamlı olmayan sonuçlar verdi. Ancak MS hastaları ile kontrol grupları arasında Taq I polimorfizminde (p:0,025) anlamlı bir fark gözlendi; bu durum, MS gelişimi için potansiyel bir genetik yatkınlık belirtecinin altını çizdi. Bu çalışma, daha büyük kohortları ve ek parametreleri kapsayan gelecekteki araştırmaların önünü açan bir ön araştırma görevi görmektedir. Serum kalsitriol seviyeleri, hastalık alt tiplerinin farklılaşması, kemik dansitometrisi ve vücut kitle indeksi korelasyonları gibi değişkenlerin dahil edilmesi, genetik yatkınlık, çevresel faktörler ve MS patogenezi arasındaki karmaşık etkileşimi anlamamızı geliştirecektir. Bilgi tabanımızı genişleterek

bu çabalar, bu zayıflatıcı nörolojik durumdan etkilenen bireyler için daha hedefe yönelik ve etkili tedavi stratejilerinin geliştirilmesini kolaylaştıracaktır.

2023, 53 sayfa

Anahtar Kelimeler: NEFL, Genetik polimorfizmler, SNP'ler, Otoimmün hastalık, PCR

PREFACE AND ACKNOWLEDGEMENTS

I would like to thank my thesis advisor, Asst. Prof. Dr. Serdal TARHANE, for his patience, guidance and understanding.

Alyaa Adeeb Sallama SALLAMA

Çankırı-2023

CONTENTS

LIST OF SYMBOLS

Ca	Calsium
Cu	Copper
dL	Deciliter
/	Divide
=	Equal
g	Gram
≤	Greater or equal to
<	Greater than
kg	Kilogram
≥	Less or equal to
>	Less than
L	Liter
Mg	Magnesium
µg	Microgram
mIU	Milli-international units
mL	Milliliter
mmol	Millimole
-	Minus
min	Minute
mol	Mole
ng	Nanogram
nm	Nanometer
NS	Non-significant
%	Percent
+	Plus
±	Plus minus
rpm	Revolutions per minute
Se	Selenium
**	Significant
m^2	Square meter
Zn	Zinc

LIST OF ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
EBV	Ebstein barr virus
EOMS	Early onset or pediatric onset
GWAS	Genome-wide association study
HTLV	Human T lymphocyte virus
MARRS	Membrane-associated rapid response steroid-binding receptor
MS	Multiple Sclerosis
PCR	Polymerized chain reaction
PPMS	Primary progressive MS
PRMS	Progressive relapsing MS
SNPs	Single nucleotide polymorphisms
SPMS	Secondary progressive MS

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1. INTRODUCTION

Multiple Sclerosis could be a inveterate, dynamic, neurodegenerative malady that influences the central apprehensive framework (CNS) and advances with myelin damage and related neurological disarranges. It is the foremost common CNS infection of youthful grown-ups (Hauser and Oksenberg 2006). It is thought to influence 2-2.5 million individuals around the world. There are four clinical subtypes of MS: Relapsing-remitting MS (MS with assaults and reductions), essential dynamic MS (essential dynamic MS), dynamic backsliding MS (dynamic backsliding MS), and auxiliary dynamic MS (auxiliary dynamic MS) (Merkt *et al.* 2017).

Multiple Sclerosis (MS) may be a central apprehensive framework illness. The central anxious framework comprises of the brain, cerebellum, brain stem, and spinal line. The title of this malady comes from sclerosis since it is seen in numerous parts of the brain, that's , numerous, solidifying within the harmed tissues. Nerve cells within the central apprehensive framework create all motivations electrically. The myelin sheath encases and protects the nerve, whereas the axon is the extended arm of the nerve cell responsible for transmitting these impulses. Myelin not as it were ensures nerve cells, but too makes a difference cells carry out their capacities (Milo and Kahana 2010).

In MS patients, the axon and myelin layer can be harmed totally different parts of the brain and cannot transmit nerve driving forces appropriately. Depending on the harm to the anxious framework, issues may happen within the faculties, discourse, vision, adjust and strolling. In expansion to natural variables (climate, living zone, etc.) and past viral diseases, hereditary inclination moreover plays an imperative part within the arrangement of Different Sclerosis. MS Illness can moreover happen as a result of a combination of hereditary and natural causes (Hauser and Oksenberg 2006).

Multiple Sclerosis (MS) is generally seen in women, particularly between the ages of 20-40, but the reason for this distinction is obscure. Researchers are trying to figure out why the incidence of MS is three times greater in countries located north of the equator

compared to those located south of it. Some have hypothesized that vitamin D deficiency could be a risk factor for multiple sclerosis (MS), citing the lower amount of sunshine days in northern countries relative to tropical regions (Motl *et al.* 2008).

The specific cause of the condition is unknown, but there is mounting evidence from a growing body of research that points to a complex interplay between genetic predisposition and environmental variables. There is mounting evidence that the illness may have a hereditary component, as shown by the disease's upsurge in certain Northern European-descended ethnic groups and civilizations as well as in some families (Gregory *et al.* 2007). Numerous family and twin studies have been conducted and have shown the presence of a strong genetic component in the etiology. As a result of twin studies, it was found to be approximately 50% hereditary (Oksenberg *et al.* 2004).

As a result of the genome-wide association study (GWAS), which was conducted for the first time in 2007 to find the genetic cause of MS disease, genomic changes, single nucleotide polymorphisms (SNPs) that are thought to cause susceptibility in many gene regions in the human genome were defined (Solomon *et al.* 2021). Some changes in the IL7R gene are also among the conditions that are predicted to increase the risk of MS. In the next period, these changes should be sought in MS patients and their relationship with the disease should be revealed (Etemadifar *et al.* 2013).

1.1 Aim of Study

The aim of this study is to investigate the relationships between NEFL genetic polymorphisms in multiple sclerosis patients.

2. LITERATURE REVIEW

2.1 History

For thousands of years, all animals, plants and humans have survived by producing offspring through reproduction and undergo genetic selection. For most of this time, humans were unaware of how species are inherited. The offspring were similar to their parents but also had differences. After a long adventure, it was understood that the reason for this was that individuals contained many genes and different versions (alleles) of these genes were found. Later, it was learned that inheritance is not only as simple as Mendel described, but also that there are complex inheritance patterns (Albers *et al.* 2013).

The elucidation of the genetic basis of diseases began after all these developments and continued to progress rapidly. The genetic basis of many diseases has been revealed. However, there are still diseases whose cause cannot be found and whose genetic basis has not been revealed. One of them is Multiple Sclerosis (MS) disease (Cariaga-Martinez *et al.* 2016). To briefly touch on the relationship and history of genetics and MS.

2.1.1 History of genetics

Although the origin of genetics is based on the work of Gregor Mendel in the 19th century for the first time, many ideas about heredity have been put forward before that, and most of these assumptions have suggested that acquired traits are inherited (De Castro 2016). According to this belief, a person could only inherit dominant traits from the parent.

In 1866, modern genetics began when Gregor Mendel revealed the basic laws of heredity, dominant and recessive inheritance patterns, and the existence of genes (Kouchaki *et al.* 2016).

In 1902, Walter Sutton and Theodore Boveri, by staining the cells, noticed objects that were stained inside the cells. The existence of these bodies showed parallelism with the heredity agents put forward in Mendel's hypothesis. Later, these objects will be called 'chromosomes' (Satzinger 2008).

In 1905, Nettie Stevens found that homologous chromosomes and these chromosome pairs were the same size except for the X-Y sex chromosomes (Hoogendoorn *et al.* 2003).

In 1909, Thomas H. Morgan discovered that white eye color is transmitted by X-linked inheritance in Drosophila. Morgan succeeded in developing genetic mechanisms and linkage analysis to be applied to all diploid organisms with his research on flies (Lappalainen *et al.* 2011).

Beadle and Tatum revealed in 1941 that each gene encodes a product/protein (Buckland 2006).

In 1944, Oswald Avery defined DNA as genetic material (Cobb 2014).

In 1953, Rosalind Franklin and Maurice Wilkins showed that DNA is double-stranded, and later in 1979, Thomas Watson and Frances Crick defined the structure of base pairs and explained the replication mechanism (Liberda and Zajkowska 2017).

In the 1960s, Barbara McClintock discovered transposable elements (Gibbs 2020).

In 1970, Temin and Baltimore found the reverse transcriptase enzyme in retroviruses, and later this enzyme was used to clone genes from RNA (Baltimore 1970).

In 1977, Maxam, Gilbert and Sanger found the DNA sequencing method (Sanger *et al.* 1977).

The first transgenic mammal was produced in 1981 (Brinster *et al.* 1981).

In 1987, the polymerized chain reaction (PCR) technique, which can be called a breakthrough, was developed by Kary Mullis (Mullis and Faloona 1987).

In 1995, the first bacterial genome (Haemophilus influenzae) was fully sequenced (Mullis and Faloona 1987).

In 1996, Ian Wilmut succeeded in cloning a sheep named Dolly from an adult mammalian cell (Campbell *et al.* 1996).

The human genome project was completed in 2000 (Roest Crollius *et al.* 2000). Also this year massive parallel sequencing was discovered and the first step towards next-generation sequencing was taken.

An enhanced version of next-generation sequencing was released by Life Sciences in 2004.

2.1.2 History of MS

The history of MS disease dates back to the 1800s. Augustus d'Este (1794-1848) published a case on MS for the first time (Firth 1941). He followed the patient from the appearance of blurred vision in 1822 until his death in 1848 and noted all the findings with their dates.

Jean Martin Charcot, considered the father of neurology in 1868, studied a patient with tremor, speech disorder and nystagmus at the University of Paris, discovered 'plaques' in the white matter of the brain, which is a distinctive feature of MS, and described the disease in general terms for the first time. In the 1980s, high-dose methylprednisolone became available for treatment. Interferons were discovered in the 1950s and took their place in therapy in 1993 (Orrell 2005).

The higher incidence in some ethnic groups and the familial increase in some cases have cast doubt on the genetic component. As a result of the genome wide association study (GWAS) conducted by Oksenberg in 2004 and Gregory in 2007, two genes, HLA-DRB1 and IL7R, were found to be associated with disease susceptibility (Gregory *et al.* 2007, Oksenberg *et al.* 2004).

Four "more GWAS-MS studies were conducted in 2007 and 2009 and again in many genes, eg; CD6, CD25 (interleukin 2 receptor, alpha, IL2RA), CD40, CD58, CD226, C-type lectin domain family 16, member A (CLEC16a), ectopic viral integration site 5 (EVI5), glypican 5 (GPC5), regulator of It has been suggested that it may be related to G protein signaling 1 (RGS1), tyrosine kinase 2 (TYK2), tumor necrosis factor receptor superfamily member 1A (TNFRSF1A), and interferon regulatory factor 8 (IRF8) etc. (Oksenberg *et al.* 2004, IMSGC 2007, Baranzini *et al.* 2009)".

2.2 Definition

MS could be a illness that advances with harm to the brain and spinal rope, which are components of the central apprehensive framework (CNS). The resistant framework harms the myelin sheath that covers the nerve filaments and disturbs the communication between the brain and body organs (Figure 2.1). Damage to nerve fibers can be permanent. Signs and symptoms are directly proportional to the amount of neuron damage and closely related to which nerve is affected. There is no cure for MS. The treatments used consist of changing the course of the disease and trying to reduce the attacks (Merkt *et al.* 2017).

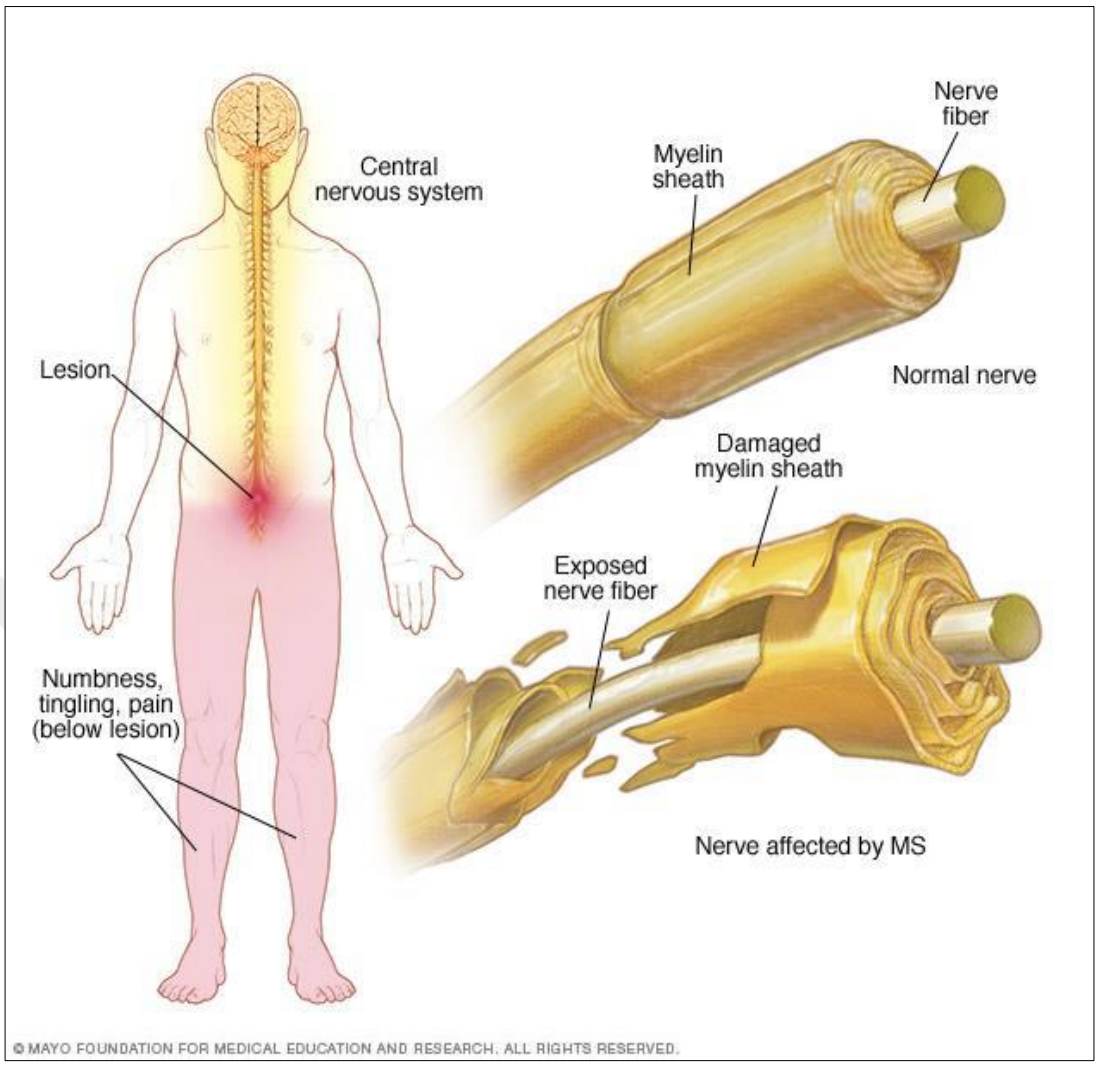

Figure 2.1 Neuron involvement and myelin damage in MS (Merkt *et al.* 2017)

2.3 Clinical Findings

The peak age of onset of the disease is around 20-40 years (Naseri *et al.* 2021, Kurtzke *et al.* 1992). It may occur more rarely in children (Ghezzi *et al.* 1997). MS can also occur after the age of 60 (Martinelli *et al.* 2004).

In the first studies, it was found that women were diagnosed with MS about twice as often as men (Hernandez-Pedro *et al.* 2016, Wallin *et al.* 2004). Recent studies have shown that the incidence of MS tends to increase, being more pronounced in women (Debouverie 2009, Orton *et al.* 2006, Ramagopalan *et al.* 2009).

Figure 2.2 shows the most prevalent clinical signs and symptoms. Forty percent of patients have a loss of feeling in their extremities. Fifteen percent have partial or total loss of vision. Thirteen percent have acute or chronic motor dysfunction in their limbs. Seven percent have diplopia. Five percent have gait disruption (Merkt *et al.* 2017). A clinical attack is defined as the presence of any of the following symptoms for at least 24 hours, which might manifest alone or in combination. It is possible to hold any anatomical posture, which varies greatly among multiple sclerosis patients (Merkt *et al.* 2017).

The course of the disease may be relapsed-remission or progressive, mild or severe, may involve the entire neuraxis, or only the spinal cord and optic nerve may be affected. The reason why the course of the disease is so variable is unknown. While individuals are stable for months or years, they may have an attack at a time. As of today, there is no biological marker to monitor the course of the disease and to be used in follow-up. Even within families, the clinic can vary considerably between individuals (Dyment *et al.* 2002).

The EDSS, which methodologically demonstrates the buildup of neurological impairment, is used to evaluate clinical progression (Kurtzke 1983). In a cohort study, more than 1000 MS patients were followed for 25 years and a great deal of clinical data was obtained (Weinshenker 1998, Weinshenker *et al.* 1989).

The collected data demonstrated that impairment escalated with the passage of time during the illness. Similar results were obtained in other cohorts. Recently, new scoring systems have also emerged. Multiple sclerosis functional composite (MSFC) and multiple sclerosis severity score (MSSS) are two ways to measure how far along the disease's course a patient is (Roxburgh *et al.* 2005, Cohen *et al.* 2001).

Multiple sclerosis (MS) was categorized into three age groups: early onset (EOMS) occurring before the age of 18, adult onset (AOMS) occurring between the ages of 18 and 50, and late onset MS (LOMS) occurring between the ages of 18 and 50 (McDonald

et al. 2001, Polliack *et al.* 2001, Pohl *et al.* 2010). In addition, there are 4 clinical subtypes of MS.

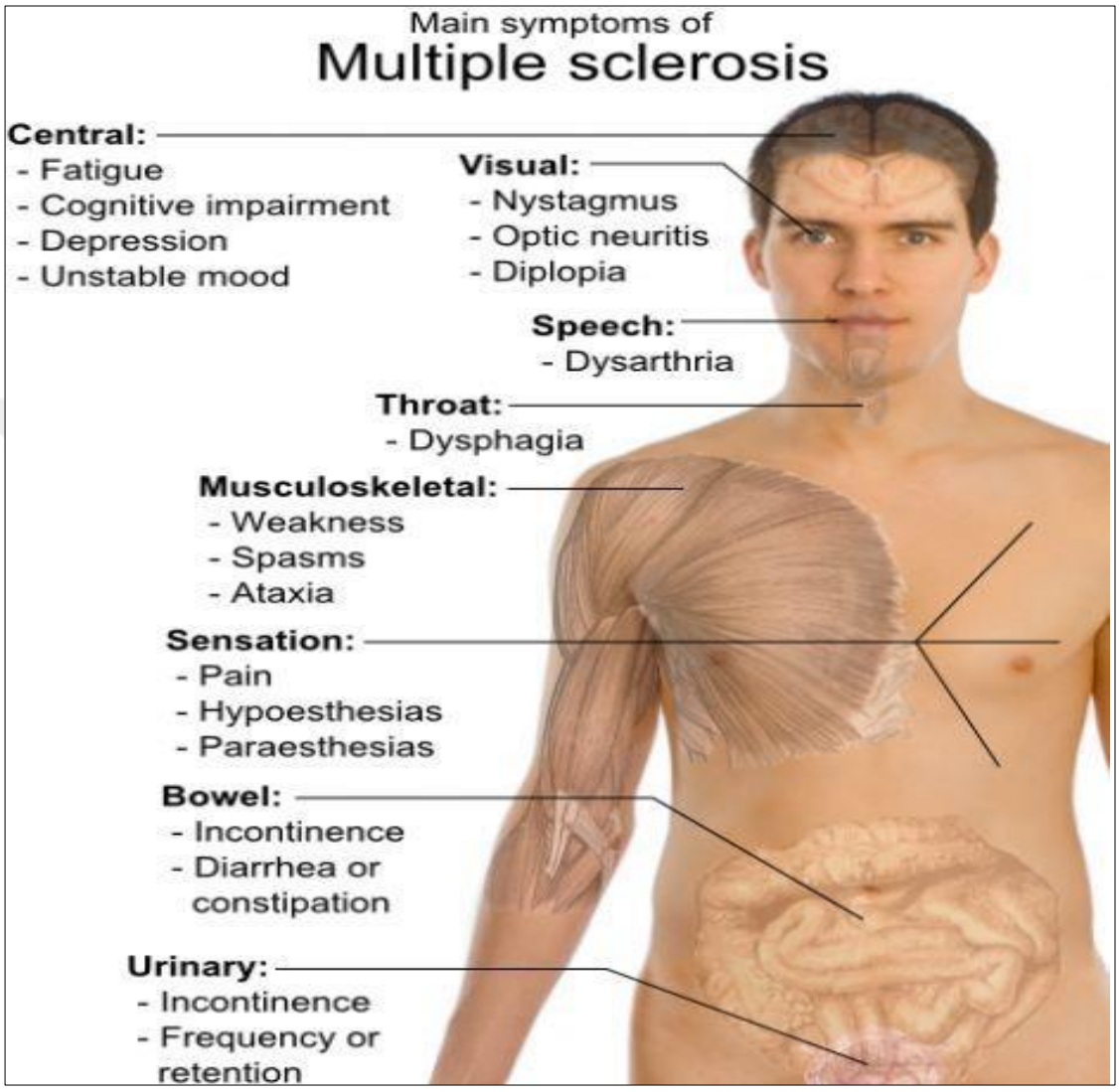

Figure 2.2 MS major clinical findings (Merkt *et al.* 2017)

2.4 Clinical Subtypes

2.4.1 Relapsing remitting MS (RRMS)

Eighty percent of the patients are in this group, and it progresses in the form of exacerbation of neurological symptoms and complete or partial remission in the following period (Lublin and Reingold 1997). About 10 years after disease onset,

almost 50% of RRMS patients progress to progressive MS. In progressive MS, there is no clinical attack and remission, but there is a progression of symptoms (Lublin and Reingold 1997, Confavreux *et al.* 1980).

2.4.2 Primary progressive MS (PPMS)

About 10-20% of the patients are in this group, and the disease progresses progressively from the onset and there are no episodes of remission (Lublin and Reingold 1997).

Their demographic characteristics differ markedly from other MS subgroups:

1. The onset of the disease is typically around the age of 40, and with this feature, it has a significant difference from RRMS (Weinshenker *et al.* 1989, Confavreux *et al.* 1980, Andersson *et al.* 1999, Bashir and Whitaker 1999).
2. Unlike RRMS, PPMS does not discriminate by gender (Weinshenker *et al.* 1989).

2.4.3 Progressive relapsing MS (PRMS)

This group of patients, which is a very rare form, initially starts as PPMS and experiences neurological exacerbations during the course of the disease (Tullman *et al.* 2004). Patients in the SPMS group whose clinical exacerbations are incomplete remission can also be mentioned in this group (Lublin *et al.* 1997).

2.4.4 Secondary progressive MS (SPMS)

This subset relates to 1 RRMS patients who never fully recover from attacks and 2) PPMS patients who suffer from acute exacerbations, while there is no agreement among specialists in this field (Lublin and Reingold 1996).

In industrialized nations, the life expectancy of people with multiple sclerosis is comparable to that of the general public (Sadovnick *et al.* 1992). A Danish nation-based

prognostic analysis found a ten- to twelve-year reduction in life expectancy relative to the whole population (Brønnum-Hansen *et al.* 2004).

2.5 Histopathology

The histopathological results (Figure 2.3) show that the plaques have lost some of their myelin sheath, which has disrupted the ion channels and caused the action potential in the demyelinated portion of the axon to elongate abnormally. Demyelinated lesions were shown to cause substantial axonal damage in the early investigations. Additional research has shown that axonal damage occurs even in the earliest phases of the illness, and that neurological diseases result from the buildup of this damage (Ferguson *et al.* 1997, Van Waesberghe *et al.* 1999). Research in histopathology has shown an abundance of damaged, dystrophic axons in areas where inflammation and demyelination are currently taking place (Trapp and Nave 2008). Cortical atrophy may develop as a result f axonal loss in multiple sclerosis (Figure 2.4) (Merkt *et al.* 2017).

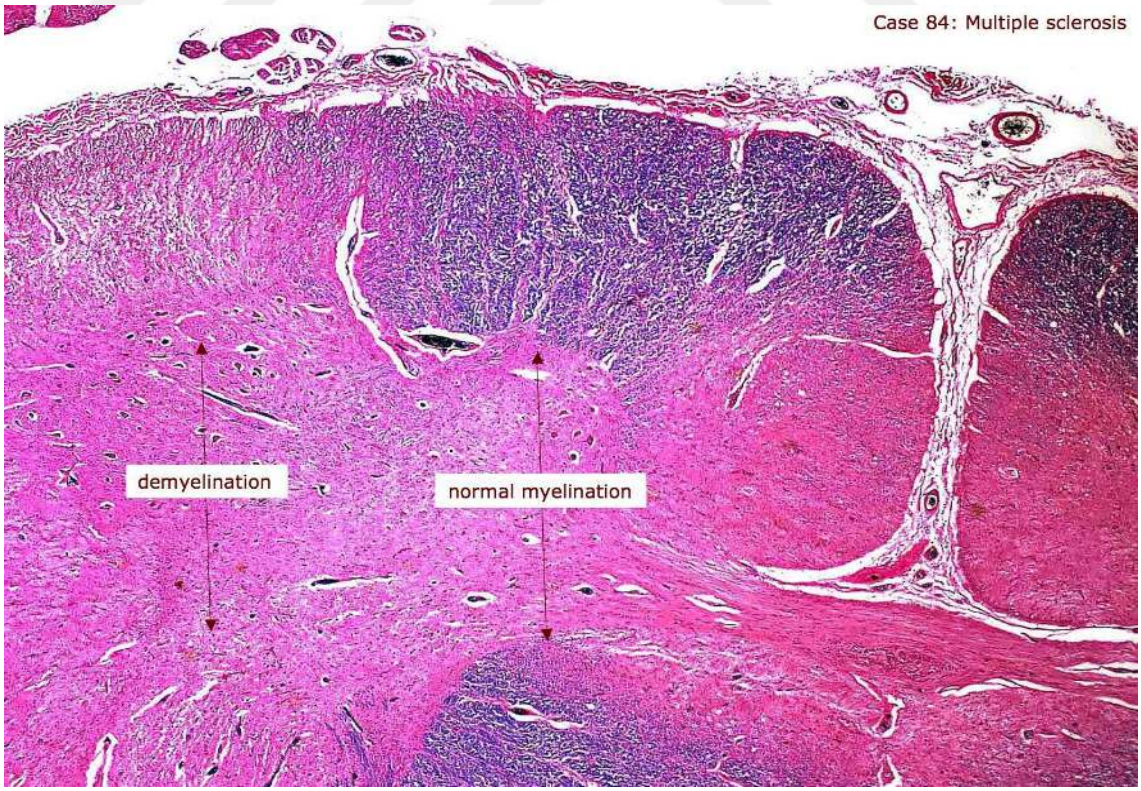

Figure 2.3 MS histopathology, brain biopsy material (Merkt *et al.* 2017)

A group of researchers determined 4 different histopathological patterns in MS biopsy and autopsy materials (Lucchinetti *et al.* 1998). All of these were detected in RRMS patients, but only one was seen in PPMS patients. Despite the fact that this question has sparked discussions about the genuine and pathogenic diversity among MS patients, a more recent histopathological study has shown that MS damage are remarkably consistent and marked by the invasion of T and B cells (Breij *et al.* 2008). Future considers will be more illuminating on whether there's without a doubt histopathological heterogeneity.

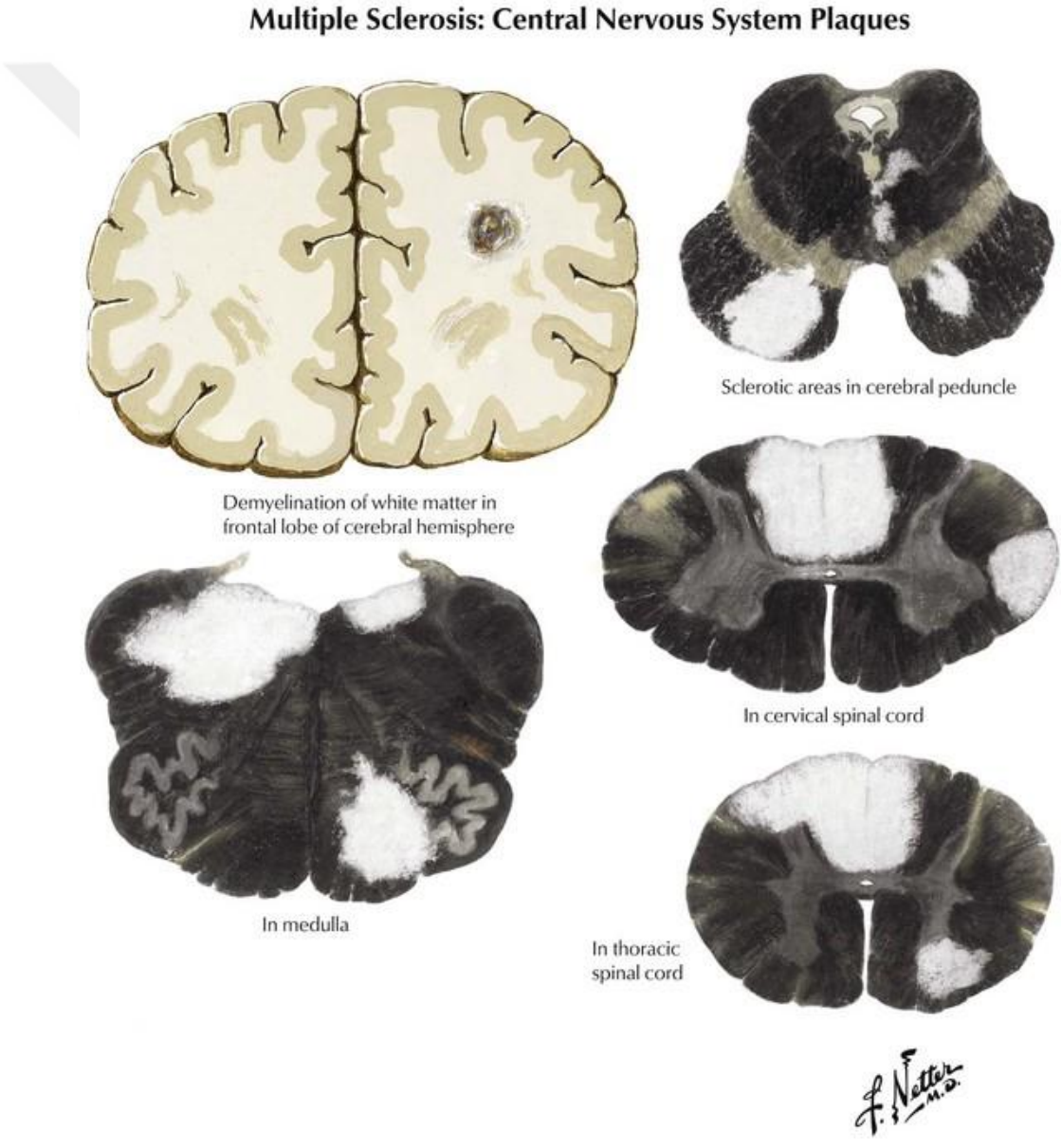

Figure 2.4 MS brain pathology (Merkt *et al.* 2017)

2.6 Diagnosis

MS is a clinical diagnosis. The 2010 revised Mc Donald Diagnostic Criteria (Polman *et al.* 2011) are used for diagnosis.

2.6.1 2010 McDonald Criteria

Distribution in space (At least 1 T2 bright lesion in at least two of their localizations)

- Periventricular.
- Juxtacortical.
- Infratentorial.
- Spinal cord.

Distribution in time

a. A new lesion with T2 and/or enhancement on follow-up MRI.
b. Simultaneous asymptomatic enhancing and non-enhancing lesions at any time.

According to the 2010 revision of the Mc Donald Criteria, the subgroups were basically renewed as follows:

1.6.2 RRMS

The diagnosis is mostly made according to the spread of the disease over time and its involvement in the body:

1. Time span: Minimum of two 24-hour clinical episodes spaced at least one month apart, or six months of escalating, slow-onset symptoms.
2. Involvement: Presence of lesions in more than one area in the white matter of the brain or spinal cord or in the functional system

The MRI technique (Figure 2.5) has been added, which greatly simplifies the diagnosis and approach with the revised criteria (Polman *et al.* 2011).

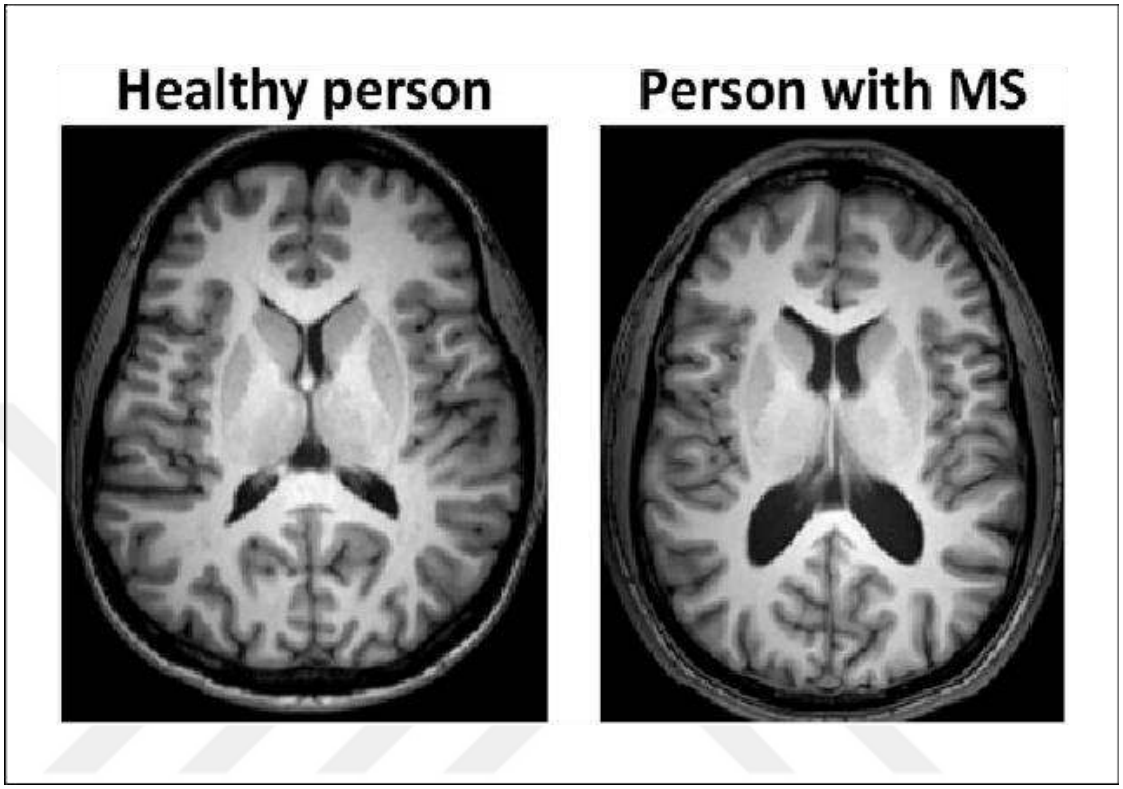

Figure 2.5 Brain MRI images of healthy individuals and MS patients (Polman *et al.* 2011)

2.6.3 PPMS

There is no internationally accepted diagnostic criterion. But the following diagnostic criteria are used by specialists:

1. **Significant PPMS:** individuals with exact clinical and laboratory findings
2. **Possible PPMS:** Individuals with strong clinical suspicion but no conclusive evidence
3. **Possible PPMS:** limited bends.
4. Intrathecal IgG production
5. One of the following MRI findings:

a. 9 Brain lesions
b. 2 Spinal cord lesions
c. 4-8 Brain lesions and 1 spinal cord lesion

2.7 Differential Diagnosis

Since the clinic is heterogeneous, the differential diagnosis is as follows:

2.7.1 Inflammatory and infectious diseases

1. Systemic lupus erythematosus.
2. Sjogren's syndrome.
3. Behcet's disease.
4. Sarcoidosis.
5. Lyme disease.
6. Antiphospholipid antibody syndrome.
7. Neurosyphilis.
8. Acquired immunodeficiency syndrome (AIDS).
9. Whipple's disease.
10. Human T lymphocyte virus (HTLV) associated myelopathy.

2.7.2 Cerebrovascular diseases

1. Recurrent stroke attacks.
2. CADASIL (leukoencephalopathy, subcortical infarcts, and cerebral autosomal dominant arteriopathy).
3. Vascular malformations.
4. Moyamoya disease.

2.7.3 Other

1. Cerebral lymphoma.
2. Mitochondrial diseases.
3. Funicular myelopathy.
4. Adrenoleukodystrophy – adult onset type.
5. Other adult-onset genetic leukodystrophy (Van Der Knaap *et al.* 1999).

2.8 Prevalence

Between 1.1 and 2.5 million people are thought to have multiple sclerosis (MS) globally (Pugliatti *et al.* 2002, Oraby *et al.* 2019).

When the topographical dispersion of MS cases analyzed by the clinician (Figure 2.6) is inspected, it is seen that they are not equitably conveyed. The most elevated recurrence is watched between 40-60 degrees south scope. Subsequently, geological locales with a tall predominance of 50-120/100.000:

1. Western Europe (Koch-Henriksen *et al.* 2015).
2. Northern Europe
3. Canada (Warren and Warren 1993).
4. Russia (Boiko *et al.* 1995).
5. Israel (Alter *et al.* 1978).
6. Part of northern United States, New Zealand.
7. South-East Australia (Barnett *et al.* 2003).

Very low frequency regions with a frequency of 5/100,000:

1. Asia (Bian and Xin 1992, Araki *et al.* 1987)
2. Sub-Saharan Africa

3. South America (Callegaro *et al.* 2001). Information on the prevalence increase in this region continues to come (Merkt *et al.* 2017).

There have been reports of rises in prevalence in several of the locations indicated above in the previous few decades. Either a genuine uptick or improved assessment might be behind this (Merkt *et al.* 2017).

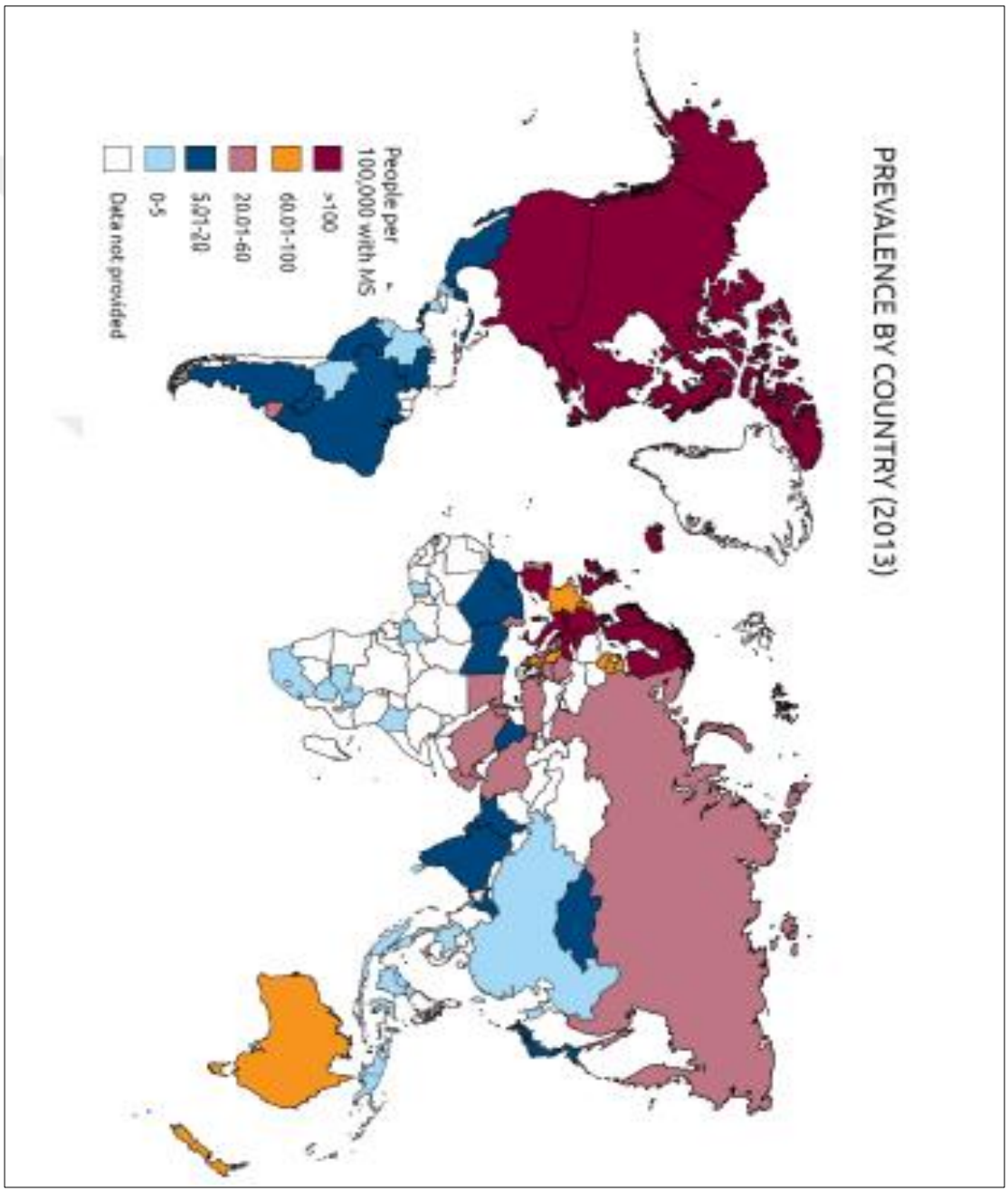

Figure 2.6 Geographical distribution and prevalence of MS disease (Merkt *et al.* 2017)

2.9 Reasons

The causes of MS disease are explained under the following headings. In addition, the reasons are summarized schematically in Figure 2.8.

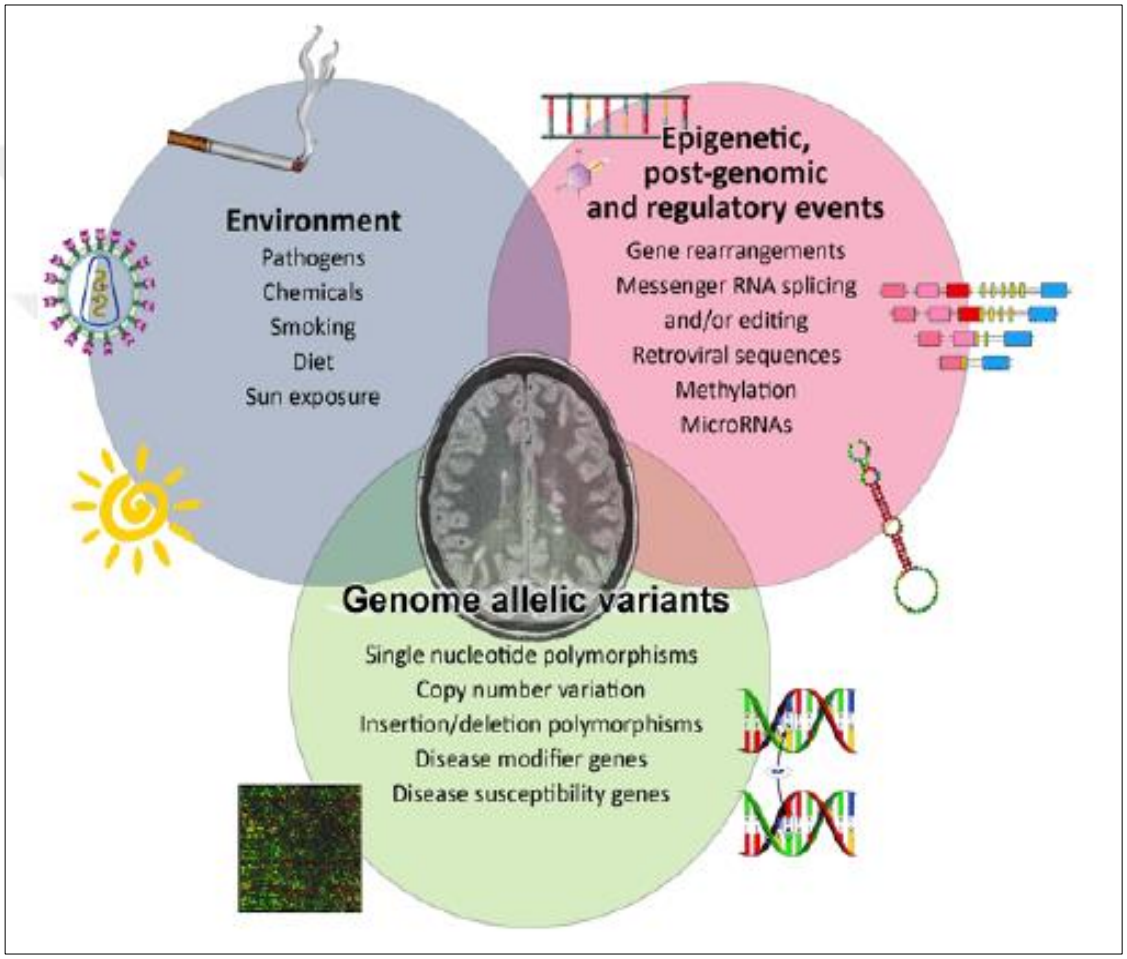

Figure 2.7 Causes of MS disease (Merkt *et al.* 2017)

2.9.1 Environmental reasons

Studies with epidemiological methods have shown that infectious pathogens and environmental factors play a role in MS disease. Environmental variables are implicated in the genesis of multiple sclerosis (MS) according to at least four criteria:

1. Geographical.
2. Seasonal (Koziol and Feng 2004).
3. Socioeconomic variations.
4. Migration studies (Alter *et al.* 1978).

Further evidence that non-genomic variables are at play is the low concordance rate of around 30% among monozygotic twins of index cases. But discordance may be associated with epigenetics or scholastics.

There is still no known cause for multiple sclerosis (MS), despite the availability of sensitive and cutting-edge diagnostic tools. It has not been considered realistic for an agent thought to be the cause to explain all cases. However, recently, strong evidence has been found regarding the relationship between Ebstein Barr Virus (EBV) (Ascherio and Munger 2007a) and Vitamin D (Ascherio and Munger 2007b) and MS.

2.9.2 Hereditary causes

MS is a disease that belongs to the group called complex genetic diseases and shows significant hereditary risks and numerous gene-environment interactions.

Familial aggregation of cases suggesting a genetic contribution to MS disease and a higher incidence in some ethnic groups (Northern European origins) than others (African, Asian origins) in relation to their origin, regardless of geographic distribution. After being identified as potential genes via investigations, two genes—HLA-DRB1 and IL7R (CD127)—were definitely linked to the condition (Gregory *et al.* 2007, Oksenberg *et al.* 2004).

Use of the GWAS technique in multiple sclerosis has distinguished additional disease-related susceptibility genes and loci, demonstrating the power of this approach (GWAS) in detecting important genetic factors contributing to this complex neurological disease. High-resolution genotyping techniques have led to significant advances in this area.

There have been four published MS-GWAS investigations so far (IMSGC 2007, BCCJRW 2007, DA 2009, Baranzini *et al.* 2009).

The GWAS investigations yielded significant evidence linking around a dozen novel loci to illness vulnerability, and these persisted with extensive meta-analyses and further research. These detected loci can be listed as follows: CD6, CD25 (interleukin 2 receptor alpha, IL2RA), CD40, CD58, CD226, C-type lectin domain family 16, member A (CLEC16a), ectopic viral integration site 5 (EVI5), glypican 5 (GPC5), G protein signal 1 regulator (RGS1), tyrosine kinase 2 (TYR2), tum ɔr necrosis receptor superfamily member 1A (TNFRSF1A) and interferon regulator factor 8 (IRF8)".

Previous research has linked certain MS-associated allelic variations to other autoimmune disorders. An underlying cause of several autoimmune disorders, such as IL2RA, has been linked to multiple sclerosis (MS), type 1 diabetes mellitus (T1DM), Grave's disease (GD), and rheumatoid arthritis (RA) (Baranzini *et al.* 2009). There may be shared causes between multiple sclerosis and other autoimmune disorders due to the nature of the disease.

Layer metallo-endopeptidase-like 1 (MMEL1), protamine 1 (PRM1), ribosomal protein L5 (RPL5), kinesin family part 21B (KIF21B), METTL1, and other ponders are other prospective features having good verify listens.

As the list of risk loci expands in this way, MS genetics remains elusive. HLA-DRB1 can explain about 7% of cases, while other genes can explain up to 3% in total. A GWAS study conducted on a high SNP density platform with 10,000 cases achieved sufficient power with an odds ratio of 1.2 or higher and will be the method to be used in MS genetics in the future (Oksenberg *et al.* 2008).

Epigenetic events and alterations in copy number have yet to have their full impact assessed (Coyle *et al.* 2014).

Genomic polymorphisms impact disease severity or phenotype in addition to propensity, as shown by the prevalence of concordance in families about certain early and late clinical findings. Although it is difficult to show this clearly, phenotypic differences indicate a true etiological heterogeneity or modifier effects of specific genes or combinations of both (Oleen-Burkey *et al.* 2011).

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Subjects and ethics statement

The files of 618 multiple sclerosis patients were analyzed. It was determined that 25 patients out of 618 patient files examined showed familial MS features. The phone numbers of these patients who met the study criteria were reached and information about the study was given.

Families and family members participating in the study were numbered. In total, 25 families were numbered; each family had at least 2, at most 3 MS patients. Blood samples were taken from all patients and from their parents, and/or siblings and relatives of MS patients in the family.

Blood samples could not be taken from the patients and their relatives who could not be reached for various reasons (the phone number could not be reached, being dead, being abroad, etc.). 29 patients with familial MS could be included in the study.

29 multiple sclerosis patients and 120 healthy individuals were included in our study. Included individuals were grouped as patient and control, female patient and female control, male patient and male control.

3.1.2 Inclusion criteria of patients in the study

1. Being between 18-60 years old.
2. Having a relative with multiple sclerosis in the family history.
3. Not being pregnant.
4. It was determined that there was no other systemic disease.

5. Healthy volunteers aged 18-60 years, non-pregnant and without any other systemic disease were included in the study as the control group.

3.1.3 Exclusion criteria

1. MS patients without a family history of multiple sclerosis,
2. Being under the age of 18, over the age of 60
3. It was determined as being pregnant.

Participants' ages, genders, and health conditions were deemed insufficient to include them in the research. Our work was carried out in 4 stages;

3.1.4 Collection of blood samples

For work; MS patients with a family history of 1 and/or more individuals with MS diagnosis, parents and/or siblings of the patients who can be reached; As the control group, 2 cc venous blood samples from healthy volunteers with the above-mentioned criteria were taken into EDTA tubes.

After the blood samples were collected, DNA isolation from the blood samples, measurement of DNA concentration and genotyping were performed.

In total, blood was drawn from 29 MS patients and 120 healthy volunteers. Samples that were able to obtain sufficient DNA concentration for each polymorphism and were responsive to genotyping were analyzed statistically.

3.2 Methods

3.2.1 DNA isolation

DNA isolation was performed using Purelink Genomic DNA kit on 200 µL blood sample. The kit was worked with the following method;

1. Blood lysate

a. It was 55 degrees in the water bath.
b. A sterile microcentrifuge tube was used to hold 200 µL of blood sample.
c. Add 20 microliters of proteinase K. 40 microliters of RNase A was included.
d. Vortexing was done for a short period of time.
e. Two minutes were spent letting it cool to room temperature.
f. Purelink Genomic Lysis/Binding Buffer, 200 µL, was added.
g. To get a uniform mixture, it was vortexed.
h. The proteins were broken down by letting it marinate at 55 degrees for 10 minutes.
i. 220 microliters of ethanol (96-100% concentration) was introduced.
j. For 5 seconds, it was vortexed to ensure it was uniform. Binding of DNA: The collecting tube was filled with columns.
k. The 640 µL blood lysate that had been produced was then transferred to the spin column.
l. To spin it at normal temperature for one minute, we used a centrifuge with a 10,000 x g force.
m. Eliminated collection tubes.
n. After cleaning the collecting tube, the spin column was set in it.

2. Washing of DNA

a. 500 microliters of ethanol-containing Wash Buffer 1 was introduced to the spin column.
b. At room temperature, it was spun in a centrifuge at 10,000 x g for one minute.
c. After disposing of the old collecting tubes, a new, clean one was used to house the spin column.

d. 500 microliters of ethanol-containing Wash Buffer 2 was introduced to the spin column.
e. For three minutes at room temperature, the column was spun at maximum speed in a centrifuge.
f. Disposal of collecting tubes.

3. Removal of DNA

a. The spin column was placed in a sterile 1.5 mL microcentrifuge tube.
b. 25-200 µL Purelink Genomic Elution Buffer was added to the column.
c. Incubated for 1 minute at room temperature.
d. The column was centrifuged at maximum speed for 1 minute at room temperature.
e. In this way, genomic DNA was isolated in the tube.
f. The same column was placed in a clean 1.5 mL microcentrifuge tube to obtain more DNA.
g. 25-200 µl of GenomicElution Buffer was added to the column.
h. It was centrifuged at room temperature at maximum speed for 1.5 minutes.

4. Storage of DNA

a. Stored at +4 degrees or divided into small volumes at -20 degrees.

3.2.2 Measuring DNA concentration

Qubit dsDNA BR (Broad-Range) Assay Kits were used for the measurement of DNA concentration.

The operating method of the kit is as follows:

1. 0.5 µL samples and standards were placed in the tubes.
2. Tube caps are labeled. Qubit working solution was prepared by diluting Qubit dsDNA BR Reagent 1:200 in Qubit dsDNA BR Buffer.

3. Clean plastic tubes were used each time in the preparation of the Qubit working solution. Glass tubes were not used.
4. A volume of at least 200 µL was obtained in each tube.
5. 190 µL of Qubit working solution was added to each tube used for the standard.
6. 10 µL Qubit standard was added to the appropriate tubes.
7. Vortexing was done for 2-3 seconds.
8. Qubit working solution was added to each sample tube and a volume of 200 µL was obtained.
9. It was incubated for 2 minutes at room temperature. DNA concentrations were measured with a Qubit device.

3.2.3 Genotyping

The following mixture was used for genotyping as shown in Table 3.1.

Table 3.1 Mixture Table Used for Genotyping

2x Master mix	8 µL
20x Assay mix	2 µL
Sd H_2O	6 µL
DNA	4 µL
Total Reaction Volume	**20 µL**

The pipetted mixture was loaded on the step one plus real time device and the genotyping reaction was carried out. The PCR profile is as follows:

1. 8 minutes at 95 degrees.
2. 0-30 sec at 95 degrees, 35 cycles.
3. It was run for 1 minute, 35 cycles at 60 degrees.

The results were taken from the step-one software of the device. Statistical analyzes were carried out.

3.3 Statistical Analysis

For the statistical analysis, the SPSS package software (version 17.0) was used. The mean and standard deviation were used to describe continuous quantitative data, whereas numbers and percentages were used for categorical variables. For the purpose of making comparisons, chi-square tests, precise chi-square tests, and continuity-corrected chi-square tests were used.

The numerical data did not fit the normal distribution, therefore we created the square with the lowest and maximum values and compared them using the Kruskal-Wallis test.

The significance level was accepted as 0.05 in all tests. The gene frequencies of the individuals in the population were calculated according to the Hardy-Weinberg rule.

4. RESULTS AND DISCUSSION

For our study, blood was taken from 29 MS patients and 120 healthy volunteers. There were eight male patients and twenty-one female patients. The age range of the patients who took part in our research was 19–54 years old, with an average age of 33.7 ± 10.7 years. Relapsing remitting MS affected 23 people, secondary progressive MS affected 5, and primary progressive MS affected 1. The mean disease year was determined as 7.6 ± 5.6.

1 patient (No. 26) in Taq I gene polymorphism analysis and 3 patients (No. 12, 19 and 22) in Apa I gene polymorphism analysis were not included in the study due to low DNA concentrations. All of our patients whose blood samples were taken for analysis of seal Igen polymorphism were included in the study.

Among those who served as controls, 61 were female and 59 were male. For the control group, the minimum age was 18, the maximum age was 58, and the mean age was 33.1 ± 8.5. In the control group, 48 individuals in Taq I gene polymorphism analysis, 39 individuals in Apa I gene polymorphism analysis, 6 individuals in Fok I gene polymorphism analysis could not be included in the study due to low DNA concentration.

In the Taq I polymorphism analysis, 72 individuals for the control group and 28 individuals for the patient group were included in the study. In the patient group, patient 26 was excluded from the study due to low DNA concentration. Twenty patients were female and 8 were male. In the patient group, the minimum age was 19, the maximum age was 54, and the mean age was 33.1 ± 10.4 years.

The mean disease year was 7.7 ± 5.7. Twenty-two of the patients were diagnosed with relapsing remitting MS, 5 with secondary progressive MS, and 1 with primary progressive MS. There were 35 women and 37 men in the control group. In the control group 48 healthy volunteers could not be included in the study due to low DNA

concentration. In the control group, the minimum age was 18, the maximum age was 58, and the mean age was 32.8 ± 8.8 years.

In the Taq I polymorphism analysis, a significant difference was found between the patient population and the control population for the GG genotype (p:0.025). While GG genotype was detected in 15 individuals, 7 female and 8 male, in the control group, GG genotype was not detected in any of the patients. 13 patients with AA genotype (6 female, 7 male), 31 controls (15 female, 16 male), 15 patients with AG genotype (14 female, 1 male), 26 controls (13 female, 13 male) available.

While a significant difference was found between the female patient and the female control group (p:0.028), there was no significant difference between the male patient and the male control group (p:0.079).

In the Fok I polymorphism study, 114 individuals for the control group and 29 individuals for the patient group were included in the study. Twenty-one patients were female and 8 were male. For the patients participating in our study, the minimum age was 19, the maximum age was 54, and the mean age was 33.7 ± 10.7 years.

The mean disease year was 7.6±5.6. Twenty-three of the patients were being followed up with a diagnosis of urealapsing remitting MS, 5 with secondary progressive MS, and 1 with a diagnosis of primary progressive MS. There were 57 women and 57 men in the control group. The minimum age for the control group was 18, the maximum age was 58, and the mean age was 32.8 ± 8.5.

In the Fok Ipolymorphism analysis, 1 patient with AA genotype (1 female), 8 controls (3 male, 5 female), 6 patients with AG genotype (5 female, 1 male), 34 controls (18 There were 22 patients (15 females, 7 males) with the GG genotype, and 72 controls (34 females, 38 males). There was no significant difference between the AA-AG-GG genotypes between the two groups (p:0.042).

There was no significant difference between the female patient-female control group (p:0.658) and the male patient-male control group (p:0.517).

In the Apa I polymorphism study, 81 individuals for the control group and 26 individuals for the patient group were included in the study. 20 of the patients were female and 6 of them were male. In the patient group, the minimum age was 21, the maximum age was 54, the mean age was 33.9 ± 10.1 years. was detected. The mean disease year was determined as 7.46 ± 5.2.

Twenty-one of the patients were followed up with relapsing remitting MS, 4 with secondary progressive MS, and 1 with primary progressive MS. There were 46 women and 35 men in the control group. The minimum age for the control group was 18, the maximum age was 58, and the mean age was 34 ± 8.7.

In Apa I polymorphism analysis, 8 patients (7 female, 1 male) with AA genotype, 28 controls (18 female, 10 male), 13 patients (11 female, 2 male) with AC genotype), 37 controls (18 female, 19 male), 5 patients (2 female, 3 male) with CC genotype, 16 controls (10 female, 6 male). For Apa I polymorphism, no significant difference was found between AA-AC-CC genotypes between control and diseased subjects (p:0.956). There was no significant difference between female patient and female control group (p:0.383) and male patient-male control group (p:0.273).

Allele distribution graphs of Fok I, Taq I and Apa I polymorphisms are shown in Figure 4.1, Figure 4.2, and Figure 4.3.

4.1 The Molecular Daignosis

Sequence of primers used for NEFL the investigation of the selected gene polymorphisms in our study are shown in Table 4.1.

Table 4.1 Sequence of primers used for NEFL the investigation of the selected gene polymorphisms in our study

Polymorphisms	Primers	PCR product size
NEFL-gene	Forward AAATTCCCTAGCCGCACCATC	415-bp
	Reverse CAGCCTGCGATCGATCACG	

The direction and variations in the regression coefficient represent a special kind of effect of the risk genotype (GG or GA) when it is matched with the reference genotype (AA) of the polymorphism and the NF gene distribution of the very light chain gene.

In the study, the Rs2979687 SNP was used, which is located or is located in the promoter region of the NEFL gene, which causes mutation and converts adenine to guanine.

The -374A is greater than the G (rs2979687) polymorphism in multiple sclerosis subjects (n = 29) and healthy controls (n = 120) Genotyping and DNA quality were confirmed in the studied samples by gel electrophoresis. 21 of the patients were female and 8 were male. For patients participating in our study, the minimum age was 19 years, the maximum age was 54 years, and the mean age was 33.7 ± 10.7 years.

Twenty-three of the patients had relapsing-remitting MS, 5 had secondary progressive MS, and 1 had primary progressive MS. In this study, we examined the selected polymorphism of patient samples using MS by PCR and sequencing methods.

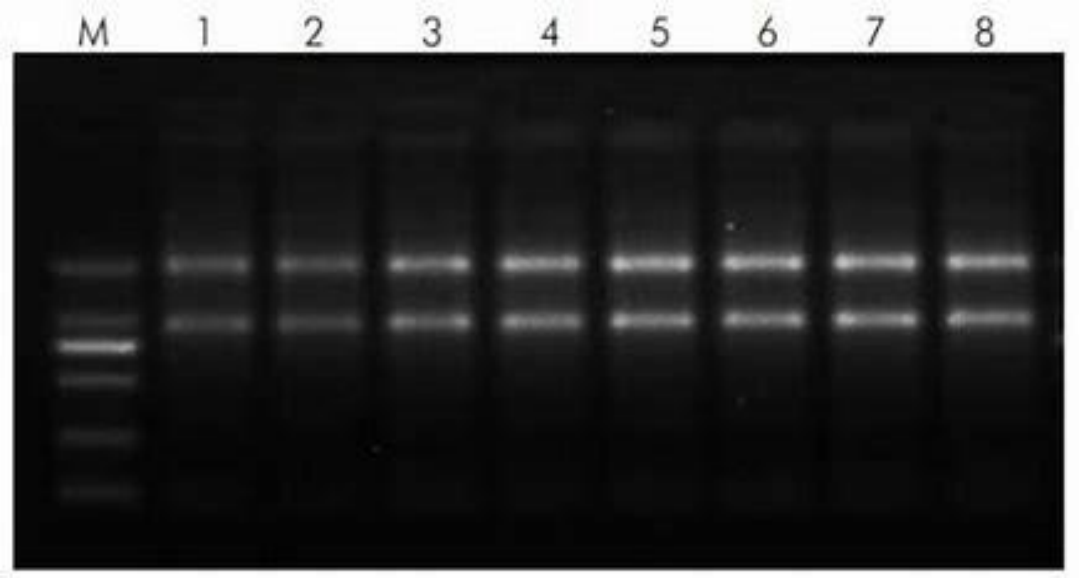

Figure 4.1 Sequence of primers used for NEFL the investigation of the selected gene polymorphisms in our study

The genotypes and allele frequencies in order to understand the results for the NEFL gene polymorphism are presented in Table 4.1. The analysis indicated that the most common and studied genotype among the case and control groups in NEFL is the GA genotype, which is the pathogen.

The frequencies of genotypes in the results of our study as follows: AA, AG and GG for NEFL rs2979687 were 12%, 67% and 21%, respectively, among the cases and 35%, 55%, and 10%, respectively, among the controls group.

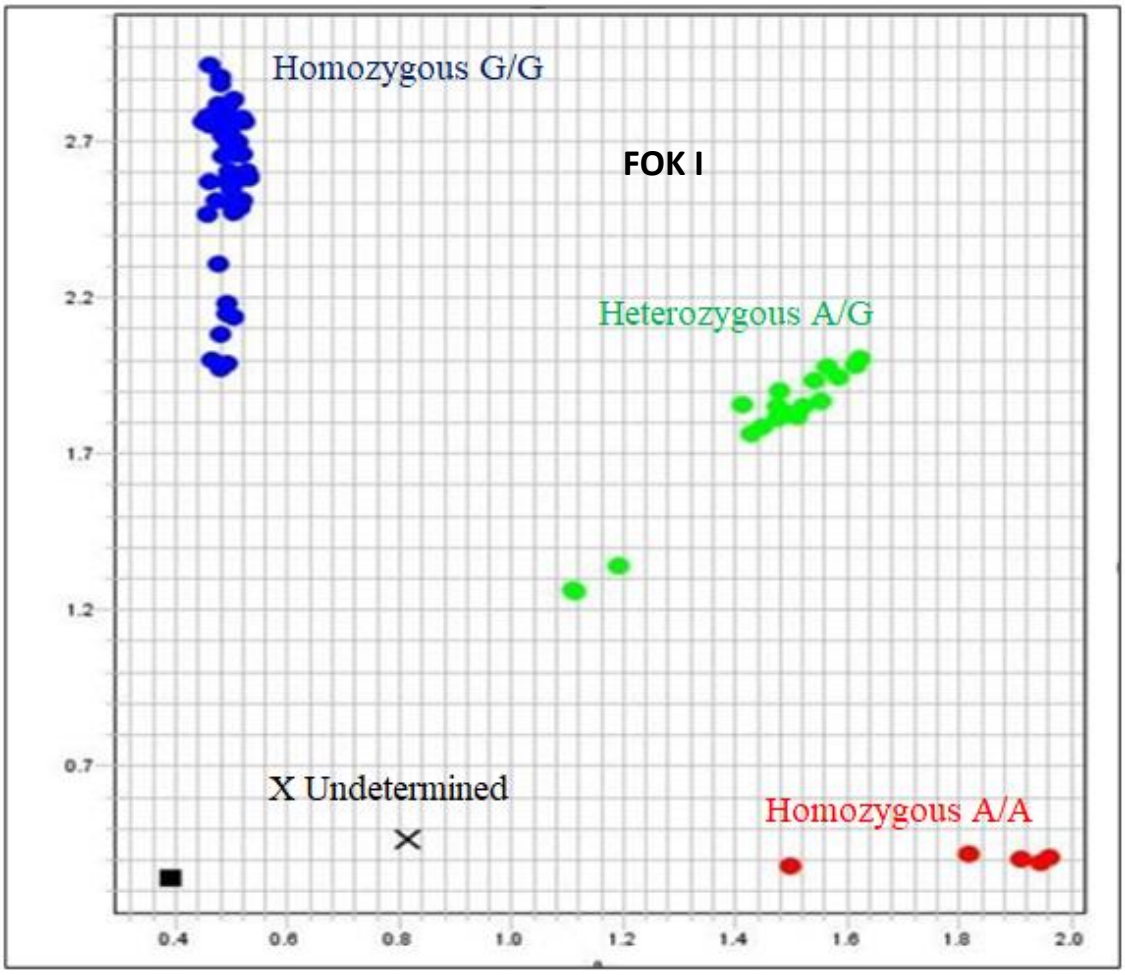

Figure 4.2 Distribution Graphic (Homozygous A/A; Homozygous G/G; Heterozygous A/G; X Undetermined)

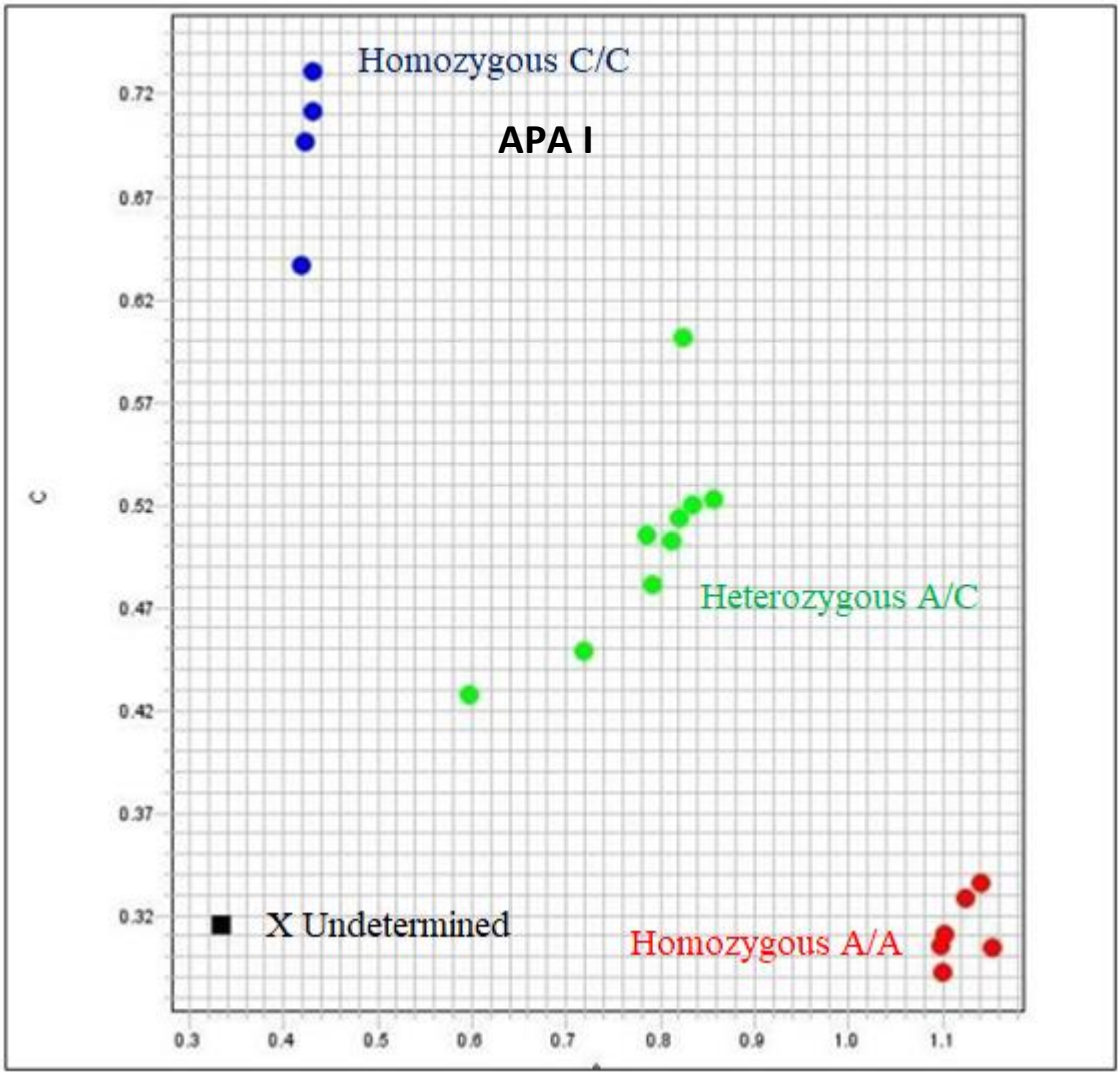

Figure 4.3 Distribution Graphic (Homozygous A/A; Homozygous C/C; Heterozygous A/C; X Undetermined)

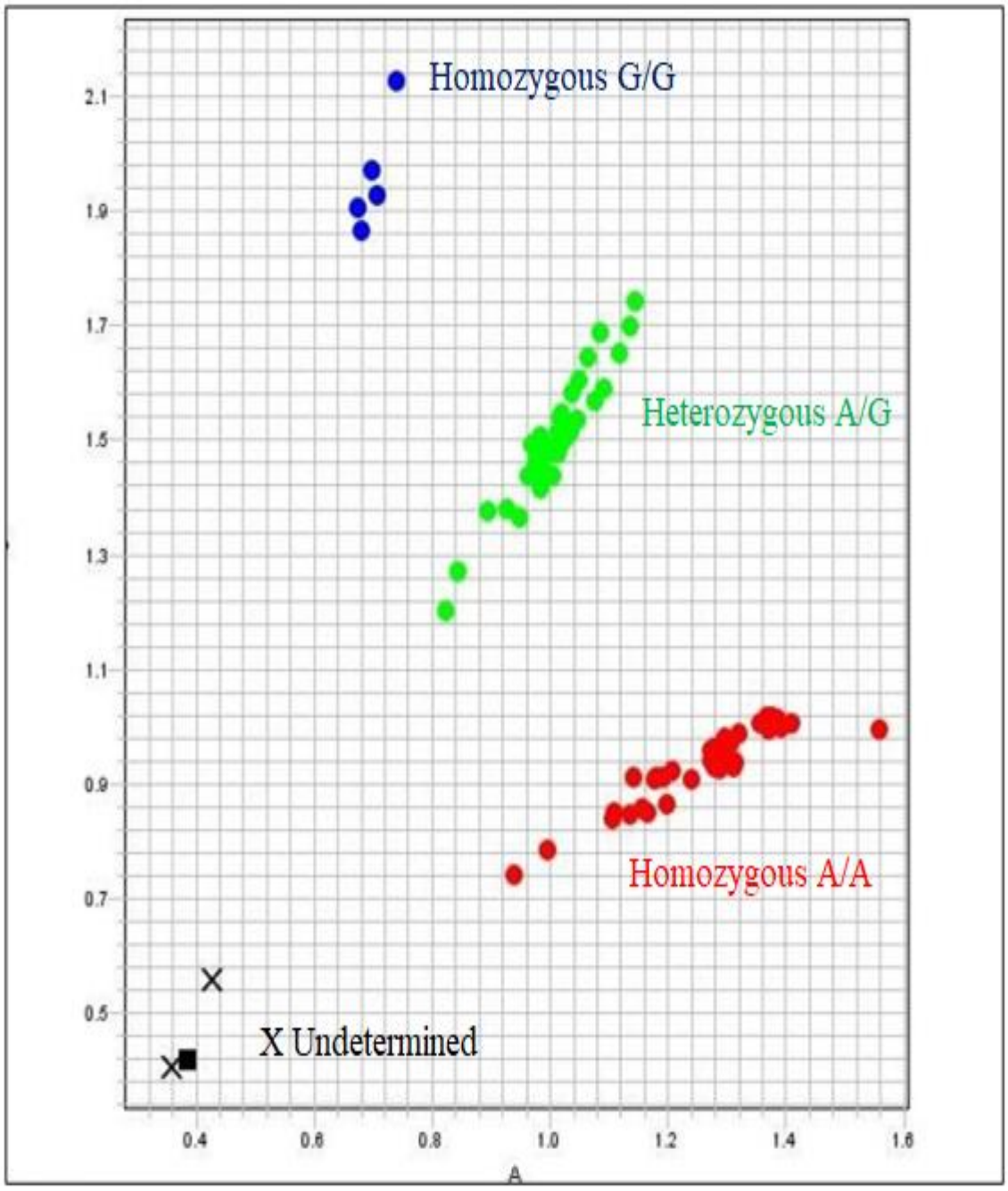

Figure 4.4 Distribution Graphic (Homozygous A/A; Homozygous G/G; Heterozygous A/G; X Undetermined)

5. CONCLUSIONS AND RECOMMENDATION

A persistent demyelinating illness affecting the central nervous system is multiple sclerosis. It ranks high among the illnesses that impede the mobility of middle-aged and younger persons. Its incidence is expected to rise steadily, impacting an estimated 2.1 million individuals globally. The etiology of multiple sclerosis is believed to entail both genetic and environmental variables, with a focus on T cell-mediated autoimmunity. Vitamin D insufficiency in MS patients has drawn attention because of its possible role as an immune system regulator (Waubant et al. 2015).

One of the several steroid hormones, vitamin D affects many bodily functions and processes. The vitamin D receptor (VDR) and the membrane-associated rapid response steroid-binding receptor (MARRS) are the mechanisms by which vitamin D exerts its effects. Vitamin D metabolism and function may be affected by certain polymorphisms in the vitamin D receptor gene (Lublin and Reingold 1996)". Many studies have been conducted on the role of VDR gene polymorphism in various ethnic groups, with conflicting results (Sioka et al. 2011).

In many "countries around the world, research on the relationship between VDR polymorphism and MS has been conducted. First, Bsm Ipolymorphism research with MS was conducted in Japan in 1999; Then, in 2000, the relationship between Graves' disease and MS was investigated in Japan. In 2005, researchers in Australia looked at the possible link between multiple sclerosis and the Taq I, Apa I, and Seal I polymorphisms. In 2011, researchers in Greece also performed a comparable study (Sioka et al. 2011).

Because they are the most prevalent single nucleotide polymorphisms in the VDR gene, researchers have focused on studying the association between MS and the Taq I, Apa I, Fok I, and Bsm I polymorphisms (Čierny et al. 2015). Since no previous research has examined individuals with familial MS features in connection to the Taq I, Apa I, and Seal I polymorphisms of the VDR gene, we set out to fill that void. Bsm Igen polymorphism could not be included in our research because the kit was not available.

In our study, Bsm I polymorphism research was not performed, and no significant results were obtained in Apa I polymorphism research. Obtaining different results may be due to the limited number of our patient population.

A statistically significant variation in the genotype distribution between the patient and control groups was observed in an MS case-controlled research comprising 104 patients and 104 controls in Australia. The study specifically examined MS patients using the Taq I polymorphism analysis (Lin et al. 2019). In our study, a significant difference was found in the Taq I genotype distribution of patients with familial MS characteristics and the control group, supporting the study conducted in the Australian population (p:0.025)". The data obtained suggested that the genetic characteristics of the Turkish population and the Australian population may be similar.

Once again, "the Apa I polymorphism was determined to be associated with an increased risk of both primary and secondary progressive MS in the same study where a statistically significant difference was also observed in MS patients" (Brønnum-Hansen *et al.* 2004). In the Apa I polymorphism analysis performed in our study, no significant difference was found in our patient group compared to the control group. In our study, analysis was not performed according to MS subtypes, when the limited number of patients were divided into subtypes, the number of patients sufficient to draw meaningful conclusions for each subtype could not be reached". Fok I polymorphism research was similar to our study, and no significant difference was found in the distribution.

Research on Taq I and Apa I polymorphisms in the Canadian population did not reveal any statistically significant differences (Ferguson *et al.* 1997). In our study, no relationship was found with Apa I polymorphism, but Taq I polymorphism was found to be significantly different in the patient and control groups.

In a study "conducted with 69 MS patients and 81 controls in the Greek population, Bsm I and Taq Ipolymorphisms were investigated, but no relationship was found. Hip and lumbar bone mineral density were shown to be decreased in MS patients compared

to the control group, according to this research. There was no discernible correlation between Bsm I and Taq I polymorphisms and poor bone mineral density (Confavreux *et al.* 1980).

In a study by Polliack *et al.* (2001) consisting of 136 patients and 235 control groups, Cdx-2, Fok I, Taq Ipolymorphism and the risk of developing MS were investigated, but no relationship was found. However, childhood sun exposure and Cdx-2 polymorphism were associated with the risk of developing MS. In the same study, a relationship was found between the G allele and reduced MS risk in patients with less than 2 hours of daily sunlight exposure". This suggests that previous exposure to sunshine may explain the association between MS and VDR gene mutations (Polliack *et al.* 2001).

In the study of Pohl *et al.* (2010) in the United Kingdom consisting of 214 patients and 428 control groups, it was determined that the risk of developing MS is high with low vitamin D intake in patients with Seal Ipolymorphism. No link was found between VDR gene single nucleotide polymorphisms (Apa I, Taq I, Bsm I and Fok I) and MS (Pohl *et al.* 2010). In our study, there was no significant result in Apa I and Fok I polymorphism analysis, but unlike this study, a significant difference was found in Taq I polymorphism (p:0.025)".

In a study conducted by Cox MB *et al.* in England in 2012, an increased risk of MS was found with Taq I polymorphism (Lublin *et al.* 1997).

In the study of Smolders *et al.* consisting of 212 patients and 289 control groups, no significant results were found between Apa I, Taq I polymorphisms, serum calcidiol and calcitrol levels and MS risk. No relationship was found between the seal I polymorphism and MS (Weinshenker *et al.* 1989).

In Tunisia, researchers studied 114 healthy controls and 60 people with relapsing-remitting multiple sclerosis. There are a total of 38 female patients and 22 male patients. There are 47 males and 67 females in the control group. Findings from this research

demonstrated that across age and gender categories, the presence of the T allele in the Taq I polymorphism protected Tunisian participants against developing multiple sclerosis. This study's findings provide credence to the idea that VDR contributes to the likelihood of acquiring multiple sclerosis. Having said that Apa Ipolymorphism and MS did not show any meaningful association (Van Waesberghe *et al.* 1999).

This "study supported the conclusion that there is a relationship between Taq Ipolymorphism performed in Australia and the risk of MS development; Similar results were obtained in our study as well. In addition, analysis was made according to gender discrimination in this study; Taq I polymorphism was associated with MS disease in both groups.

In our study, it was determined that the presence of the G allele in the Taq I polymorphism analysis was protective against the risk of developing MS in female patients in familial MS patients. In the literature, it has been thought that the discordant results related to Taq I polymorphism analysis may be due to the ethnic origin of the patients, sample size, study design, or the role of many genes in the pathogenesis of MS disease. Again, in this study, no correlation was found between Apa I polymorphism and the risk of developing MS, which is consistent with our study (Van Waesberghe *et al.* 1999).

A study was conducted in the Kuwaiti population that included 50 MS patients and 50 controls. Of the MS patients, 33 were female, 17 were male, 31 of the control group were female and 19 were male. Vitamin D level, vitamin D supplementation, VDR polymorphism, sun exposure, and risk of multiple sclerosis were all examined in the research (Al-Temaimi et al. 2015). Results showed no statistically significant difference in vitamin D levels between MS patients and healthy controls, and treatment with vitamin D had no effect on MS patients' vitamin D levels either. It was shown that there was a substantial difference in the distribution of Taq Igenotype between the control group and the MS patients (Trapp and Nave 2008).

No significant difference was found between MS patients and control group in Apa Igenotype distribution. Regarding Apa I and Taq I polymorphism; In this study conducted in the Kuwaiti population, similar data were obtained in Tunisia (Van Waesberghe *et al.* 1999) and our familial MS patient population".

The study conducted in Australia (Brønnum-Hansen *et al.* 2004) supports the association of Taq I polymorphism with MS, but the fact that Apa I polymorphism was found to be associated with MS in Australia is incompatible with the work.

The study was conducted with 113 MS patients (88 women, 25 men) and a control group of 122 healthy individuals, 94 women and 28 men, in the Southeast Iran region. In this study, it was planned to investigate the relationship between Taq Ipolymorphism and Apa I polymorphism and MS susceptibility. A relationship was found between both polymorphisms and MS; these results are in agreement with Japan (Cohen *et al.* 2001), Australia (Brønnum-Hansen *et al.* 2004).

In our study, significant results were obtained in the analysis of Taq I polymorphism, but inconsistent results were obtained with Apa I polymorphism. No difference was found between the levels of calcidiol and calcium in the blood and cerebrospinal fluid of MS patients and the levels of the control group (Lucchinetti *et al.* 1998)".

The role of VDR gene polymorphism on VDR functions could not be evaluated clearly with this study; It may be due to ligand-receptor affinity, signaling pathway (Breij *et al.* 2008) or gene expression (Polman *et al.* 2011), which indirectly affects vitamin D functions.

In a "study by Cierny et al in Slovakia, in which 270 MS patients and 303 controls were included, it was aimed to investigate MS susceptibility with VDR gene Seal I polymorphism. MS patients were divided into 3 subgroups according to their clinical disability progression (slow-moderate-fast progressing). Neither the Fok I genotype nor the allele frequency ratio showed any statistically significant difference between the

control group and MS patients. It was discovered that the Fok I polymorphism was more prevalent in female MS patients when comparing them to the female control group (p:0.042), but no significant findings were achieved when comparing males to males in the control group. It was thought that this might be the result of the fact that MS is more common in the female population. No significant difference was found in the comparison between subgroups (Van Der Knaap *et al.* 1999).

In addition, analysis was made according to gender discrimination in this study; MS disease has been associated with the Seal I polymorphism in female MS patients. In our study, Fok I polymorphism in familial MS patients was female patient-female control group.

A male patient-male control analysis was performed and no significant results were obtained in both genders. Fok I genotype was thought to be a possible predictor for calcitriol plasma level. Low serum calcitriol plasma level and Fok I polymorphism were detected in 150 Canadian cases (Polman *et al.* 2011) and 212 Dutch cases (Roest Crollius *et al.* 2000). Due to the complexity of the etiopathology of MS, it is difficult to establish a definite relationship between VDR gene polymorphism and MS (Van Der Knaap *et al.* 1999).

The research included 180 individuals with Graves' disease (48 men and 132 females) and 195 healthy controls (67 men and 128 females) in its investigation of the link between VDR gene variation and the illness in the Japanese population. In Japanese women, Graves' illness was shown to be related with the Bsm I and Apa I polymorphisms, particularly the VDR Fok I polymorphism. Predicting osteoporosis risk by the Apa I polymorphism was previously believed to be possible (Pugliatti *et al.* 2002, Oraby *et al.* 2019). Our study did not examine the potential link between Bsm I polymorphism and familial MS. Similarly, when we analyzed the associations between Taq I, Apa I, and Fok I polymorphisms and familial MS, we did not find any evidence of a link between the two in Graves patients with an autoimmune disease etiology similar to MS.

As a result of the literature review, regarding the results of Taq I polymorphism that we found to be associated with MS in our patients with familial MS; We found that results supporting our study were obtained in England (Lublin *et al.* 1997), Australia (Brønnum-Hansen *et al.* 2004), Tunisia (Van Waesberghe *et al.* 1999), and Southeast Iran (Lucchinetti *et al.* 1998) regions. Also our findings were consistent with study conducted by (Moosavi et al. 2021), where demonstrated found that the frequency of FokI and TaqI polymorphisms significantly differed between the patients and the controls ($p = 0.0127$ and $p = 0.0236$, respectively). Also this study suggest that FokI and TaqI polymorphisms of VDR are associated with MS risk and TaqI polymorphism is associated with Vitamin D levels in MS patients.

Additionally, in study by Yucel et al. (2018) revealed that the Taq I polymorphism analysis indicates that the GG genotype may protect against familial MS and may be a positive marker.

In many studies, when analyzing the polymorphism relationship with MS patients, comparisons were made between the control and patient groups. In our study, we compared control and patient groups, as well as female patient-female control, male patient-male control comparison. In studies conducted in Kuwait (Van Waesberghe *et al.* 1999) and Slovakia (Van Der Knaap *et al.* 1996), we found that patients were compared with the control group according to gender differences. This detail was not included in other studies. In the Kuwait study, the analysis of the relationship between Taq I polymorphism and MS was found to be significant in female patients, and in our study, Taq I polymorphism was found to be significant in female patients. In the study conducted in Slovakia, Fok I polymorphism analysis was found to be significant in female patients, but in our study, no difference was found between female patient-female control, male patient-male controls in the Fok I analysis.

The incidence of familial MS in the community is low (4% in case of a first-degree relative with MS (Andersson *et al.* 1999); In our study, 29 familial MS patients were included in the study. The reason why the Seal I and Apa I polymorphism analysis data obtained from the study were insignificant may be that they do not predispose to the

disease or the number of our patients is limited. In our study, MS patients could not be differentiated according to disease subtypes, and when the limited number of patients were divided into subtypes, the number of patients sufficient to draw meaningful conclusions for each subtype could not be reached.

In our study concluded that there are a significant difference was found in the Taq I genotype distribution of patients with familial MS characteristics, where the Taq I acossiated with the risk of developing MS. In the Apa I polymorphism analysis performed in our study, no significant difference was found in our patient group compared to the control group. Also, there was no significant result in Apa I and Fok I polymorphism analysis.

This study we have done is preliminary; Additional studies we recommend are needed to make detailed associations (serum calcitriol level, differentiation according to disease subtypes, association of bone densitometry, and body mass index). Further studies with a larger number of patients will help us gain better knowledge.

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