CURRICULUM VITAE

Tzu-Yun C. McDowell

Education

2005- Ph.D. Department of Epidemiology and Preventive Medicine
University of Maryland, Baltimore (expected graduation date: May, 2010)

2003 M.A. Kinesiological Sciences
University of Maryland, College Park, MD

2000 B.S. School of Physical Therapy, College of Medicine
National Taiwan University, Taipei, Taiwan

Certifications

2003, Dec. Sensory Integration and Praxis Test Certification, U.S.A.

2000, Nov. Physical Therapy License by the Department of Health, Taiwan, R.O.C.

Professional Experience

Research

2007 - Research Analyst/Programmer, full-time position
Under grants:
Examining Healthcare Patterns and MS Outcomes with VHA and Medicare Data
A National Survey of Veterans with Multiple Sclerosis
Department of Epidemiology and Preventive Medicine
University of Maryland, Baltimore

Responsibilities: Develop, collect and manage the national survey for the Multiple Sclerosis Surveillance Registry (MSSR) under the Multiple Sclerosis Center of Excellence East, Baltimore VAMC (MSCoE); analyze MSSR survey data for presentation and publication; perform statistical consulting, analysis and programming (SAS) for projects using Veteran Health Administrative database; work with MSCoE personnel to design and implement MS related research and to establish quality indicators and benchmarkers for MS care; present research and represent the MSCoE at national and international meetings.

2003 - 2007 Study Coordinator/Research Analyst, full-time position
Under grants:
Adaptive sensorimotor control in children with developmental coordination disorder
Bilateral arm training in patients with chronic stroke
Bilateral vs. Unilateral arm training in chronic stroke
Department of Physical Therapy and Rehabilitation Science
University of Maryland, Baltimore

Responsibilities: Coordinate personnel and testing schedule for multi-site studies for children with developmental coordination disorder (DCD) and patients with stroke; serve as primary contact for parents of children with DCD regarding recruitment, testing and seminar; serve as primary tester for the sensory integration and praxis test; setup and collect data with various laboratory equipment; perform programming, data analysis (Matlab) and statistical analysis (SAS); provide technical assistant and statistical consulting; develop agendas and instruct at the DCD parent seminar; perform administrative tasks related to the day-to-day operation of the laboratory. Other duties include: serve as primary trainer for intervention studies for patients with chronic stroke, assist various performance tests related to arm function and balance; and support functional MRI and transcranial magnetic stimulation tests.

2001 - 2003 Graduate School Fellowship
Under grant: *Posture development and perception-action coupling*
Department of Kinesiology
University of Maryland, College Park

Responsibilities: Assist with data collection, reduction, and analysis; train undergraduate assistants for lab related activities; participate and present projects at national meetings.

2000 - 2001 Research Assistant, full-time position
Under grant: *Kinematic analysis of treadmill walking in pre- and full-term infants*
Infant Development Research and Motion Analysis Laboratory
School of Physical Therapy
National Taiwan University, Taipei, Taiwan, R.O.C.

Responsibilities: Assist with data collection, support writing grant applications; and perform administrative tasks related to the day-to-day operation of the laboratory

Clinical
2000 Physical Therapist, part-time position
National Taiwan University Hospital, Taipei, Taiwan, R.O.C.

Responsibilities: Evaluate, treat, and design training programs for patients at an outpatient clinic

Teaching Experience

2007 - 2008 Teaching Assistant for Graduate Course –
Survival Analysis (2007)
Longitudinal Analysis (2008)
Department of Epidemiology and Preventive Medicine
University of Maryland, Baltimore, MD
Responsibilities: Grade weekly assignments; assist and advise students on their questions related to SAS programming, assignments, and projects; evaluate and generate questions for students' final presentations.

2008 Guest Lecturer for Graduate Course – Longitudinal Analysis
Topic: Covariance Structures
Department of Epidemiology and Preventive Medicine
University of Maryland, Baltimore, MD
Responsibilities: Prepare and organize lecture materials with the course instructor; give two lectures and demonstrate related SAS programs in class; Stimulate questions and discussion from students

2002 - 2003 Teaching Assistant for Undergraduate Course
Laboratory Instructor – Motor Development
Department of Kinesiology
University of Maryland, College Park, MD
Responsibilities: Develop and prepare materials for the lab; instruct weekly lab class to students; grade students' exams, assignments and lab performance; stimulate discussions and assist students' understanding of course materials.

2002 - 2003 Guest Lecturer for Undergraduate Course
Laboratory – Topical Investigations: Introduction to Kinesiology
Department of Kinesiology
University of Maryland, College Park, MD

Responsibilities: Demonstrate research related to Kinesiology

Fellowships/Grants/Awards

2009	Service Recognition Award, School of Medicine, University of Maryland, Baltimore
2008	Young Investigator Travel Award, from the Americas Committee for Treatment and Research in Multiple Sclerosis
2008	Scholarship, from the Consortium of Multiple Sclerosis Center
2001 – 2003	Graduate School Fellowship award, University of Maryland, College Park
2000	Highest Class GPA & Scholarship, National Taiwan University, Taipei

Journal Publications

- Olugemo OA, Desai T, Krumholz A, **McDowell T-Y**, Zhan M, Royal W, Wallin M, Bever C, Culpepper WJ. Sleep Disorders in Multiple Sclerosis (submitted).

- Viswanathan P, **McDowell T-Y**, Kagerer F, Contreras-Vidal JL, Whitall J. Modulation of bilateral finger tapping to subliminal and perceptible auditory change in the phasing of sensorimotor coupling (submitted).

- Culpepper WJ., Cowper-Ripley DC., Litt ER, **McDowell T-Y**, Hoffman, PM. Using GIS tools to improve MS specialty care access in the Veterans Health Administration. Journal of Rehabilitation Research and Development 2010 (in press).

- **McDowell T-Y,** Amr S, Langenberg P, Royal W, Bever C, Culpepper WJ, Bradham DD. Time of birth, residential solar radiation and age at onset in multiple sclerosis. Neuroepidemiology 2010; 34:238-244

- Whitall J, **Chang T-Y**, Horn CL, Jung-Potter J, McMenamin S, Wilms-Floet A, Clark JE. Auditory-motor coupling of bilateral finger tapping in children with and without DCD compared to adults. Hum Mov Sci. 2008 Dec;27(6):914-31

- Chen L-C, Metcalfe JS, **Chang T-Y**, Jeka JJ, Clark JE. The development of infant upright posture: sway less or sway differently? Exp Brain Res. 2008 Mar;186(2):293-303.

- Metcalfe JS, McDowell K, **Chang T-Y**, Chen L-C, Jeka JJ, Clark JE. Development of somatosensory-motor integration: an event-related analysis of infant posture in the first year of independent walking. Dev Psychobiol. 2005 Jan;46(1):19-35.

- Metcalfe JS, Chen L-C, **Chang T-Y**, McDowell K, Jeka JJ, Clark JE. The temporal organization of posture changes during the first year of independent walking. Exp Brain Res. 2005 Mar;161(4):405-16.

Published Reports

- Cowper Ripley DC, Culpepper WJ, Hoffman PM, Litt ER, **McDowell T-Y**.
  Geographic Access to Treatment for VHA Patients with Multiple Sclerosis: MSCoE-East.
  Management Report, Rehabilitation Outcomes Research Center REAP, Gainesville, FL, 2008.

- **Chang T-Y**. The relationship between touch and infants' upright posture during the first year of walking. University of Maryland, College Park, Master thesis. 2004

Presentations

- **McDowell T-Y,** Amr S, Langenberg P, Royal W, Bever C, Culpepper WJ, Bradham DD. Past sun exposure, vitamin D intake and age at onset in multiple sclerosis. Consortium of Multiple Sclerosis Center, San Antonio, TX 2010.

- Culpepper WJ, **McDowell T-Y,** Wallin MT, Royal W, Bever CT. Therapy-related Acute Leukemia in Mitoxantrone-treated Veterans with MS. Consortium of Multiple Sclerosis Center, San Antonio, TX 2010.

- **McDowell T-Y,** Culpepper WJ, Bever C, Royal W, Bradham DD. Timing of Birth, Residential Solar Radiation and Age at Onset in Veterans with Multiple Sclerosis. Consortium of Multiple Sclerosis Center, Atlanta, GA, 2009

- Culpepper WJ, **McDowell T-Y,** Wallin MT, Bever CT, Bradham DD. Cardiac Disease After Mitoxantrone in Veterans with MS. Consortium of Multiple Sclerosis Center, Atlanta, GA, 2009

- Cowper Ripley DC, Culpepper WJ, Hoffman PM, Litt ER, **McDowell T-Y**. Geographic Access to Treatment for VHA Patients with Multiple Sclerosis. HSR&D, Baltimore, Maryland, 2009.

- **McDowell T-Y**, Culpepper WJ, Bever C, Bradham DD. Osteoporosis in Veterans with Multiple Sclerosis. the Americas Committee for Treatment and Research in Multiple Sclerosis, Montreal, Canada, 2008

- Culpepper WJ, Bever C, **McDowell T-Y**, Bradham DD. DMT Compliance in the VHA MS Surveillance Registry. the Americas Committee for Treatment and Research in Multiple Sclerosis, Montreal, Canada, 2008

- **McDowell T-Y**, Culpepper WJ, and Bradham DD. Age and Comorbidity in Veterans with Multiple Sclerosis. Consortium of Multiple Sclerosis Center, Denver, CO, 2008.

- Ajayi OF, **McDowell T-Y**, Culpepper WJ, Bever C, Royal W. High Prevalence of Sleep Disorders in Veterans with Multiple Sclerosis. American Academy of Neurology, 2008

- Whitall J, McComber-Waller S, Liu W, **Chang T-Y**. Changing motor control and coordination in persons with chronic stroke. Motor Control and Human Skill. Australia, 2007.

- Metcalfe JS, Chen L-C, **Chang T-Y**, McDowell K, Jeka JJ, Clark JE. The structure of postural sway in the first year of independent walking: Effects of experience and somatosensory cues. International Conference on Infant Study, Chicago, IL, 2004.

- Viswanathan P, **Chang T-Y**, Horn C, Roche R, Whitall J. Adaptation to gradual and abrupt changes in sensorimotor coupling: Auditory cues and bilateral finger tapping. North American Society for the Psychology of Sport and Physical Activity, Vancouver, British Columbia, 2004.

- Roche R, Horn C, **Chang T-Y**, Viswanathan P, Whitall J. Auditory motor processing in typically developing children: A cross-sectional study. North American Society for the Psychology of Sport and Physical Activity, Vancouver, British Columbia, 2004.

- **Chang T-Y** and Clark JE. The relationship between touch and infants' upright posture during the first year of walking. North American Society for the Psychology of Sport and Physical Activity, Savannah, GA. 2003.

Computer Skills

Standard Windows-Based Word-processing, Spreadsheet and Illustration Programs. Programming level use of Matlab and SAS. Operational level use of Labview and SPSS. Microsoft Office Access and AutoData (a software package used to design questionnaires/surveys and automates data entry via scannable/faxable forms).

ABSTRACT

Title of Dissertation: Ultraviolet Radiation, Vitamin D Intake and Multiple Sclerosis

Tzu-Yun McDowell, Doctor of Philosophy, 2010

Dissertation directed by: Sania Amr, MD, MS, Associate Professor
Department Epidemiology and Preventive Medicine
University of Maryland, Baltimore

Background: Multiple Sclerosis (MS), an inflammatory and neurodegenerative disease, has an elusive etiology that is thought to be an interaction between genetic and environmental risk factors. Ultraviolet Radiation (UVR) and/or vitamin D have been consistently shown to be protective against MS development; however, their roles in modulating the clinical course of this disease remain unclear.

Objectives: The overall objective of this dissertation was to examine the effects of UVR and vitamin D related exposures on the ages of disease onset and progression to disability among a national cohort of Veterans with MS.

Methods: We conducted a cross-sectional study, using a questionnaire designed to assess the different parameters that contribute to UVR exposure and vitamin D synthesis. We examined the dual influence of (1) timing and geographical location of birth and (2) sun exposure and vitamin D-related intakes from childhood to MS onset on the clinical course of this disease. All the analyses were conducted by disease subtype (Relapsing vs. Progressive MS). Multiple linear regression and Cox proportional hazard models were used to analyze the data.

Results: Among Veterans with Relapsing MS (N=731), those born in winter and in low solar radiation areas, had their disease symptom onset on an average 2.8 years earlier (p = 0.02) than those born during other seasons in areas with medium to high solar radiation. Among 948 veterans with Relapsing MS, we found that low sun exposure in fall/winter seasons during childhood and early adolescence was also associated with early MS onset (p = 0.01); whereas regular use of cod liver oil in childhood was associated with later disease onset (p = 0.01). Among Veterans with progressive MS (N=151), low average fall/winter sun exposure before symptom onset was associated with an increased risk of disability (p = 0.01); while regular intake of cod liver oil during childhood and early adolescence decreased the risk (p = 0.04).

Conclusions: Our findings suggest that environmental exposures before MS onset, primarily related to UVR and/or vitamin D status, early in life and during childhood and early adolescence have significant effects on the clinical course of MS.

Ultraviolet Radiation, Vitamin D Intake and Multiple Sclerosis

by

Tzu-Yun McDowell

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, Baltimore in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2010

ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

Dedication

To all the patients who suffer with multiple sclerosis, their families, and all those dedicated to helping overcome this disease.

Acknowledgments

I would like to extend my gratitude to all the participants for contributing their time and effort for this study.

My deep appreciation goes to my committee members: Dr. Sania Amr, Dr. Chris Bever, Dr. Douglas D. Bradham, Dr. William J Culpeper, Dr. Patricia Langenberg, and Dr. Walter Royal for their guidance and valuable input into this dissertation. I would like to especially thank Dr. Sania Amr, the chair of the dissertation committee, for her time, patience and constructive advice that sharpened my scientific thinking and writing; Dr. Douglas Bradham for the opportunities and support he has given me and Dr. William J Culpeper for sharing his experience in survey research and friendship during my research.

I would like to thank Dr. Jill Whitall for her early support and encouragement that helped me to join the graduate program at University of Maryland, Baltimore.

I would like to extend my thanks to many staff of the Cooperative Studies Program Coordinating Center of the VA Maryland Health Care System at Perry Point, Maryland for their assistances and hard work on the survey production. I would like to thank Josh Moore and Shan Jin for their assistance on survey data entry and reduction.

My deepest gratitude goes to my dear and loving family and friends in Taiwan and the United States for their endless support, especially my husband Kaleb, for his slave-driving (encouragement) to complete this long journey.

Table of Contents

List of Figures

List of Tables

CHAPTER I: LITERATURE REVIEW

1.1 Multiple Sclerosis

1.1.1 Introduction to Multiple Sclerosis

Multiple Sclerosis (MS), a chronic, immune-mediated inflammatory and neurodegenerative disease, is the most common neurological disorder as well as the leading non-traumatic cause of disability among young adults. The signs and symptoms of MS occur as the consequence of underlying neuropathologic changes within the central nervous system (CNS). Acute inflammatory demyelination initiated by autoimmune attacks, and axonal loss that results from chronic demyelination are the mechanisms underlying CNS damage and neurodegeneration. They can lead to either slowing of neural conduction or complete disruption of conduction[1]. Demyelination resulting from acute focal inflammation is often what causes MS symptoms to appear at early stages of the disease. When inflammation subsides, partial or complete recovery of the clinical symptoms (remission) often occurs with repair of the axonal structure (re-myelination), especially in the early phases. However, once a pathological threshold is reached (chronic demyelination and accumulated axonal loss), disease progresses under the primary mechanism of neurodegeneration that aggregates the clinical disease and results in irreversible neurological disability [2]. Brain atrophy, in addition to cortical lesions, is another major contributor to disease burden in patients with MS; causing a variety of signs and symptoms that depend on the size, number, and location of the CNS lesions. The most common symptoms include: optic neuritis, nystagmus, weakness, sensory loss, fatigue, ataxia, bowel dysfunction and cognitive impairment [1, 3].

Figure 1.1 illustrates a natural history of MS clinical presentation and disability (Black line), underlying mechanism (Blue shaded areas on the bottom of the diagram) and neurological disability measured from MRI (gadolinium enhancing [GD] or 'active' lesions – Green; T2 lesions – Purple; and brain volume – Blue). During the inflammatory phase, which is the dominant disease process in the early stage of the disease, relatively frequent relapses (black line) and GD lesions (green line) occur. During this phase, varying degrees of recovery can take place as depicted by the stepped pattern in black and green line representing decrease of disability along with reduction of GD lesions, respectively. As time progresses, there is an accumulation of axonal loss (neurodegeneration) with fewer and fewer relapses and GD lesions, a plateau in the number of T2 lesions and an ever increasing loss of total brain volume. Once neurodegeneration becomes the dominant disease process, patients transit their MS from relapsing-remitting (RRMS) type of disease to secondary progressive (SPMS) type associated with constant worsening of the symptoms and functions.

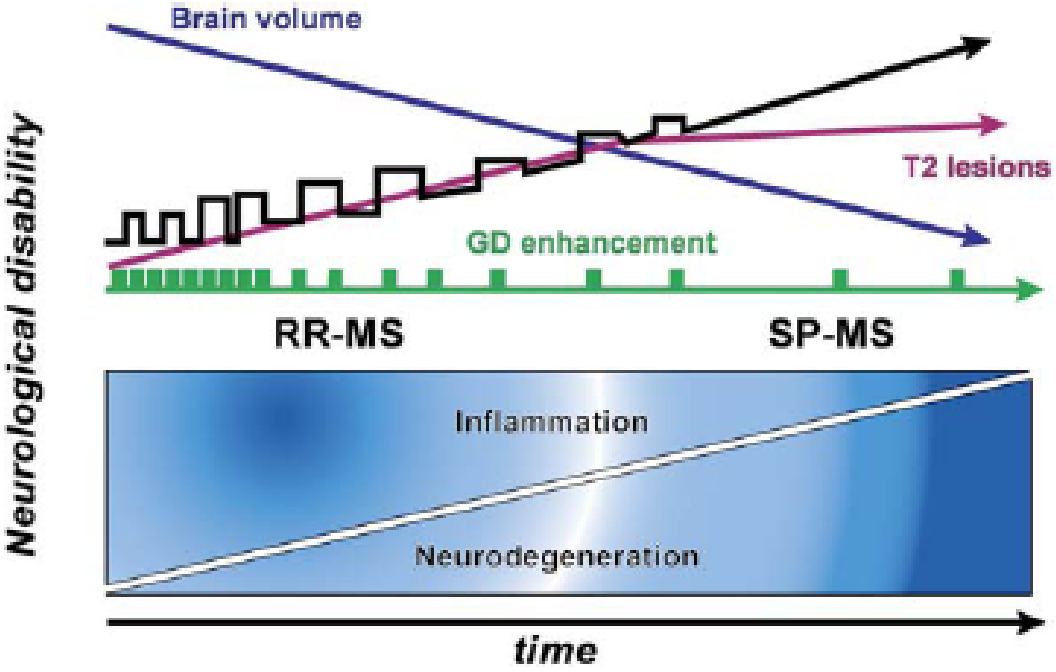


Figure 1.1 Natural history of MS in terms of clinical presentation, underlying mechanism and neurological disability measured from MRI. [2]

The unpredictable pattern in type, frequency, and severity of symptoms as well as progression to disability is the hallmark characteristic of MS. As a consequence of these clinical characteristics, MS presents as a complex disease that is very difficult to manage [3, 4]. MS patients, not only have to deal with current symptoms and the resulting disability, but also live with fear about progression of the disease. To date, there is still no cure for MS. Most pharmacotherapy is for symptom management (e.g., fatigue, spasticity), except for a subgroup of patients for whom there are medications, such as interferon- β (INF- β), which are approved by the FDA as primary disease modifying therapies (DMT). However, the long-term effect and impact of DMT on MS progression has yet to be determined.

1.1.2 Clinical Course of MS: Subtype and Disease Progression

The clinical course of MS consists of two forms: (1) relapses of acute neurological symptoms followed by a partial or complete remission, and (2) progression with irreversible worsening of symptoms and signs [3-5]. Four main subtypes for MS are defined based on whether clinical symptoms are relapsing or progressive in nature: (1) relapsing-remitting (RRMS), (2) secondary progressive (SPMS), (3) primary progressive (PPMS) and (4) progressive relapsing (PRMS) (see Figure 1.2)[1]. RRMS, the most common subtype that affects about 85% of MS cases, is characterized by relapses (symptom exacerbations) followed by varying degrees of recovery with a stable course between relapses. Approximately 50-80% of RRMS patients progress to SPMS within 10 years. SPMS is characterized by relapses with incomplete recovery and a progressive course (e.g. increasing disability) between relapses.

The primary distinction between RRMS and SPMS is what happens between relapses. There is a stable course (little if any worsening) between relapses in RRMS; whereas there is observable progression of disability between relapses in SPMS. In contrast to the relapsing forms of MS, about 10-15% of new MS cases have a steady progression of symptoms from onset without any exacerbation and remission, termed PPMS. A small number of PPMS patients will go on to develop PRMS, which is characterized by a progressive disease course from onset with appearance of relapses[1], which are usually rare and occur earlier in the disease history.

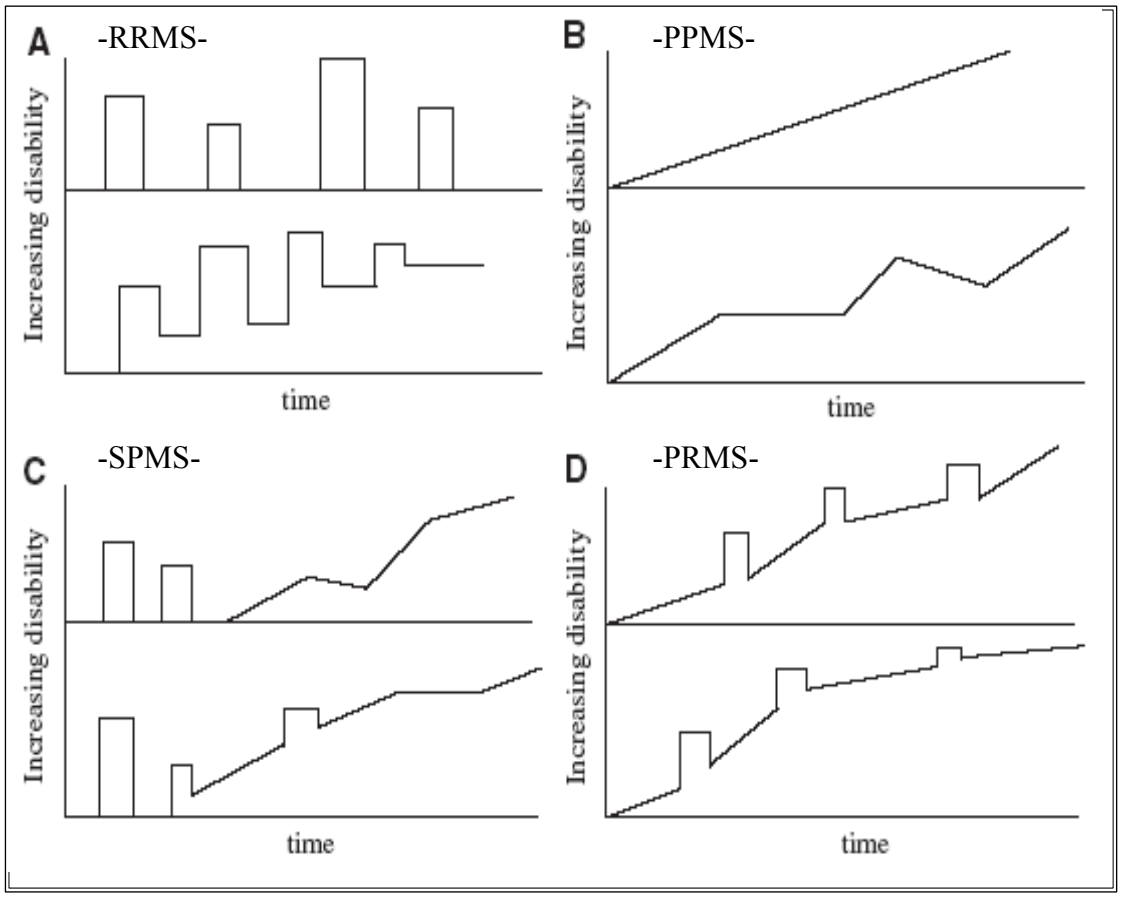


Figure 1.2 Graphical summary of MS subtype A: Relapsing-remitting MS (RRMS), B: Primary-Progressive MS (PPMS), C. Secondary-Progressive MS (SPMS), and D. Progressive-Relapsing MS (PRMS)[1]

Because the clinical course of MS is variable between and within patients, identifying reliable prognostic factors has been challenging. Lubin [1] summarized the broad clinical guidelines for favorable and unfavorable indicators in MS (Table 1.1). Characteristics of relapses in the first year (low number, little residual disability after each relapse and long time between first and second relapse), early age of onset, female sex, and monosymptomatic onset with optic neuritis or sensory symptoms have consistently been associated with a better prognosis. On the other hand, a

progressive course from onset is associated with the worst outcome [1, 6]. Although these indicators have been widely accepted for clinical applications, whether or not they are actual predictors for progression of MS is debatable.

In a recent systematic review, Langer-Gould et al. reported that early disease characteristics (degree of remission after relapses, frequency of relapses) but not age of onset nor patient gender, are the most consistent predictors of long-term disability in RRMS patients[6]. Other investigators examined the age at which patients reached disability milestones according to their clinical subtypes. They found that patients, with either RRMS or PPMS, were comparable with respect to the age at which they reached an assigned disability milestone, especially a more severe one [5, 7-10]. They suggested that clinical subtype and course of MS may be mainly age-dependent. The degree of long-term disability increases with age, which reflects an age-related degenerative process that is independent of previous relapses and age at onset [5, 7-11]. Their findings also support the concept that MS should be viewed as a single disease with different clinical phenotypes, rather than several distinct diseases.

Although the course of MS is very difficult to predict, there are important clinical stages associated with disease progression that can be recognized by neurologists and patients, such as physical disability. Various studies show that 50 to 80% of MS patients are unable to perform work tasks and usual housework after 10 years of disease. Approximately 32 to 76% of MS patients require a walking aid; and 11 to 29% are bedridden after 15 years of illness [12-16]. Patient Determined Disease Steps (PDDS) is a simple and reproducible assessment of functional disability in MS[17, 18]. It primarily evaluates ambulation of MS patients on a scale of 1 to 9 (from a

stage of normal motor function to bedridden, see Appendix A); a broad scale of disability categories that is particularly useful for studies targeting long-term outcomes of MS.

Table 1.1 Favorable and Unfavorable prognostic indicators in MS

Favorable indicators	**Unfavorable indicators**
Early age of onset	Later age of onset
Female sex	Male sex
Optic neuritis as presenting episode	Progressive course from onset
Sensory symptoms as presenting episode	Involvement of cerebellar or motor function
Little residual disability after each exacerbation (i.e. Excellent recovery)	Poor recovery from exacerbations
Long inter-exacerbation period	Frequent exacerbations
Acute onset of symptoms	

1.1.3 Epidemiology and risk factors of MS

The prevalence of MS is approximately one per 1000 with female to male ratio of 1.5 to 2.5 [19]. MS symptoms generally appear in early adulthood with the diagnosis peaking between the ages of 20 and 45 [3, 4, 12, 20-22]. Because of the chronic nature of this disorder, there are 250,000 to 350,000 cases of MS in the United States at any point in time, with 45% of these being older than 55 [23]. The disease contributes to about 92,000 hospitalizations per year with an estimated annual medical cost of $2.5 billion in the U.S. [4, 24].

The etiology of MS remains elusive and is thought to be a complex interaction between genetic and environmental risk factors [25-27] . It is acknowledged that there is a strong genetic component in development of MS. This is supported by evidence of

increased MS incidence in immediate family members, and association with certain HLA allotypes [28-30]. Further, Caucasians, particularly of European/Scandinavian descent, are more likely to develop MS compared to people of African and Oriental descent [12, 24, 31]. However, monozygotic twin and familial studies consistently show that genetic factors contribute to approximately 30% of the risk [12, 32, 33]; the remainder is thought to be associated with non-inherited factors.

Parallel to the ongoing research on the genetic risk for MS, research for possible environmental risks has investigated various infectious agents, toxins, and vaccinations. Infectious agents have been suggested to be the most plausible candidates among non-inheritable factors [26, 34]. Many latent viruses, certain herpes and influenza viruses, which have antigens close to the structure of myelin basic protein (MBP), can trigger an autoimmune process by activating T-cells specific to MBP[35]. Infection with a certain common viruses in late childhood and adolescence is associated with increased risk of MS when compared to the same infection in early childhood[36, 37].

In this context, "hygiene hypothesis" was originally proposed [38]. According to the hygiene hypothesis, lack of intense infections (not a specific agent) in industrialized countries due to improved hygiene and advanced medicine may alter the human immune system and leads to autoimmunity or allergy. It was postulated that exposure to several infectious agents early in life is protective against the development of MS in susceptible individuals. On the other hand, there is compelling evidence from epidemiological and biological research implicating Epstein-Barr virus (EBV) as a specific infectious agent that triggers MS[26, 27, 36, 37, 39, 40]. Higher EBV

antibody titers have been found in MS patients compared to controls, in a number of cross-sectional studies[39, 41-43]. Levin et al. [44] conducted a nested case-control study using serum samples stored in the Department of Defense Serum Repository to demonstrate a temporal relationship between elevated EBV titers and risk of MS. They also found an age-dependent relationship between EBV infection and development of MS. That is, infection during adulthood (over 25 years) exhibited a greater risk for MS, compared to infection at the age of 20 or younger. The age of EBV infection was also investigated in the form of infectious mononucleosis (IM) that is a strong marker of late age EBV infection. A meta-analysis using 14 studies of IM and risk for MS showed that the pooled relative risk of MS was 2.3 (95% CI: 1.7-3.0) in patients with IM [36]. The authors concluded that EBV infection manifesting as IM in adolescents and young adults is a risk factor for MS. A recent study, in which postmortem brain tissue (brain-infiltrating B cells and plasma cells) was examined, showed evidence of EBV markers in 21 of 22 examined MS cases[40]. Overall, the current evidence supports EBV infection as a strong risk factor for MS and suggests that other environmental risk factors, such as smoking, might also play a role in disease development [27, 34].

Data from a variety of studies have suggested that other environmental factors might reduce, not increase, the risk for MS. One of the most striking epidemiological features of MS is the positive gradient of MS prevalence with increasing latitude. That is, MS prevalence increases with increased distance from the equator in both hemispheres. The national prevalence survey in Australia demonstrated a six-fold increase in age-standardized MS prevalence from tropical Queensland to Tasmania at

latitudes 41-42˚S[45, 46]. Similar findings of a positive gradient of MS prevalence with increasing latitude were also reported within the United States and Europe [31, 47]. These marked geographical differences were thought to be associated with environmental factors that vary with latitude, such as sunlight intensity and dietary habits. Cumulative evidence from epidemiological, genetic, biological and experimental studies points to ultraviolet radiation (UVR) directly or via synthesis of Vitamin D as an important protective factor for MS development (discussed in detail in the following sections).

In summary, the etiology of MS is a complex or multi-factorial process that does not result from a single genetic or environmental factor. Although there are some good candidate genes under investigation, the specific MS genetic susceptibility has not been isolated. Among a variety of environmental risk factors, infectious agents have emerged as leading triggers for MS development. And more recently, UVR/vitamin D appears to provide some degree of protection against MS. There is no doubt that more work has to be done to identify genetic, environmental factors and their interactions for developing MS.

1.2 Ultraviolet Radiation and MS

Solar ultraviolet radiation (UVR) is well known for its links with skin cancer. The adverse outcome of excessive exposure to UVR on human health has been of concern and led to many health promotion activities aimed at reducing human UVR exposure. Apart from the adverse effects of UVR exposure, there is a rapidly evolving body of evidence for its beneficial effect in preventing a variety of diseases

in humans, including many cancers and autoimmune diseases[48-51]. UVR-induced immnosuppression was proposed as the mechanism to attenuate the autoimmune process underlying MS[50-55]. The following sections review the general background of UVR and evidence for the potential protective effect of UVR on MS.

1.2.1 UVR, risk factors, and skin pigmentation

UVR forms part of the spectrum of electromagnetic radiation emitted by the sun. It can be divided into three categories according to wavelength: UVA 400-315 nm, UVB 315-280 nm and UVC 280-100 nm[56]. UVR is absorbed by atmospheric ozone, with maximum absorption for UVC band and minimum absorption for the longer-wavelength UVA band. Thus, almost all UVA passes through the atmospheric ozone, while about 10% of UVB and almost no UVC reach the surface of the earth[57]. As a result, UVA and UVB are the two main sources of solar UVR to which humans are exposed. The depth of penetration of UVR into the skin increases with increasing wavelength. Thus, UVA can penetrate into the skin more deeply than UVB. While UVA is involved in the etiology of some diseases, recent studies show that it is UVB (the middle range of the wavelength) that has particular effects on DNA and cutaneous chromophores, which subsequently have effects on carcinogenic processes and the immune system[58].

The amount of UVR impinging on the body depends on several environmental and behavioral factors[55]. First of all, since UVR is absorbed by atmospheric ozone, the concentration of ozone affects the amount of UVR reaching the earth, particularly UVB. Secondly, the intensity of UVR varies with latitude, i.e. the angle through which the sun rays pass through the atmosphere. There is more intense UVR and a

greater proportion of shorter wavelengths at low latitude (close to the equator). Annual UVR expresses a down gradient of changes in erythema dose from a minimal 6,000 in Hawaii (latitude 20 degrees North) compared to 2,500 in Spain (latitude 40 degrees North) and 1,500 in Belgium (latitude 50 North) [59]. In addition to latitude, increasing altitude is associated with increased UVR intensity resulting from decreased air mass. Other environmental factors such as time of day, seasons, cloud cover, air pollution, dust, haze and many organic compounds can further alter the amount of UVR reaching ground level and thus human skin [55]. Scotto and his colleagues[60] studied UVR measurements in the United States and found that, in a multiple regression model, 70% of the variation in UVR intensity is accounted for by latitude, and 90% after including altitude or cloud cover in the model. As a result, while latitude provides a rough approximation to variation in UVR intensity worldwide, considering other environmental factors may further increase accuracy of UVR measurements.

When assessing human UVR exposure, not only do we have to consider different environmental factors for ambient UVR, but we also need to take into account behavioral factors at an individual level. Time spent outside and the amount and frequency of sunscreen used contribute to the variation in UVR exposure among individuals. Gies et al [61] found that personal UVR exposure varies between 5 to 15% of daily total ambient UVR. Within population groups, individual exposure may vary from one-tenth to ten times the average exposure at a particular location. Thus, it is possible to have high individual UVR exposure even in the areas of low ambient UVR and vice versa.

In addition to the magnitude of UVR exposures, individual skin pigmentation plays an important role in the effect of UVR on human health. That is, any UVR-induced biological activities through skin exposure are altered by the level of the skin pigmentation. Deeply pigmented skin provides strong protection against the sun and reduces harmful effects of excess UVR exposure. On the other hand, beneficial effects of UVR, such as UVR-induced vitamin D synthesis, decrease with increased pigmentation. In order to obtain sufficient UVR dose for vitamin D synthesis, a certain exposure time is required; such exposure times are six-fold and two-fold longer for very brown and Asian skin, respectively, when compared to fair skin. Therefore, to examine any health outcome associated with UVR, one should control for the type of skin. The most common classification of skin type for UVR sensitivity is the Fitzpatrick scale [62](Table 1.2). The scale classifies skin type according to skin color, ability to burn and tan.

Table 1.2 The Fitzpatrick scale of skin type

Type	Description
I	Extremely fair skin, always burns, never tans
II	Fair skin, usually burns, tans slowly and with difficulty
III	Medium skin, sometimes mild burns, gradually tans
IV	Olive skin (typical Mediterranean Caucasian skin) , rarely burns, tans with ease
V	Moderately pigmented brown skin (mid-eastern skin types), never burns, tans very easily
VI	Markedly pigmented black skin, never burns, always tans

1.2.2 UVR and the immune system

New insights into the area of photoimmunology have suggested that UVR could attenuate the autoimmune process that underlies MS [52]. Recent research indicates that UVR has inhibitory function on both local and systemic immune systems [50, 51]. The mechanisms of immunosuppression by UVR are diverse. Enhanced vitamin D synthesis is one of the postulated pathways for the beneficial effect of UVR exposure; it is the subject of current novel research in the field of, not only autoimmune diseases, but also cancer and cardiovascular diseases (discussed later). Both UVA and UVB were found to have direct immunosuppressive effects in humans [53, 54]. Specifically, UVB modulates several cytokines and upregulates T regulatory cells [50] that are protective against the development of autoimmune diseases. Second, the immunosuppression of UVR may result from inhibition of melatonin production[63]. Melatonin has been shown to have an adverse role in autoimmune diseases through promoting serum level of Th1 cytokines, which may subsequently enhance Th1 cell-mediated immune responses[50, 54]. Skin exposure to UVR or light itself was found to be associated with suppression of melatonin production and this decrease is hypothesized to modulate the autoimmunity underlying MS[50, 54].

In summary, current immunological evidence supports the potential role of UVR in protecting against MS development; however, the mechanism(s) through which UVR affects the immune function needs further investigation.

1.2.3 UVR and MS prevalence/Incidence

The link between MS and UVR originated from early ecological studies showing a marked geographical gradient of increased MS prevalence with increased

distance from the equator in both hemispheres. Within the United States, there is at least a two- to three-fold gradient of increasing MS prevalence with increasing latitude [31, 47]. This striking observation has suggested that certain MS-determining environmental risk factors are somehow linked to latitude.

Acheson and his colleagues [64] were the first to report latitude-linked risk factors and suggested that solar radiation might be protective against developing MS. They found a strong inverse correlation ($r = -0.8$) between MS prevalence and the average annual hours of sunlight among US veterans with MS. Other ecological studies also showed the same inverse correlation between MS risk and solar radiation[65, 66], and in one of these studies the inverse relationship between UVR and MS risk was stronger than the positive correlation observed for UVR and malignant melanoma incidence [65]. The association between MS risk and solar radiation was also supported by the finding of an inverse correlation between MS prevalence and altitude (a marker of sunlight intensity) in Switzerland [67]. Kurtzke (1967) found that in the high altitude ($\geq$ 2000 m) districts MS prevalence was lower than that in the low altitude ($\leq$ 1000 m). However, the potential protective role of sunlight exposures in MS was not intensively investigated until the past twenty years, when a rapidly developing body of evidence indicated the function of ultraviolet (especially UVB irradiation) as an immunosuppressant in humans and other mammals.

1.2.4 Current Evidence for association between UVR and MS risk

In 1997, McMichael and Hall [52] hypothesized that UVR attenuates the autoimmune process underlying MS through suppression of immune function, and its effect is maximal at low latitude. Several recent studies with different approaches

have further supported and strengthened this hypothesis. Freedman et al.[68] used death certificates to study the association between sunlight exposure and MS mortality. They found that residence in high sunlight areas (based on annual mean of solar radiation) and outdoor occupation were associated with lower mortality. A few case-control studies, in which MS patients and controls were asked to recall the time they spent in the sun at different age periods before MS onset, allowed estimation of individual UVR exposure. In Tasmania, Van der Man et al. studied 136 MS cases and 272 community controls and found a strong protective role of past childhood sun exposure in MS risk [69]. Specifically, they observed a stronger effect of winter sun exposure at the ages of 6-15 years. In another study of 152 MS cases and 402 matched controls in northern Norway, the time spent in the sun during childhood and adolescence, particularly at ages 16-20 years, was associated with a reduced risk of MS [70].

In a study of twins, the authors compared childhood sun exposures among 79 disease-discordant monozygotic twin pairs, using a sun exposure index generated from self-reported exposure. They found a strong inverse association between frequency of sun exposure and the occurrence of MS, and sun exposure-related activities during childhood seemed to be protective against MS within twin pairs. This study indicates that the protective effect of UVR is independent of genetic susceptibility to MS [71].

1.3 Vitamin D and MS

As discussed above, one postulated biological mechanism, and perhaps the most likely one, is that UVR attenuates MS risk through the biosynthesis of vitamin D. That is, increased solar UVR exposure enhances the body's own production of vitamin D, which is protective against developing MS. Earlier epidemiological studies reported lower MS prevalence in the Atlantic coast of Norway where there was high consumption of fish/fish oil, a rich source of vitamin D, compared to inland [72]. Agranoff and Goldberg observed that regions with low-intensity solar radiation and inadequate dietary vitamin D correlate with regions of high MS prevalence [73]. Their group was the first to propose that a vitamin D derived metabolite might have a role in reducing MS risk. Recently, compelling evidence from biological and experimental studies has recognized vitamin D as a physiologically important immune modulator, which accordingly might have a beneficial effect on an autoimmune disease such as MS. Consistent with epidemiological observations (MS prevalence and sunlight), along with strong biological plausibility, Hayes et al [74, 75] hypothesized that vitamin D is a natural inhibitor of autoimmune-mediated processes underlying MS. Since then, growing evidence, discussed below, supports this hypothesis and a role for vitamin D in MS; not only its ability to prevent the occurrence of the disease, but in modifying its clinical course [50, 69-71, 76-84].

1.3.1 Vitamin D Metabolism [76, 80, 85, 86]

Vitamin D is produced by two distinct pathways: 1) the majority (80-90%) is synthesized in the skin tissue after exposure to direct sunlight and UVR; and 2) the remaining (10-20%) is provided by direct intake of vitamin D through diet that includes fish liver oil, fatty fish like herring, mackerel, and sardines, fortified foods

and vitamin D supplement. Although the major determinant of vitamin D status is skin exposure to UVR, the dietary intake of vitamin D and/or its supplement is particularly important during the winter season in the high latitude areas where, the ambient level of UVR is insufficient for the cutaneous production of vitamin D [87].

Radiation in sunlight, mainly the UVB radiation, photolyzes provitamin D in the skin to previtamin D, which is then converted to Vitamin D through a thermal process. Vitamin D is then converted by 25-hydroxylase enzyme in the liver to 25-hydroxy vitamin D (25(OH)D), which is the major circulating metabolite and a biomarker of vitamin D in human serum.

25(OH)D is biologically inert and is further converted, mainly in the kidney, by the enzyme 1α-hydroxylase to 1,25-dihydroxy vitamin D ($1,25(OH)_2D$), the biologically active form of vitamin D. This hormone has been reported to have a variety of biological impacts, including modulation of calcium homeostasis and immune responses [75, 76, 80, 88-91]. Ultimately, excessive amounts of 25(OH)D and $1,25(OH)_2D$ are metabolized by 24-hydroxylase and excreted in the form of calcitroic acid by the kidney.

Vitamin D, a lipid-soluble vitamin, is best known for its role in modulating calcium homeostasis and being beneficial for the skeletal system. Its long-term deficiency is linked to conditions such as osteomalacia and osteoporosis [85]. Recent evidence also points toward UVR exposure/vitamin D as having beneficial effects on susceptibility to and outcome of a variety of cancers as well as many autoimmune diseases, including MS and type 1 Diabetes.[48, 50, 92-94].

1.3.2 Vitamin D and the Immune System

Vitamin D metabolites are transported by vitamin D-binding protein (DBP) to a number of target organs. Once in a target cell, $1,25(OH)_2D$ dissociates from DBP and binds to the vitamin D receptor (VDR) to exert its biological actions through VDR-mediated gene expression [95]. The fact that many constituent cells in the CNS were found to have VDR suggests some function of vitamin D in the CNS [96]. VDR has been reported in the oligodendrocytes of the MS patient brain [97, 98]. VDR expression was also reported in several cells of the immune system including T-lymphocyte, monocyte, and antigen presenting cells such as macrophages and dendritic cells [99, 100]; suggesting that $1,25(OH)_2D$ has a role in the regulation of the immune system.

The mechanism(s) by which $1,25(OH)_2D$ exerts its biological actions on the immune system have been extensively studied both *in viv*o and *in vitro*. Although multi-leveled and complex, what is generally acknowledged is the inhibitory effects of $1,25(OH)_2D$ on: 1) T lymphocyte proliferation; 2) oligodendrocytes maturation; 3) B cells antibody secretion; and 4) pro-inflammatory cytokine production including IL-12. In addition, $1,25(OH)_2D$ enhances T regulatory cells activities [76, 80]. Therefore, $1,25(OH)_2D$ appears to be a potent immune modulator and potentially an anti-inflammatory mediator, the benefit of which in suppressing MS activity and reducing its severity, if proven, would be unequivocal.

1.3.3 Experimental autoimmune encephalomyelitis (EAE) and UVR/ Vitamin D-related treatment

Experimental autoimmune encephalomyelitis (EAE) is a useful animal model, often using rodents, to study patho-physiology of human MS. Administration of $1,25(OH)_2D$ before EAE-induction, or after immunization but before the appearance of symptoms, has been found to prevent the onset and development of EAE in rodents in several studies [89, 101-103]. Even in animals with EAE and clinically active disease, $1,25(OH)_2D$ was able to slow down the progression and reduce the severity of the disease [89] Additionally, a diet without or with inadequate vitamin D aggravated clinical symptoms of EAE [89, 104]. Calcium may play an important role in the immunosuppressive function of $1,25(OH)_2D$ in EAE. It was found that the lower the calcium intake, the higher the $1,25(OH)_2D$ dose needed to prevent EAE [90]. Treatment directly with vitamin D, a precursor of $1,25(OH)_2D$ was found to effectively inhibit EAE, though in female mice only, not the males. These data suggest potential gender differences in vitamin D metabolism and/or function[79]. Several reports also indicated that treatment with synthetic $1,25(OH)_2D$ analogs inhibited and prolonged the time to EAE onset, and it decreased disease progression and severity. A 2003 article reported that the combination of the $1,25(OH)_2D$ analogs and INF- β had a synergistic effect in protecting against EAE, hence having a potential therapeutic use for clinical intervention in MS[103].

1.3.4 Vitamin D and MS risk

Most of the earlier evidence in support of the protective effect of vitamin D in MS development resulted from a variety of observational studies of MS and UVR. In a recent case-control study in Norway, increased outdoor activity during summer, and

cod liver oil and fish consumption (≥three times a week) in childhood and adolescence, were found to decrease MS risk [70]. Two recent longitudinal studies provide additional evidence to support the protective effect of vitamin D on reducing MS risk. The first study investigated the effect of vitamin D intake on MS risk in two large cohorts of U.S. nurses [83]. They found that women with vitamin D intake of 400 IU/d or more had about 41% reduction of risk for developing MS, compared to those without intake of vitamin D. In a subset of their population, where blood samples were available, the authors also found a positive correlation between blood levels of 25(OH)D and vitamin D intake from supplement and food; evidence for the validity of the dietary questionnaire [105]. Another study investigated 25(OH)D levels in healthy young military adults from the Department of Defense Serum Repository [106]. The investigators compared 25(OH)D levels before MS onset in the cases and their two matched controls. Among Caucasian men, individuals with high 25(OH)D levels (greater than 100 nmol/L) had low risk of developing MS, and the association was stronger for 25(OH)D level measured before compared to after age 20. Similar results were not found among African Americans or Hispanics soldiers, and that was attributed to the small size of these subgroups.

Evidence from genetic studies also supports the association between vitamin D and MS risk. As discussed above, $1,25(OH)_2D$ exerts its biological function only after binding to the VDR. Fukazawa and his group [107, 108] found that Japanese MS patients have over representation of one allele at the VDR locus, suggesting that the VDR gene may be associated with MS susceptibility. Such an association was also found in an Australian population[109], but it was not confirmed by other studies [110-112].

Recently, the expression of the MS-associated HLA allele, the major determinant locus in genetic susceptibility, was found to be regulated by vitamin D[113]. This study provides evidence of a connection between vitamin D and genetic features of the disease.

1.3.5 Vitamin D and MS progression

Although 1, 25-$(OH)_2$D has been found to block disease progression using the EAE model, no large clinical trials have been completed to examine the potential effects of vitamin D in patients with MS. One reason might be concerns about the safety of vitamin D supplementation. It is known that excess intake of vitamin D will result in hypercalcaemia and its subsequent complications, including renal and heart failure. Only a few case series studies of vitamin D therapy in MS patients have been conducted, and those were to test safety instead of effectiveness of vitamin D supplementation. Two of these studies found that the use of supplemental vitamin D appeared to reduce relapse rate and delay clinical progression of the disease [114, 115]. Other small sample studies also showed an increase of serum 25(OH)D and anti-inflammatory cytokines as well as a decline in MRI lesions in MS patients who received vitamin D supplements [84, 116]. Several efforts have been made to correlate serum 25(OH)D levels with clinical MS episodes. Soilu-Hannime et al [117] found that 25(OH)D level was lower during relapses, compared to during remission, in RRMS patients and suggested that vitamin D could be involved in regulating the clinical disease activity of MS. In their recent longitudinal study [118], Soilu-Hannime et al further suggested that regulation of vitamin D and calcium homeostasis are both

likely to be important in MS. Lower levels of 25(OH)D was found to be strongly associated with increasing disability in MS patients in Australia; decreased recent sun exposure was suggested to be a main contributor to the observed low vitamin D level in severe patients [77]. Another recent study investigated associations between both 25(OH)D and $1,25(OH)_2D$ and relapse rate and disability in 267 MS patients[119]. The authors found that high 25(OH)D, but not $1,25(OH)_2D$, levels were associated with greater odds of remaining relapse-free in a subgroup of RRMS patients with disease duration less than or equal to 5 years. The authors also found a negative correlation between 25(OH)D and disability scores. Because these cross-sectional studies only examined disability level and serum samples at one time, it is difficult to determine if vitamin D status truly influences MS progression.

1.3.6 Timing of Vitamin D Influences

A number of epidemiological observations have indicated that environmental factors influence MS pathogenesis and clinical course at different time periods extending from gestation and the neonatal period, to childhood and adolescence and later to adulthood[120-123]. There is evidence in support of vitamin D, or the lack of it, early in life, as well as during childhood and adolescence, being an environmental contributing factor to MS[69, 70, 120, 124].

The most direct evidence with respect to early life events in MS susceptibility comes from studies of month of birth in several northern countries[121, 124-126]. In these studies, significantly more patients with MS were born in May compared to the population based controls; whereas fewer patients with MS were born in November. Maternal environmental events, related to low sun exposure during pregnancy, thus

low maternal vitamin D levels, have been speculated as a plausible explanation for the month of birth effects[124]. A recent study observed interaction effects of MS susceptibility gene and month of birth on MS risk, suggesting potential gene-environment interactions that could occur during gestation or shortly after birth[127]. A possible influence of month of birth on the clinical course of the disease has also been investigated[128-130]. MS phenotype and potentially disease progression were found to be associated with month of birth[128, 129].

Several studies investigating the effects of past sun exposure and vitamin D intake on MS risk have pointed to exposures during childhood and adolescence as being particularly important[69-71]. Van de Mei et al. [69] found that the protective effect of sun exposure in southern Australia was the greatest when it occurred between the ages of 6-15 years. In line with these findings, some early migrant studies and space-time clustering research also pointed to early childhood and/or adolescence before the age of 15 years as critical periods where environment affects MS susceptibility[131-134]. However, whether or not environmental exposures during childhood and adolescence could influence the clinical course of the disease remains unclear.

1.4 Skin Type and MS Progression

It is known that skin pigmentation influences vitamin D synthesis through UVR. Increased pigmentation (darker skin tone) leads to reduction of vitamin D synthesis [135]. When examining a potential effect of UVR exposure/vitamin D on MS progression, it is important to take into account the skin type of an individual. Several studies have suggested that African American patients with MS suffer worse outcomes compared to Caucasian patients [136, 137]. Whether or not sub-optimum

vitamin D synthesis due to increased skin pigmentation contributes to this difference in clinical presentation of MS requires further investigation. Woolmore et al. [138] conducted a cross-sectional study to examine if MS outcome is associated with skin type and UVR exposure. They found that lighter skin type is associated with less disability in female MS patients with disease longer than 10 years, but no association between sun exposure and disability. Small sample size, measurement errors and residual confounding [cumulative sun exposure was measured in only two age periods with a relatively wide range (≤16 and 16-40 years) were significant limitations of this study.

1.5 Summary of Backgrounds and Study Rationale

In summary, vitamin D is a potent immune regulator that has been shown to prevent the occurrence and delay progression of the disease in animal models of MS [76, 80, 89, 101-104]. In humans, there is evidence to support the protective effect of UVR/vitamin D against developing MS, but little is known about its role in modulating the clinical manifestation of this inflammatory and neurodegenerative disease. Table 1.3 summarizes the observed effects of UVR/vitamin D in reducing MS risk and progression. Vitamin D might have an effect for short-term clinical activity of disease, such as reducing the frequency of and delaying relapses. However, evidence about the effects of UVR/vitamin D on age at onset and long-term outcomes of MS is not available and research is warranted to examine this important topic that may have significant clinical application.

Although a longitudinal study or a randomized clinical trial would be the optimal epidemiological design to prospectively determine whether UVR exposure or vitamin D status mediates the clinical course of MS, both designs are very costly and time-consuming, especially to study long term disability. A cross-sectional study with questions retrospectively investigating major exposures and outcomes would help us examine this important question more efficiently and could provide support for the more costly designs.

Therefore, we investigated UVR/vitamin D exposures before symptom onset, with focus on exposures in very early life and during childhood and adolescence, and assess their impact on the clinical course of the disease and its long term disability in Veterans with MS. Skin pigmentation and many environmental and behavioral factors associated with UVR exposure/vitamin D synthesis have to be taken into account and analytically investigated. Figure 1.3 illustrates the conceptual models for association between UVR/vitamin D exposures and MS onset and progression and major variables that were collected for the project. We conducted a large cross-sectional study, using a questionnaire designed to assess the different parameters that contribute to UVR/vitamin D synthesis (timing of birth, residential solar radiation, sun exposure behaviors, skin type, and vitamin D intake) and the outcomes of interests (age at MS onset and age at major disability stages) retrospectively. The specific aims of this project were to:

(1) Examine the effect of timing of birth and residential solar radiation of birthplace on age at MS onset.

Hypothesis:

a. Timing of birth is associated with the age of MS onset.

b. The lower the birth residential solar radiation, the younger is the age of MS onset

(2) Examine the effect of estimated UVR/vitamin D before symptom onset (residential solar radiation, past sun exposures, and vitamin D intake) on age at MS onset.

Hypothesis:

The lower the sun exposures and vitamin D intake are, the younger is the age of MS onset.

(3) Examine the effect of estimated UVR/vitamin D before symptom onset (residential solar radiation, past sun exposures, and vitamin D intake) on MS progression.

Hypothesis:

. The lower the sun exposure and vitamin D intake are, the shorter is the time to the defined stages of disability in patients with MS; stages are defined by Patient Determined Disease Steps (PDDS)

Table 1.3 Evidence for effect of UVR/vitamin D on (a) MS risk and (b) MS progression

(a) MS RISK†

Author, year, country	Study Type	Study population	Outcome	Main Exposures	Exposure Ascertainment	OR/RR (95% CI)
van der Mer et al., 2003, Australia	Case-control	136 cases: 272 controls	MS defined by clinical and MRI criteria	Sun exposure (time in sun) at childhood/ adolescent	Interview/questi-onnaire	0.31 (0.16-0.59)
Kampman et al., 2007, Norway	Case-control	152 cases: 402 controls	MS defined by Poser or McDonald criteria	Frequency of outdoor activities[1], sun exposure behaviors, fish consumption[2]	Questionnaire	0.55[1] (0.39-0.78) 0.55[2] (0.33-0.93)
Islam et al., 2007, USA	Twin study	79 monozygotic twin pairs	MS identified through self report	Nine ranked sun exposure behaviors at childhood, e.g. which one spent more time in suntanning[3]	Questionnaire	0.42[3] (0.19-0.83)
Munger et al., 2004, USA	Longitudinal	187,563 female nurses	MS identified through self report with confirmation by neurologist	Diet and vitamin D supplementation[4] (comparing intake of 400 IU/day with no supplemental vitamin D intake)	Questionnaire	0.59[4] (0.38-0.91)
Munger et al., 2006, USA	Longitudinal (nested case-control)	257 cases: 512 controls	MS identified by military physical evaluation boards	Serum 25(OH)D	Serum samples from the Department of Defense Serum Repository	0.38 (0.19-0.75)

(b) MS PROGRESSION

Author, year, country	Study Type	Study population	Outcome	Treatment/ Intervention	Exposure	Results
Goldberg et al., 1986, USA	Case series	16 RRMS patients	Number of relapses	Vitamin D supplement, calcium and magnesium	----	↓ relapse rate
Nordvik et al., 2000, Norway	Case series	16 RRMS patients	Number of relapses/ Expanded disability status scale (EDDS)	Fish oil and vitamin supplementation with dietary advice	----	↓ relapse rate ↓ EDDS score
Mahon et al., 2003, USA	Clinical trial	39 MS patients	Cytokine profile	Vitamin D supplemented vs. Placebo	----	↑ serum 25(OH)D ↑ serum TGF-β1
Samantha et al., 2007, Canada	Case series	12 MS patients	Serum 25(OH)D & calcium/MRI	Calcium/progressively increasing dose of vitamin D	----	↑ serum 25(OH)D ↓ MRI lesions
Soilu-Hänninen et al. 2005, Finland	Cross-sectional	40 MS patients/controls	25(OH)D	------	MRI/EDDS/ Relapse and remitting status	↓25(OH)D among patients with relapses
Soilu-Hänninen et al. 2007, Finland	Longitudinal	23 MS patients/ controls	25(OH)D Intact parathyroid hormone (iPTH)	------	MRI/Relapse and remitting status	↓25(OH)D and ↑ iPTH during MS relapses
Smolders et al., 2008, Netherlands	Cross-sectional	267 MS patients	EDDS/relapse rate/relapses (yes/no)	------	25(OH)D/1,25$(OH)_2$D	Association between relapse (Y/N) and each 10 nmol/L increase of 25(OH)D: OR = 0.49 (0.09-0.91) in RRMS patient with disease duration ≤ 5 years

† Evidence supporting UVR/vitamin D as protective against development of MS also comes from numerous ecological and experimental studies which are not listed here.

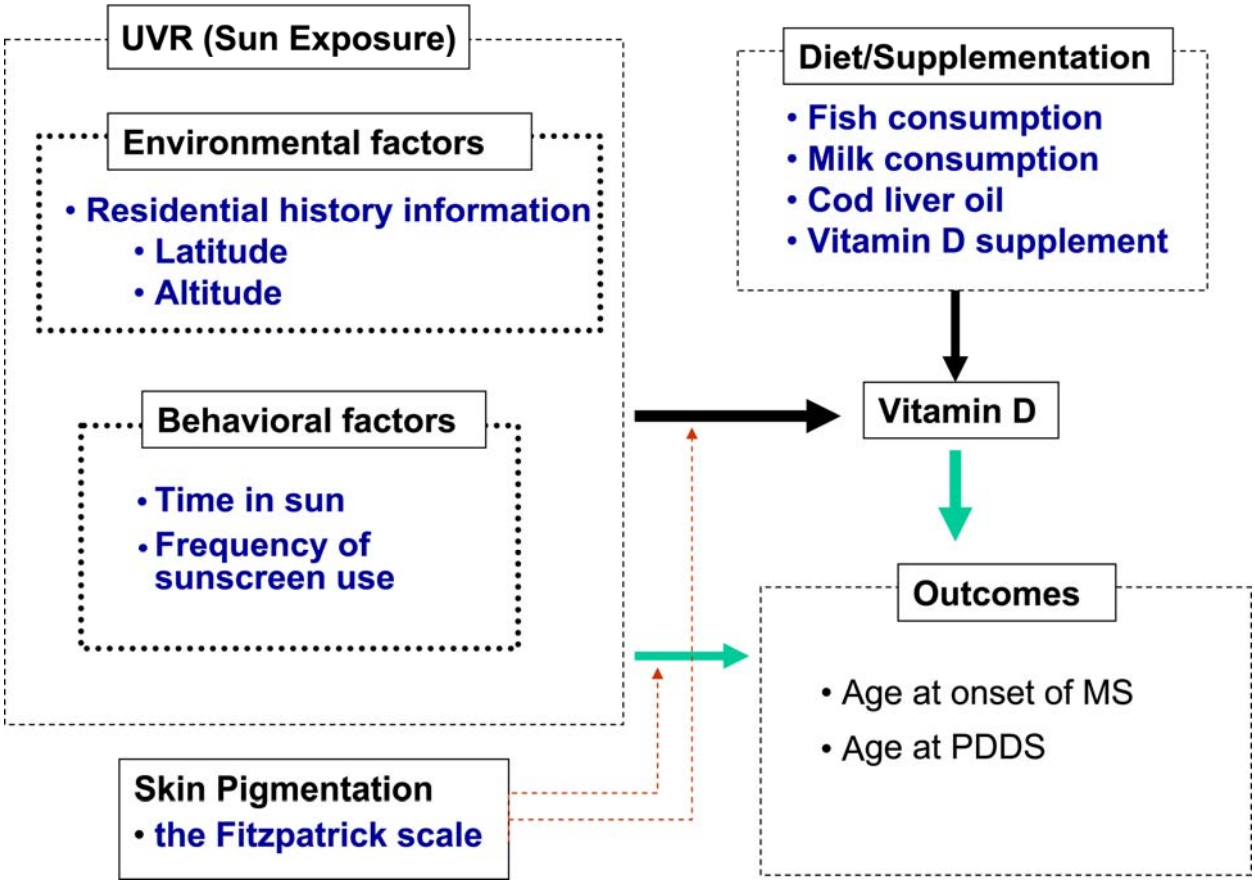


Figure 1.3 Conceptual model with variables collected in the project

1.6 Multiple Sclerosis Surveillance Registry

The Veteran's Health Administration (VHA) funded two MS Centers of Excellence (MSCoE) in 2003. One is located at the Baltimore Veteran Medical Center (VAMC) and the other is shared between the Seattle and Portland VAMCs (MSCoE-East and MSCoE-West). The objectives of these two centers were to improve the access to and quality of care for veterans with MS as well as to conduct

MS-related research to further our understanding of pathology, etiology and treatment of MS.

The Multiple Sclerosis Surveillance Registry (MSSR) was established under the VHA's MSCoE– East in 2007. The MSSR was designed to target a nationally representative and random sample of veterans with MS (oversampling of female veterans with MS) and to obtain detailed MS-specific information directly from the participant-information that is not included in the routine VHA databases. To target a cohort of Veterans with MS for the MSSR, Culpepper and colleagues[139] developed an algorithm to identify MS cases from the VHA database. They first defined a larger MS User Cohort using a 3-digit diagnostic code (ICD-9) from the VHA database for the fiscal years period from1998 through 2006 (N = 34,743). The application of the case finding algorithm helped ascertain cases and refined the MS User Cohort to a more specific cohort – MS Patient Cohort (N = 16,808), which served as the initial population of the MSSR (see Figure 1.5). Details of this method can be found in Culpepper, et al[139].

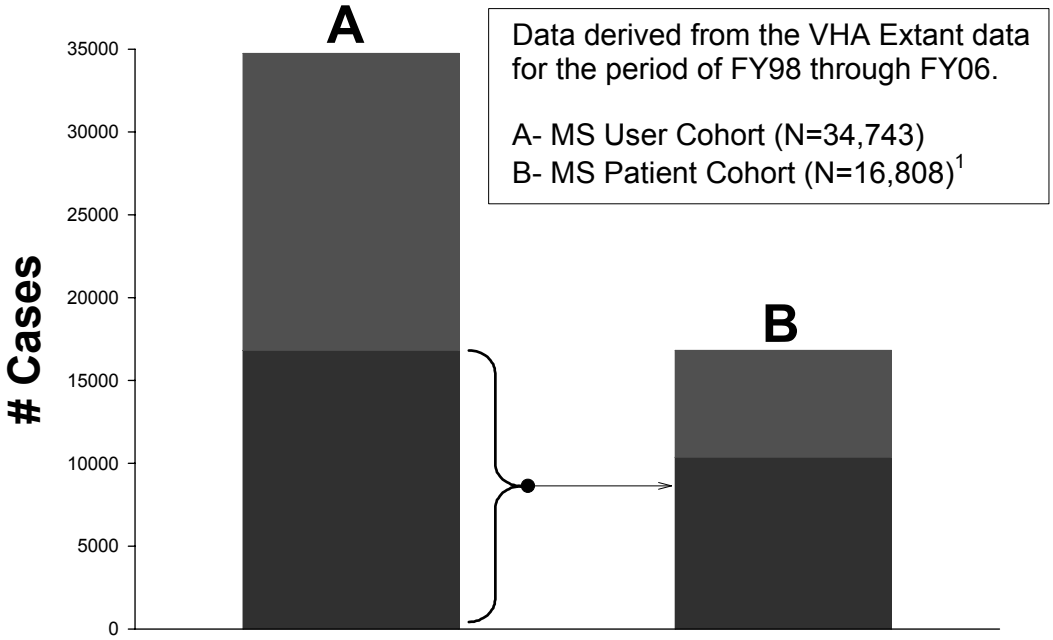
To further identify a sampling pool for the MSSR, Veterans with the following categories, deceased, did not have a valid address, had only one year of extant data, older than 74 years, or were institutionalized, were excluded from the MS Patient Cohort. This process yielded a total of 9,372 veterans who were classified as having MS. Because of budget constraints, for the baseline survey of the MSSR and in order to have sufficient power for the planned analysis, 3,905 Veterans were targeted . A stratified random sample (by geographic region) and an oversampling strategy (sampling all female veterans) were applied to achieve a nationally

representative MS Veteran cohort and to partially control for gender and geographic variations in MS prevalence.

As of August 2008, a total number of 1,393 MS Veterans have responded to the MSSR baseline survey, and 1,368 of them completed it, bringing the overall response rate to approximately 35%. Although the response rate for the baseline survey was lower than anticipated, our preliminary analysis showed that there were no meaningful differences between participants and non-participants that could introduce bias into the study[140]. In addition, the MSSR participants appear to be very similar to the MS Patient Cohort with respect to their demographic characteristics. Thus, MSSR participants are a representative sample of Veterans with MS and the findings from the baseline survey can be generalizable to the MS Patient Cohort, of 34,743 in 2006.

The MSSR baseline survey [140] obtained detailed information on demographics, MS clinical data and MS-related outcomes. MS clinical data included the respective ages at onset of symptoms and at diagnosis, MS subtype and detailed reports on usage of DMT (type of DMT, pattern of the usage and side effects). Patient-reported symptoms, functional status, disability at the time of the baseline survey and quality of life were also included to provide primary and secondary outcomes for MS-related research. All the MSSR participants were willing to assist with future research and agreed to be re-contacted with follow-up questionnaires. To enrich and expand the MSSR database for further research, follow-up surveys will be administered annually to collect longitudinal data on clinical outcomes and data from other emerging areas

of MS research. The one-year follow up survey used in this project was distributed between March and October 2009 (see 2.2 Study Population).



1- The sampling pool for the MSSR comprised 10,372 cases identified by the algorithmwho had a valid address. There were 6,442 cases eliminated from the sampling pool: deceased - 2,986; no address - 2,490; entered cohort in FY2006 or had only 1-yr of data - 1,368; greater than 74 yo - 409; institutionalized - 189.

Figure 1.4 Summary of VHA MS cohorts as classified by the statistical algorithm[139, 140]

CHAPTER II: GENERAL METHODOLOGY

2.1 Study Designs

This study was a retrospective cross-sectional, mail-based survey of participants in the Veterans' Health Administration (VHA)- MS Surveillance Registry (MSSR). The MSSR, established in 2007 under VHA's MS Center of Excellence –East (MSCoE-East), consists of a cohort of MS patients who completed the baseline survey and agreed to be re-contacted regarding participation in future survey and MS-related studies. The baseline survey was designed to obtain data on demographic/clinical history, functional and quality of life outcomes as well as DMT use. To collect longitudinal data on the original cohort of MSSR participants as well as to obtain information for this project, the one-year follow up survey was mailed in 2009. Along with this follow up survey, we sampled another new cohort for MSSR to recruit un-approached MS Veterans, to expand the size of the MSSR, and to increase the sample size for this study. The Institutional Review Board (IRB) of the University of Maryland, Baltimore and the Baltimore VA Medical Center's Research and Development Committee approved this study.

2.1.1 Study questionnaire

We prepared a set of questions (Appendix A) regarding the main exposure and outcome variables (see sections 2.5 and 2.6). Timing of UVR/Vitamin D exposures and outcomes were recorded retrospectively at specific age periods. Important covariates, such as history of mononucleosis infection and smoking, were included.

Several other variables, including demographic, were extracted from the baseline survey of the MSSR.

2.2. Study Population

The study population for this project was all participants of the MSSR who have completed the designed questionnaire. The original MSSR cohort with the baseline survey was used for the specific aim #1 with respect to the associations between very early life exposures and age at symptom onset (see 1.6 Multiple Sclerosis Surveillance Registry). The study population for the specific aims #2 and #3 assessing the effects of UVR/vitamin D exposures on the clinical course of MS came from two cohorts, those who completed the one-year follow-up survey from the original MSSR cohort and the new cohort of MS Veterans, which is discussed below.

2.2.1. Original MSSR cohort with one-year follow-up

The one-year follow-up survey, which included all the questions relevant to the present study, was mailed to 1,346 participants who were in the MSSR and had completed the baseline survey. The mailing took place in three phases between March and October, 2009. The mailing was closed on October 15th for purpose of the dissertation. Table 2.1 provides a summary of the response status from this cohort as of October 2009.

Table 2.1 Responses from the original MSSR cohort to the one year follow-up survey

Original MSSR Cohort Response Status	N	%
Total Survey Recipients	**1,346**	**100**
Non-Responders	**598**	**44.4**
Responders	**748**	**55.6**
Participants		
Completed the one-year follow-up survey	702	93.9
Non-Participants		
Declined participation	29	3.9
Undeliverable	2	0.3
Deceased	6	0.8
Blind/unable to complete survey	1	0.1
Did not have MS	4	0.5
MSSR registry only	4	0.5

The response rate was 55.6% among these 1,346 survey recipients, much lower than expected, considering that this cohort had previously completed the baseline survey and agreed to be re-contacted again for a follow-up survey. Among the responders, about 94% completed the survey and some have declined to participate due to various reasons.

2.2.2. New MSSR cohort

In addition to the original MSSR cohort, we sampled another representative cohort of 3,000 MS Veterans from the larger MS Patient Cohort using the same sampling strategies (see section 1.6). The rationale for sampling another cohort is to continuously recruit un-reached MS Veterans and to expand the size of the MSSR. In a disease with such a variable clinical course as MS, it is necessary to have a large number of survey participants to ensure sufficient statistical power for the MS-related research. The mail-survey for this cohort was also conducted between March and October, 2009, simultaneously with the one sent to the original MSSR cohort. Table

2.2 summarizes the response rate for this cohort. As of October 15th, 2009, the response rate for this cohort was 29%, which was slightly lower than our expectation of 30 to 35%. Among the responders, only 72% have completed the survey.

Table 2.2 Response from the new MSSR cohort to the mailed survey

New MSSR Cohort Response Status	N	%
Total Survey Recipients	**3,000**	**100**
Non-Responders	**2,131**	**71.0**
Responders	**869**	**29.0**
Participants		
Completed one-year follow-up survey	626	72.0
Non-Participants		
Declined participation	107	12.6
Undeliverable	29	3.3
Deceased	14	1.6
Blind/unable to complete survey	16	1.8
Did not have MS	64	7.5
MSSR registry only	7	0.8
Not veterans	4	0.4

2.2.3 Total Population from the original and new MSSR cohorts

We combined both cohorts to generate the study sample of 1,328 participants (overall response rate: 30.6%) who had completed the survey. Because of lower than expected response rate, we compared "Participants" and "Non-Participants" for meaningful differences that could potentially introduce response bias into our study findings. Participants are responders who returned and completed the survey documents. Non-participants included those who declined to participate as well as those who never responded. We also assessed how representative our study population was of the general MS Patient Cohort in the VHA system. The findings are summarized in Table 2.3.

Because we are dealing with large study samples, any small differences in a given studied variable between participants and non-participants would lead to a statistical significance. Therefore, we reported actual difference in measures between the two samples and used an absolute difference of 5% or more as criterion for meaningful difference between participants and non-participants. Accordingly, among non-participants, there were more African Americans, fewer males and fewer married, when compared to participants. The actual mean difference of age between groups was 1.6 years, which is probably not clinically meaningful in a cohort of MS patients.

Compared to the larger VHA MS Patient cohort, our study population was very similar with respect to demographics except for gender; our sample had a higher percentage of female Veterans with MS, which is what we expected. That is because our study oversampled female Veterans with MS in the VHA system to ensure a sufficient sample size for gender comparisons with other published cohorts. Overall, although we observed some differences between the Participants and Non-participants in our study population, the magnitude of these differences was not substantial. Most importantly, our study participant sample appears to provide a representative sample of veterans with MS as compared to the larger VHA MS Patient Cohort.

Table 2.3 Comparisons of study participants versus non-participants in the MSSR versus VHA Patient Cohort.

Variable	Participants[1]	Non-Participants[2]	Difference	VHA MS Patient Cohort
N	1,328	3,049		14,215
Age (mean ± SD)	55.9±10.4	54.3±11.9	1.6 years	58.2 ± 11.9
Sex (% male) *	52.3	42.6	9.7%	82.7
Marital Status				
% Never Married	17.3	20.9	-3.6%	16.1
% Married	60.5	52.9	7.6%	61.5
Race				
% Caucasian	71.4	65.7	5.7%	71.2
% African American	11.8	17.8	-6.0%	12.5
Income ($1,000)	33.5±59.1	30.9±44.2	2.6	35.6 ± 58.9
Region				
% Northeast	16.9	16.8	0.1%	18.8
% Southeast	21.2	23.0	-1.8%	21.2
% Midwest	16.9	15.9	1.0%	18.3
% Midsouth	12.5	13.9	-1.4%	14.5
% Northwest	18.7	16.1	2.6%	13.7
% Southwest	13.8	14.2	-0.4%	13.6

1- Participants were those veterans who returned a completed survey and consented to be in the MSSR.
2- Non-participants include veterans who did not respond at all and those who declined participation.

2.3 Mail-Based Survey

We conducted the survey in three phases following the methods of Edward and Dillman [141, 142] to maximize response rate, as was done for the original baseline survey of the MSSR. A packet, consisting of a cover letter describing the project, consent and HIPAA forms, the survey and a self-addressed, postage-paid envelope, was mailed to the study population. During the first phase, all the MSSR participants who previously completed a baseline survey (n = 1,346) and a newly selected random sample of 3,000 MS Veterans were the recipients. Another complete packet (the

second wave of the survey) was originally scheduled to be mailed to all-non-responders approximately one month after the initial mailing. Due to unexpected administrative delays, the packets were sent out three months after the initial mailing. Before the third mailing, to optimize generalizability and response rate, a replacement (n = 161) was randomly selected from the larger MS Patient Cohort to match age, sex and geographic region for all respondents who declined participation in response to the initial and second mailing, including ones who declined, were deceased, or had the wrong mail address. The complete packet was then sent to the replacements and non-respondents about one month after the second wave. Figure 2.1 shows the flow chart of this three-phase mail-based survey.

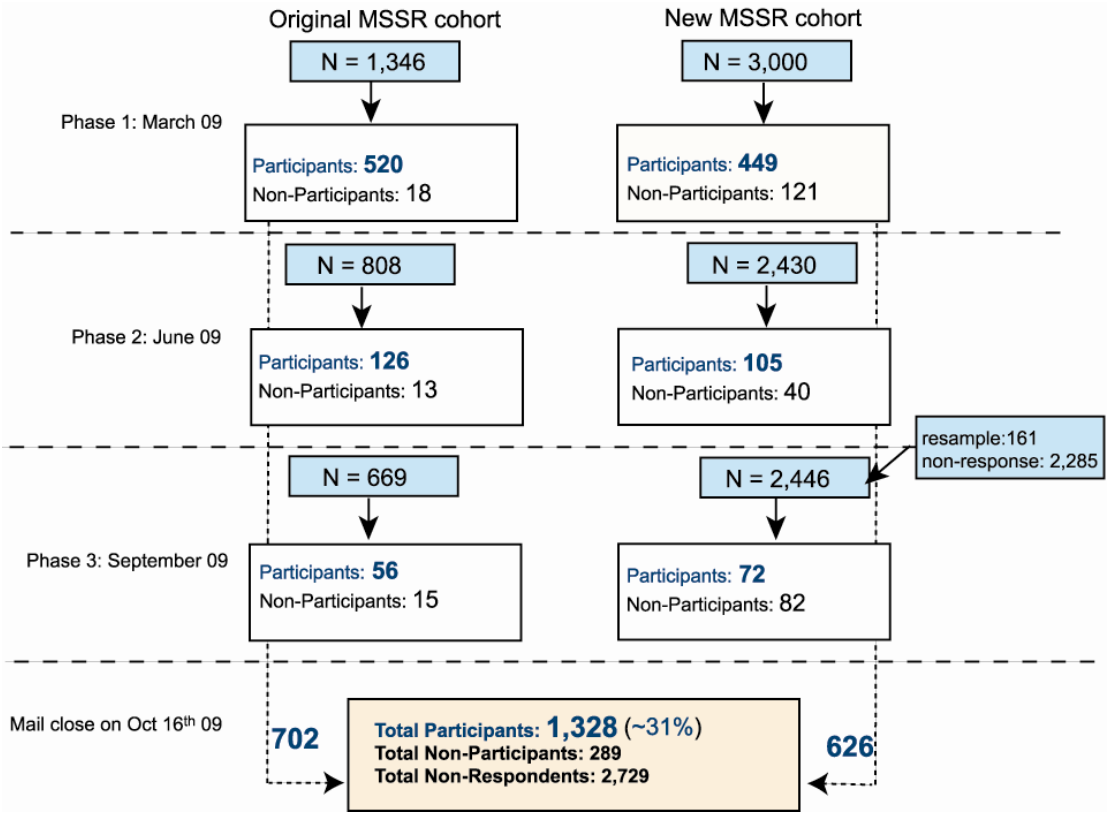


Figure 2.1 A flow chart of the MSSR mail survey conducted between March and October 2009

2.4 Data Entry and Confidentiality

All survey forms were scannable to minimize data entry error and to help data cleaning processes. This provided a paper and electronic record of all responses. All data were maintained in compliance with HIPAA requirements and IRB procedures as specified by the University of Maryland Institutional Review Board and the VHA Research and Development Committee at the Baltimore VAMC.

2.5 Exposures of Interest

All the main exposures for this project were collected through the survey of the MSSR. Participants reported date of birth and four domains of questions regarding UVR/Vitamin D exposures: (1) Residential History Information, (2) UVR exposure (3) Diet and Vitamin D Supplement Intake, and (4) Skin Type.

2.5.1 Timing of Birth

Month and season of birth were used as indicators of timing of birth. The season of birth variable was generated as follows: Spring (March-May), Summer (June-August), Fall (September-November) and Winter (December-February).

2.5.2 Residential Information

We obtained patients' city and state of residence from birth to current age. There are several methods to estimate UVR exposure based on residential data. Geographic latitude of residence is a routinely used proxy measure for UVR exposure. Accounting for altitude may further increase the accuracy in the estimation of UVR exposure[60]. In addition, published data of estimating solar radiation level reaching the

ground has been used in previous studies. Thus, we proposed three methods to estimate the residential solar radiation/UVR exposure level:

(1) Solar Radiation level of birth state: we obtained solar radiation level for each participants' state of birth from a published map of annual mean solar radiation by the United States Weather Bureau [143, 144]; a measure that factors in latitude and climate conditions. We further categorized the variable into three levels: low, medium and high solar radiation.

(2) Latitude alone: we translated city and state data to latitude data as a surrogate for residential solar radiation. The latitude of birth place and the weighted average latitude (weighted by years of residence from birth to age of 15 years.) were calculated. We used the latitude as continuous and categorical variables.

(3) UV counts: We obtained both latitude and altitude from the residential city and state data. We used an index of UVR radiation – UV counts with the following formula [145, 146]:

$$\text{UV counts} = 3220000 - 49613.9\,(\text{Latitude}) + 104.3\,(\text{Altitude})$$

We calculated weighted average UV counts to which each participant was exposed between birth and age 15 years. Similar to latitude alone, we treated this variable as continuous and categorical.

2.5.2 Participants UVR Exposure

To assess UVR exposure, we quantified participants' responses to sun exposure-related questions in the survey.

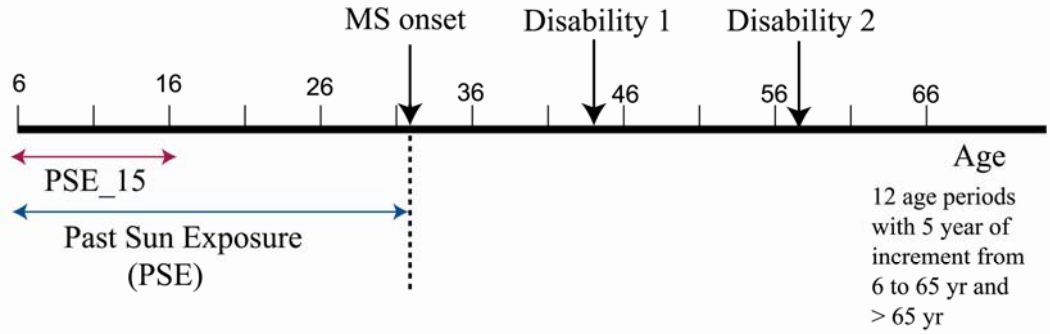
Time in Sun: MS Veterans reported on average the length of time they normally spent in the sun (<1 hour (h) a day, 1-2 h a day, 2-3 h a day, 3-4 h a day, or >4 h a day) for 12 age-periods in 5-year increment between the ages of 6 and 65 years, and older than 65 years. Weekdays and weekends/holidays as well as winter and summer seasons were considered separately. We combined these data and generated several variables as follows (Figure 2.2):

(1) Past Sun Exposure (PSE): We used the middle point of each time interval as a proxy for the length of time the participant spent in the sun in each age-period (e.g. 0.5 hour for < 1 hour, 1.5 hours for 1-2 hour and so on). Cumulative sun exposure from age 6 to MS onset for each individual was then calculated in weeks.

(2) PSE_15: Past Sun Exposure was calculated from age 6 to age 15, which indicated cumulative sun exposure between 6 and 15 years of age. The cut-off age of 15 is based on the fact that the time between childhood and early adolescence has been suggested as the critical window of exposure for MS risk.

(3) Average PSE: Average UVR exposure per year up to the age of onset was calculated by using the formula: Past Sun Exposure/years from age 6 to age at onset

.



Past Sun Exposure (PSE): Cumulative Sun Exposure before MS symptom onset

PSE_15: Cumulative Sun Exposure between age 6 and 15 years

Average PSE: PSE/year from age 6 to age at symptom onset

Figure 2.2 Variables related to Time in Sun

Frequency of sunscreen use: MS patients were asked to report the frequency of sunscreen use in the specified age periods with a scale varying from 0 (never) to 10 (always). We then explored the distribution of this variable and categorized it into three levels: never, 10-50% and 60-100%.

2.5.3 Diet and Vitamin D Supplement intake

MS Veterans indicated how often on average (rarely, occasionally, often or very often: ordinal scale 1 to 4) they ate a particular food (fish and milk) in the specified 12 age-periods. The responses were used separately for each age-period or combined by summing all the responses up to the age of the targeted time points (e.g. up to age at symptom onset or between age 6 and 15 years of age). Whether or not they took any of the supplements (i.e. cod liver oil, multi-vitamin, calcium/vitamin D, and fish oil) was also recorded in the 12 specified age-periods.

2.5.4 Skin Type

MS patients indicated their skin type according to skin color, tendency to burn and ability to tan using the Fitzpatrick classification [62] (six skin types, see Table 1.2).

2.6 Outcomes of Interest

The primary outcomes for this project, collected through the mail-survey, are listed as follows:

(1) Age at onset of MS **(AGE_{ONSET})**: MS Veterans recorded at which age they first experienced MS symptoms.

(2) Age at successive disability stages **($AGE_{PDDS1-9}$)**: MS Veterans reported at which age they reached each disability stage using the Patient Determined Disability Scale (PDDS, scale of 1 to 9, see Appendix A). We chose age at PDDS 4, PDDS 6 and PDDS 8 as the primary outcomes for assessing MS progression.

2.7 Other Covariates (potential confounders and effect modifiers)

2.7.1 Demographic characteristics:

Age, Race, Sex, Education, Income, Marital status

2.7.2 Clinical disease variables:

MS subtype, Disease duration, and Clinical Symptoms (sensory/motor) at onset

2.7.3. History and age at mononucleosis infection (glandular fever): Participants were asked to report whether or not they had ever been diagnosed with mononucleosis and their age at diagnosis, and we used this information to generate a variable for mononucleosis diagnosis prior to symptom onset (yes/no).

2.7.4. History of smoking: Several questions regarding smoking were included in the survey. We specifically derived variables to address i) whether or not

participants smoked prior to symptom onset, and ii) the average number of cigarettes smoked per day before MS symptoms onset, among smokers.

2.8 Statistical Analysis

Descriptive exploratory analysis was performed to evaluate the distributions of all variables and select potential variables that should be included in statistical models. All the analyses were performed using SAS 9.1.

2.8.1 Multiple Regression analysis

We performed multiple regression analysis to examine the effects of (1) timing of birth and residential solar radiation of birth place; (2) UVR exposures (residential information, time in sun, and sun exposure behaviors) and vitamin D intake (food and supplement intake) on $\mathbf{AGE_{ONSET}}$. For UVR exposures, we specifically generated cumulative sun exposure during ages 6 to 15 years old. The potentials of the covariates (e.g. skin type) as effect modifiers were assessed in these models. Regression models were fitted according to MS subtype to assess associations between $\mathbf{AGE_{ONSET}}$ and (1) early life exposures and (2) UVR/vitamin D during childhood and adolescence while controlling for potential covariates (e.g. demographic variables, history of smoking and history of mononucleosis infection).

2.8.2 Survival analysis

We conducted survival analysis to examine the associations between UVR/vitamin D and $\mathbf{AGE_{PDDS}}$. We specifically chose the three clinical stages: (a) Gait Disability ($\mathbf{AGE_{PDDS4}}$), (b) Late Cane ($\mathbf{AGE_{PDDS6}}$) and (c) Wheelchair / Scooter ($\mathbf{AGE_{PDDS8}}$). The survival data were calculated based on the time from age of

symptom onset to assignment of the chosen disability scores. Data were right-censored at age at the survey response when the PDDS end-points had not been reached. Kaplan-Meier analysis was used for estimating distributions and medians of $\mathbf{AGE_{PDDS}}$. The log-rank test was applied to examine unadjusted cumulative survival rates among groups according to exposure status (e.g. low, middle and high sun intensity exposed groups).

The interaction term between skin type and UVR exposures was included in the model to examine the role of skin type as an effect modifier of relationships between UVR/vitamin D and MS progression. Cox proportional hazards models were used to estimate adjusted hazard ratios and 95% CIs for the associations between UVR/vitamin D exposures and each endpoint (adjusting for demographics, clinical disease variable and history of smoking).

CHAPTER III: TIMING OF BIRTH, RESIDENTIAL SOLAR RADIATION AND AGE AT ONSET OF MULTIPLE SCLEROSIS

Timing of Birth, Residential Solar Radiation and Age at Onset of Multiple Sclerosis

Tzu-Yun McDowell[1,2], Sania Amr[2], Patricia Langenberg[2], Walter Royal[1,3], Christopher Bever [1,3], William J. Culpepper[1,2,], and Douglas D. Bradham[1,4]

1. MS Center of Excellence-East, Baltimore VAMC

2. University of Maryland, Baltimore, School of Medicine
Department of Epidemiology and Preventive Medicine

3. University of Maryland, Baltimore, School of Medicine
Department of Neurology

4. University of Kansas, School of Medicine-Wichita
Department of Preventive Medicine & Public Health

Correspondence concerning this article should be addressed to:
Tzu-Yun McDowell
MS Center of Excellence – Baltimore VAMC
100 N. Greene Street
Baltimore, MD 21210
e-mail: Tzu-Yun.Chang-McDowell@va.gov

3.1 Abstract

Backgrounds/Aim: Gestational and early life events have been suggested to contribute to multiple sclerosis (MS) susceptibility. We assessed the effects of timing and place of birth on the age at onset of MS symptoms.

Methods: We selected a national cohort of 967 Veterans from the Multiple Sclerosis Surveillance Registry for whom month and season (timing) of birth, and birthplace city and state were available. Multiple linear regression analyses were used to examine the association between timing of birth, birthplace latitude and solar radiation, and the age at onset of MS symptoms among the study sample.

Results: Patients with a relapsing form of the disease, who were born in winter and whose birthplace was in low solar radiation areas, had disease symptom onset on an average 2.8 year earlier than those born in other than winter season and in medium- and high-solar radiation areas ($p = 0.02$).

Conclusions: These results suggest that exposure early in life to geographical and seasonal factors possibly related to the protective effect of sunlight, and thus vitamin D, is associated with delay in MS symptom onset. Other larger studies are required to examine the period-specific (from conception to adulthood) environmental factors that are associated with MS susceptibility.

Keywords: Multiple sclerosis, month of birth, age at onset, solar radiation, latitude

3.2 Introduction

A growing body of evidence supports the concept that life events during gestational and postnatal periods have long lasting effects on the risk for and progression of a variety of chronic diseases, including multiple sclerosis (MS), which is an immune-mediated inflammatory and neurodegenerative disease [120, 147]. The etiology of MS is thought to be a complex interplay between genetic and environmental risk factors [25-27] with a well documented geographical distribution across populations and low prevalence in areas at low latitude [31, 148].

Geographical location at birth has been found to be associated with age of MS onset. In one study, both monozygotic and dizygotic twin born in the northern areas (at or above 41°N) were diagnosed on an average 2 to 3 years earlier than their twin pairs born elsewhere [149]. Timing of environmental exposures has also been found to relate to MS risk. A number of studies have suggested that the risk of developing MS was associated with the affected individual's place of residence at birth [149] and early in life [125, 150]. The month of birth was suggested to play a role in the development and clinical course of MS [124, 128, 129]. A large population-based study found that fewer MS patients were born in November compared to population- and family-based controls [124]. This finding, consistent with other studies of different populations in northern countries [122, 132, 151], supports the concept o f seasonally-varied and maternally-mediated environmental exposures as contributing risk factors for MS.

Accordingly, sunlight/vitamin D has gained considerable support for their potential role in determining MS susceptibility [25, 26, 36, 39, 69, 106]; seasonal variation in maternal concentrations of vitamin D, which is mainly produced in the skin after exposure to sunlight and ultraviolet radiation that are known to be related to latitude,

might have a bearing on the development of the fetal immune system, central nervous system, or both, and hence on MS susceptibility.

Although plausible, the associations between month and place of birth, age at MS onset, and related outcomes (phenotype, and clinical course) remain unconfirmed. We used a national cohort of Veterans with MS to examine the associations between age at disease symptom onset and 1) timing of birth, and 2) solar radiation level of the birthplace.

3.3 Methods

Study Population

The Veterans Health Administration (VHA)-Multiple Sclerosis Surveillance Registry (MSSR) is a population-based, self-report registry of Veterans with MS that has been described in detail elsewhere [140]. Briefly, the MSSR cohort was established in 2007 through a mail-based survey under the supervision of the VHA's Multiple Sclerosis Center of Excellence-East (MSCoE-East) in Baltimore, Maryland. From all veterans identifiable in the VHA system with possible MS, confirmed MS cases were selected using the statistical algorithm of Culpepper et al [139]. From this effective sampling pool, a regionally-stratified, random sample of MS cases (n = 3,905) was then targeted for the MSSR. Female Veterans were oversampled to make it more representative of the general (non-Veteran) population. The goal of the survey that established the registry membership was to obtain directly from the participants detailed MS-specific information that is not included in the routine VHA databases, such as year of onset of MS symptom and MS subtype.

The survey was mailed in three phases using the method of Edwards et al [142] to maximize the overall response rate. Survey packets consisted of: a cover letter describing the project; a consent form acknowledging their willingness to participate in the VHA MS Surveillance Registry (MSSR); a HIPAA form, and the survey. The University of Maryland Institutional Review Board and the VHA Research and Development Committee at Baltimore VAMC approved the present study.

As of March 2009, a total of 1,381 MS Veterans have responded to the survey (35% response rate), from which we obtained information on demographics, and MS-related clinical data and outcomes. Our preliminary analysis showed that demographic and clinical characteristics of the MSSR are comparable to those of various published MS cohorts [7, 152, 153]. Thus, the MSSR can serve as a useful cohort to study MS-related risk factors and outcomes [140].

We included only participants who were born in the United States and for whom we had complete data regarding date and place of birth, age at symptom onset, and age at diagnosis (n = 1,257). To limit heterogeneity due to childhood or very late onset of MS, or diagnostic delay, we restricted the study sample (n =967) to those whose onset of symptoms occurred between 18 and 64 years of age and for whom the MS diagnosis was made no later than 10 years after onset of symptoms.

Age at onset

Participants reported the year when they first experienced symptoms that could be related to MS and the year when they were diagnosed, and we calculated the respective ages using their date of birth.

Timing of Birth

Month and season of birth were used as indicators of timing of birth. The season of birth variable was generated as follows: Spring (March-May), Summer (June-August), Fall (September-November) and Winter (December-February). We examined the association between each indicator and age at MS symptom onset, separately.

Place at Birth

Using the city and state in which participants were born, we derived the latitude [154] and the solar radiation level of the birthplace. Latitude was used as a continuous, as well as a categorical variable. Latitude was either dichotomized into "Northern" ($\geq$41°N) and "not northern" (<41°N), or categorized as Southern ($\leq$37°N), Middle (>37 to <41°N), and Northern ($\geq$41°N). For each participant's state of birth, solar radiation level was obtained from a previously published map of annual mean daily solar radiation by the United States Weather Bureau [143, 144] and expressed in Langley as thermochemical calories/cm^2; a measure that factors in latitude and climate conditions. Such a method to estimate solar radiation reaching the ground has been previously used [68, 155]. We assigned one of three solar radiation levels: low, medium or high to each participant's birth place (Figure 3.1) [144].

Other Covariates

Participants reported their general smoking history as one of several choices, which were further grouped into three categories of smokers: never/social (infrequently smoke, at parties/bar), light-regular ($\leq$1 pack/day), and moderate-heavy

(> 1 pack/day). Other important variables such as sex, race, MS subtype and years of education were obtained from the survey.

Statistical analyses

The main outcomes were age at onset of MS symptoms and age at diagnosis. The main study variables were timing of birth (month and season), and birthplace latitude and solar radiation level. Univariate and bivariate analyses were performed to examine the variables' distributions and the unadjusted associations between the main study variables and outcomes. T-test and one-way ANOVA were used to determine the statistical significance of such associations. We used multivariable linear regression models to examine the relationship between timing and place of birth, and age at MS symptoms onset, while controlling for covariates such as sex, race, and history of smoking. The potential effect modifier of the covariates was assessed in these models. We performed stratified analysis and fit separate regression models for each MS subtype. All analyses were performed using SAS version 9.1.

3.4 Results

Table 3.1 summarizes the demographic and clinical characteristics of the study sample. The average age at onset of MS symptoms was 34.6 years (SD=9.9 years; range = 18-62 years; and > 70% of patients reporting symptoms onset before age 40) and mean age at diagnosis was 36.9 years (SD=9.7 years). We divided the patients into two groups based on disease characteristics at onset: 1) relapsing MS (R-MS) for those who experienced relapses/exacerbations at onset and during early course of the disease including patients with relapse-remitting and secondary

progressive MS; and 2) progressive MS (P-MS) for those with progressively worsening disease with or without experiencing relapses later in the disease course. The 731 (76%) patients with R-MS, had significantly earlier onset of disease symptoms (33.8±9.5 years) than those with the P-MS (36.9±10.6 years) (p<0.001). Fifty three percent of the patients with R-MS were female, whereas males represented 70% of those with P-MS. Forty one to 44% of MS patients were born in low solar radiation (<350 Langley) and northern (latitude ≥ 41°) areas. The season of birth was evenly distributed across the study sample.

Given that fewer patients were born in areas with high solar radiation (>400 Langley), we combined medium and high versus low levels. Because similar results were observed using age at diagnosis or age at onset of MS symptoms as the main outcome, we only reported results for the latter. R-MS patients born in medium/high solar radiation areas were on average 2 years older at the onset of their MS symptoms, compared to those born in low solar radiation areas (p=0.009). Those born in the northern part of the US (latitude ≥ 41^{o}) were slightly younger (33.3±8.9 years) when they started experiencing MS-related symptoms than those born elsewhere in the country (34.3±9.9 years); although the difference was not statistically significant (p = 0.17) (Table 3.2). Evaluation of latitude as three levels or as quartiles showed similar results: the higher the latitude, the younger the age at symptom onset. Among patients with P-MS, the mean age at onset of MS symptoms did not differ, either by the latitude or the solar radiation level of the birthplace. Neither month nor season of birth was significantly associated with age at onset for either relapsing or progressive patients.

We then stratified data by solar radiation levels to examine the effects of month and season of birth, respectively, on the age at MS symptom onset among R-MS patients. A significant trend for the month of birth was found ($p = 0.047$) among those whose birthplace suggested exposure to medium/high solar radiation; the R-MS patients born in winter were on an average younger at MS symptom onset (32.7±1.0 years) than those born in other seasons (35.3±0.6 years), only when their birthplaces were in median/high solar radiation areas (≥350 Langley) ($p = 0.02$).

Multiple Regression Models

A regression model was fitted for R-MS patients to further examine the significant association between season of birth, birthplace solar radiation and age at onset, while adjusting for other known MS risk factors and covariates. Timing of birth and solar radiation data were combined and re-categorized into four levels: winter born/low solar radiation (reference group), winter born/medium/high solar radiation, other seasons/low solar radiation and other seasons/medium/high solar radiation. Among R-MS patients, those born between March and November (not in winter) and whose birthplace was in medium/high solar radiation area had symptom onset on average 2.8 years later than those born in winter in low solar radiation area ($p = 0.02$, 95% CI: 0.36–5.22) (Table 3.3). Females and patients with higher education levels had earlier onset of symptoms. Patients who reported being regular/heavy smokers had symptom onset on average 2.5 years later than those who never smoked or smoked only socially ($p = 0.002$, 95% CI: 0.91–4.14).

Different models were fitted to examine associations between latitude (continuous and categorical variables) and age at onset. Latitude (continuous variable)

was found to be negatively associated with age at onset of MS, although not significant ($p = 0.10$), in patients with R-MS; each 10 degree increase in latitude was associated with an earlier onset of MS symptoms by an average of 1.3 years; 95% CI: -2.8–-0.3 years. We found no associations between either birthplace solar radiation level or latitude and age at MS symptoms onset among patients with P-MS.

3.5 Discussion

We found that R-MS patients, who were born in winter and whose birthplace was in low solar radiation areas, had disease symptom onset on an average 2.8 year earlier than those born in other than winter season and in medium/high solar radiation areas. Similar associations, although not statistically significant, were observed between high latitude birthplace (reflecting low solar radiation) and early onset of MS symptom. No association was found between age of onset and timing or birthplace among patients with progressive disease.

This is the first study to address the age at MS symptom onset and both latitude and solar radiation level of birthplace, as well as the timing of birth. Previous studies have investigated the associations either between age at MS onset and latitude [149, 156] or between month of birth and MS-related outcomes [124, 128, 129]. Our data showed an average age at MS symptom onset that decreases with increasing latitude that is consistent with prior reports [149, 156]. Associations between the month of birth and either the risk of developing MS [122, 124, 132] or the clinical course of the disease [128, 129] have been previously reported. These studies suggest that some environmental factor(s) during gestational periods might have a role in determining MS susceptibility and potentially the course of the disease [120, 124, 128, 129]. Levels of vitamin

D in humans, mainly produced through skin synthesis following exposure to solar radiation and thus subject to geographical and seasonal variations, were proposed to be one of the possible explanations for the association between timing of birth and MS risk[124] as well as for the geographical distribution of MS prevalence [31, 65]. Cumulative evidence has supported vitamin D as a strong immune modulator and protective against development of MS [25, 74, 80, 106]. In our study, the association of early MS onset with low solar radiation birthplace and winter season supports the concept that sun exposure before or at birth, thus maternal vitamin D level, influences disease course; possibly by modulating the development of central nervous system and/or immune system in the fetus. Whether or not, our findings relate to maternal or perinatal vitamin D levels or some other seasonally varied environmental factors in early life, remains to be established.

We did not observe any associations between timing of birth, birthplace solar radiation and age of MS onset among patients with P-MS. These patients are known to have later onset of the disease, and possibly exposure to other environmental factors later in life. Indeed, Pugliatti et al. [131] suggested that MS clinical heterogeneity might depend on different causative mechanisms. Consistent with our results, Sadovnick at al. [128] found that a month of birth effect was observed in relapsing-remitting MS but not primary progressive MS patients. Furthermore, the sample size of P-MS patients in our study was relatively small to allow examination of complex interactions among variables.

We found that patients who classified themselves as a regular or heavy smoker had later onset of the disease compared to those who were never or social

smokers. This result should be interpreted with caution. This is a cross-sectional study the scope of which was not to address smoking as risk factor for MS, and participants were only asked to describe their smoking status in general, but not their smoking history before their disease onset. We did not have sufficient information to investigate whether or not smoking affects the age of MS symptom onset. An alternative explanation of the observed findings is smokers' health behavior that is different from non-smokers; e.g., patients who smoked regularly and heavily might be less health conscious and not aware of clinical symptoms at an earlier stage than those who never smoked. Indeed, in a large cohort of MS patients, Marrie et al [157] reported increased delay in MS diagnosis among smokers, compared to non smokers.

This study has several limitations, some of which are inherent to its cross-sectional design. Despite oversampling female Veterans with MS, our female to male ratio was lower than that of the MS population in the US; hence our study might not be generalizable. Furthermore, we did not examine actual levels of solar radiation at the birthplace or maternal level of vitamin D; instead, we used proxies for these variables. Future studies are needed to examine more comprehensively the effects of multiple environmental and behavioral risk factors on MS related outcomes.

In summary, our study suggests that exposure early in life to geographical and seasonal factors, possibly related to the protective effect of sunlight and thus vitamin D, is associated with delay in the age at MS symptom onset. Other larger studies are required to examine the period-specific (from conception to adulthood) environmental factors that are potentially associated with MS susceptibility.

Table 3.1 Demographics and clinical characteristics of the study population

	Study Population† (N = 967)	Relapse-Remitting MS (N = 731)	Progressive MS (N = 236)
Demographic Characteristics			
Age years (Mean± SD)	53.2 ± 9.7	52.0 ± 9.8	57.0 ± 10.6)
Race, n (%)			
1.White	831 (85.9)	627 (85.7)	204 (86.4)
2. Black	136 (14.1)	104 (14.2)	32 (13.6)
Sex, n (%)			
1. Female	459 (47.8)	389 (53.5)	70 (29.9)
2. Male	502 (52.2)	338 (46.5)	164 (70.1)
Education			
Average years (range)	14.7 ± 2.7 (0-21)	14.8 ± 2.6 (0-21)	14.4 ± 2.8 (7-21)
Smoking history, n (%)			
1. Never-social smoker	417 (44.6)	328 (46.0)	424 (44.4)
2. light- Regular smoker	210 (22.4)	166 (23.3)	213 (22.3)
3. Moderate-Heavy smoker	309 (33.0)	219 (30.7)	317 (33.2)
Birthplace			
Solar Radiation, n (%)			
1. Low: < 350 Langley	417 (43.1)	313 (42.8)	104 (44.1)
2. Medium: 350-400 Langley	329 (34.0)	255 (34.9)	74 (31.4)
2. High: >400 Langley	221 (22.9)	163 (22.3)	58 (24.6)
Latitude, n (%)			
1. Northern: ≥ 41°	407 (42.1)	309 (42.3)	98 (41.5)
2. Middle: 37- <41°	345 (35.7)	261 (35.7)	84 (35.6)
3. Southern: <37°	215 (22.2)	161 (22.0)	54 (22.9)
Average Latitude° (range)	39.7±4.5 (21.2-64.8)	39.8±4.5 (21.2-64.8)	39.4±4.4 (25.8-48.2)
Season of birth			
1. Spring	241 (24.9)	182 (24.9)	59 (25.0)
2. Summer	247 (25.5)	188 (25.7)	59 (25.0)
3. Fall	246 (25.5)	185 (25.3)	61 (25.9)
4. Winter	233 (24.1)	176 (24.1)	57 (24.2)
Clinical Characteristics			
Age at MS (Mean± SD)			
Symptoms onset	34.6±9.9	33.9±9.5	37.0±10.6
Diagnosis	36.9±9.7	36.1±9.4	39.3±10.3

† Includes only MS patients with 18 ≤age at onset of symptom <65 years, and ≤ 10 years interval between symptom onset and diagnosis

Table 3.2 Mean age at onset of MS symptoms by seasonal and birthplace characteristics among Veterans with MS

	Relapsing MS (N = 731)		Progressive MS (N = 236)	
Solar Radiation	**Symptom onset**	***p* value†**	**Symptom onset**	***p* value†**
1. Low	32.8± 8.8	0.009	37.2±11.1	0.87
2. Medium-High	34.7±9.9		36.8±10.3	
Latitude				
1. Northern (≥ 41° N)	33.3±8.9	0.17	37.2±11.9	0.80
2. Other	34.3±9.9		36.8±9.7	
Season				
1. Spring	34.1±10.3	0.31	36.5±11.9	0.99
2. Summer	34.4±9.2		37.4±10.1	
3. Fall	34.3±9.4		37.2±11.2	
4. Winter	32.7±9.2		36.9±9.4	

† t-test and one-way ANOVA were used to compare dichotomized and multilevel variables, respectively.

Table 3.3 Multivariable linear regression of age at MS symptom onset on birthplace solar radiation levels and season of birth adjusted for important covariates in patients with R-MS

	Relapsing MS (n = 731), R^2=8.9		
Variables[†]	**Estimate (SE)**	**95% CI**	***p* value**
Intercept	42.16 (2.42)	37.41, 46.91	<.0001
Birth season and solar radiation			
Winter/low solar radiation	0	---	---
Winter/medium-high solar radiation	0.91 (1.54)	(-2.12, 3.9)	0.56
Other seasons/low solar radiation	0.07 (1.26)	(-2.41, 2.55)	0.95
Other seasons/medium-high solar radiation	2.79 (1.24)	(0.36, 5.22)	0.02
Race			
Black	-0.80 (1.01)	(-2.80, 1.19)	0.43
Sex			
Male	2.68 (0.71)	(1.29, 4.07)	0.0002
Education (year)	-0.48 (0.14)	(-0.75, -0.21)	0.0005
Smoking history			
Never/social smoker	0	---	---
Light- Regular smoker	0.92 (0.88)	(-0.81, 2.65)	0.30
Moderate-Heavy smoker	2.52 (0.82)	(0.91, 4.14)	0.002

[†]All the variable listed above were included in the final model

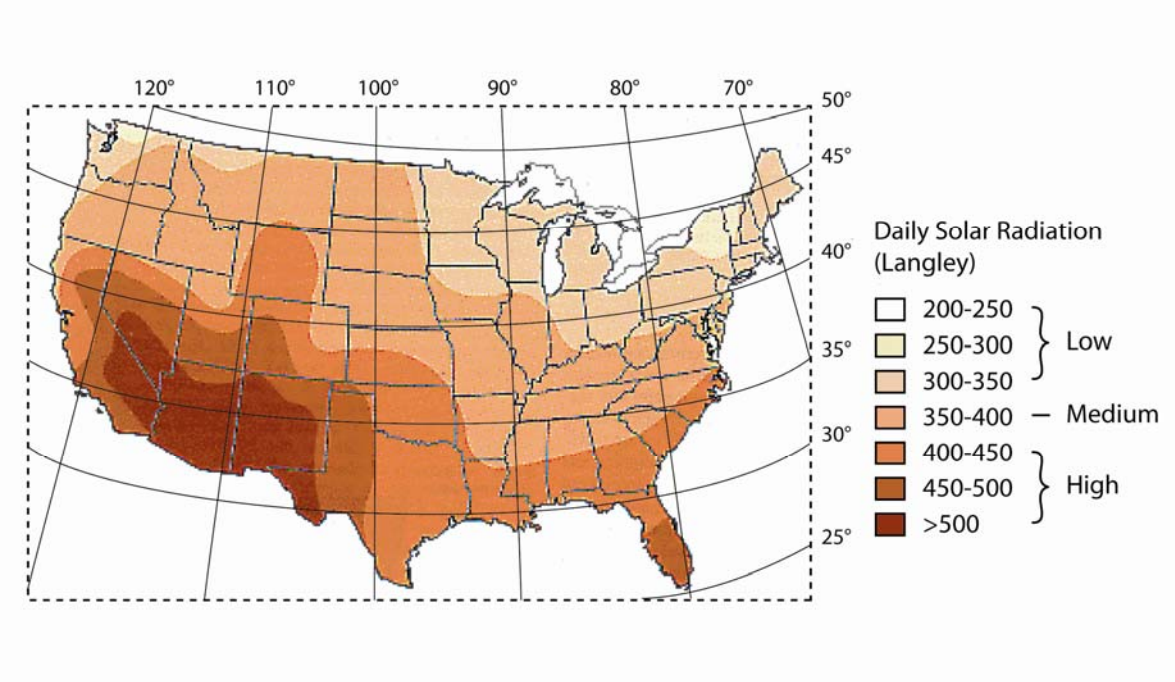


Figure 3.1 Mean daily solar radiation (Source: Data from the U.S. Department of Commerce, 1968) superimposed on the longitude and latitude map of the United States [144]. Low-level solar radiation (<350 Langley) states include: AK, CT, ME, MA, MI, MN, NH, NY, OH, OR, PA, RI, VT, WA and WI. Medium-level solar radiation (≥350-400 Langley) states include AR, DE, DC, ID, IL, IN, IA, KS, KY, MD, MO, MT, NE, NJ, NC, ND, SD, TN, VA, and WV. High-level solar radiation (>400 Langley) states include AL, AZ, CA, CO, FL, GA, HI, LA, MS, NV, NM, OK, SC, TX, UT, WY, and PR.

CHAPTER IV: CHILDHOOD SUN EXPOSURE AND VITAMIN D INTAKE AND AGE AT DISEASE ONSET AMONG VETERANS WITH RELAPSING MULTIPLE SCLEROSIS

Childhood Sun Exposure and Vitamin D intake and Age at Disease Onset among Veterans with Relapsing Multiple Sclerosis

Tzu-Yun McDowell[1,2], Sania Amr[2], William J. Culpepper[1,2], Patrician Langenberg[2], Walter Royal[1,3], Christopher Bever[1,3], and Douglas D. Bradham[1,4]

1. MS Center of Excellence-East, Baltimore VAMC

2. University of Maryland, Baltimore, School of Medicine
Department of Epidemiology and Preventive Medicine

3. University of Maryland, Baltimore, School of Medicine
Department of Neurology

4. University of Kansas, School of Medicine-Wichita
Department of Preventive Medicine & Public Health

Correspondence concerning this article should be addressed to:
Tzu-Yun McDowell
MS Center of Excellence – Baltimore VAMC
100 N. Greene Street
Baltimore, MD 21210
e-mail: Tzu-Yun.Chang-McDowell@va.gov
PHONE: 410.605.7414
FAX: 410.605.7705

4.1 Abstract

Background: Several studies have demonstrated that sun exposure and cod liver oil and fish consumption, both of which are sources for Vitamin D, during childhood and adolescence, were associated with a reduced risk of multiple sclerosis (MS). However the role of these environmental agents in the timing of disease symptom onset is not known.

Objective: To examine if sun exposure and vitamin D intake (diet and supplements) during childhood and adolescence were associated with delayed onset of multiple sclerosis in a national cohort of Veterans with MS.

Methods: Veterans with MS were recruited from the Veterans Health Administration (VHA) to participate in the VHA Multiple Sclerosis Surveillance Registry, a nationally representative sample of veterans with MS. Participants reported their histories of residential locations, sun exposure, and vitamin D related food and supplement intake by 5-year age-periods. Cumulative past sun exposure was estimated for fall/winter and spring/summer seasons. Solar radiation levels were estimated based on the latitude, altitude and UV count of self-reported residence. Multiple regression analysis was used to examine the association between these variables and the age at MS onset, controlling for known covariates.

Results: Among patients with relapsing MS, (N=948) low sun exposure in the fall/winter during the age-period of 6 to 15 years was significantly associated with earlier onset of disease symptoms for those who resided in low-medium solar radiation areas (an average of 2.3 years; $p = 0.01$). Whereas, intake of cod liver oil during childhood was associated with delayed onset of MS symptoms by 3 years ($p = 0.01$).

Conclusions: This study provides the first evidence that low sun exposure during childhood and early adolescence is associated with earlier onset of MS symptoms, while cod liver oil intake is associated with delayed onset of the disease; both variables contribute to vitamin D status.

Keywords: Multiple sclerosis, sun exposure, vitamin D, age at onset

4.2 Introductions

Multiple Sclerosis (MS), an immune-mediated inflammatory and neurodegenerative disease, is thought to have complex etiological origins with interplay between genetic and environmental risk factors [1, 2, 25, 26, 32]. Timing of environmental exposures has also been found to be related to MS risk [36, 37, 69, 120, 123, 124, 158-160]. Several studies have suggested a critical window of exposure during childhood and adolescence for the environmental factors to play a role in MS pathogenesis. [36, 37, 69, 123, 131, 132, 158-162] This concept was first developed from observations related to environmental influence among migrant populations**.** Specifically, when individuals moved from a region with high prevalence of MS to a low prevalence area before age 15 years, their risk of having MS is similar to that of the original countries from which they moved [159]. These studies imply that the environmental influence may be strongest sometime between birth to young adolescence.

Among the environmental factors implicated in MS susceptibility, increasing evidence from both experimental and epidemiological studies support a role for ultraviolet radiation (UVR) or vitamin D [74, 75, 80, 88, 89, 101, 163]. The immunosuppressive function of UVR, directly or through enhancement of vitamin D production, has been postulated as the underlying mechanisms for decreasing MS risk and potentially modulating its course [74]. Several studies have demonstrated that high sun exposure, particularly during childhood and adolescence, was associated with a reduced risk of MS [69-71]. Van de Mei et al. [69] found that the protective effect of sun exposure in southern Australia was the greatest when it occurred between the ages of 6-15 years. Another

study conducted in Northern Norway found that sun exposure between the ages of 16-20 years was particularly important [70]. However, the potential importance of sun exposure later in life has also been suggested [150]. In addition to childhood sun exposure, cod liver oil and fish consumption (equal or greater than three times a week) in childhood and adolescence, other major sources of vitamin D, were also found to decrease MS risk [70]. However the role of either sun exposure or vitamin D supplement intake in determining the course of MS clinical manifestations is not known. The aim of this study is to examine whether sun exposure or vitamin D intake (diet and supplements) during childhood and adolescence are associated with delayed symptom onset in a national cohort of Veterans with MS.

4.3 Methods

The University of Maryland Institutional Review Board and the VHA Research and Development Committee at Baltimore VAMC approved the present study.

Study Population

The study population was recruited from the Veterans Health Administration (VHA)-Multiple Sclerosis Surveillance Registry (MSSR). The MSSR, a population-based, self-reported registry of Veterans with MS, has been described in detail elsewhere [164]. Briefly, the MSSR cohort was established in 2007 through a mail-based survey under the supervision of the VHA's Multiple Sclerosis Center of Excellence-East (MSCoE-East) in Baltimore, Maryland. From all veterans identifiable in the VHA system with possible MS, confirmed MS cases were selected using the statistical algorithm of Culpepper et al [139]. From this effective sampling pool, a regionally-stratified, random sample of MS cases was then targeted for the MSSR. Female Veterans were oversampled to make it more

representative of the general (non-Veteran) population. The goal of the survey that established the registry membership was to obtain directly from the participants detailed MS-specific information that is not included in the routine VHA databases, such as year of onset of MS symptom and MS subtype._To date, two questionnaires have been distributed to the participants to obtain detailed demographic and MS-related information for both clinical outcomes and epidemiological research purposes.

The questionnaire designed for the present study was distributed to the previous MSSR participants (n = 1,346) and a new cohort of Veterans with MS (n = 3,000) between March and October 2009. The rationale for targeting a new cohort of veterans with MS was to extend the current size of the MSSR. As of October 2009, a total of 1,328 participants had completed the questionnaire (response rate ~31%). In the present study, we included only participants who were born and raised in the United States and those whose symptom onset occurred between 18 and 60 years of age to limit heterogeneity due to differences in childhood onset, and very late age of MS onset (n = 1,181).

Study variables

The outcome for this project was the self-reported age at which each MS patient first experienced MS symptoms. The predictors included solar radiation and Vitamin D exposures based on the participants responses to questions regarding (1) residential areas, (2) sun exposure (3) dietary and supplement intake, and (4) skin type.

Residential Solar Radiation Estimates

Participants were asked to report their city and state of residence from birth to their current age. We obtained latitude and altitude data for each city and state through

the U.S Geographical database. UV count was calculated for a particular residential location based on the previously reported formula[145, 146]:

$$\text{UV counts} = 3220000 - 49613.9\ (\text{Latitude}) + 104.3\ (\text{Altitude})$$

We calculated latitude and UV counts of the areas of residence from birth up to 15 years of age for each participant, weighted by years of residence. The rationale for the cut-off age of 15 is that the critical window of exposure regarding MS risk has been suggested to be during late childhood and adolescence [69, 123, 132]. Latitude and UV count, as estimates of residential solar radiation, were analyzed both as continuous variables and as categorical variables (based on tertile and quartile distributions).

Sunlight Exposure Estimates

All the participants reported the average length of time they normally spent in the sun per day (<1 hour (h), 1-2 h, 2-3 h, 3-4 h, or >4 h) during 12 age-periods in 5 year increments from 6 to 65 years. Sun exposures on weekdays and weekends/holidays, as well as during fall/winter and spring/summer seasons were considered separately. For this study, we only used each participant's reported exposure prior to his/her onset of MS symptoms. For each age period, we used the middle point of each response interval as an estimate for the average length of time the participant spent per day in the sun (e.g. 0.5 h/day for a response of <1 h/day and 1.5 h/day for a response of 1-2 h/day). Cumulative sun exposure during age 6 to 15 years, as an indicator of sunlight exposure during childhood and early adolescence was calculated by summing the hours of sun exposure on weekdays and weekends for the age-periods of 6-10 and 11-15 years and then converting it to a unit of total weeks of sun exposure. We calculated separate variables of

cumulative sun exposure from 6-15 years for three seasonal periods: (1) fall/winter, (2) spring/summer, and (3) a combined seasons. Participants also reported the frequency of sunscreen use in the specified age-periods with a scale varying from 0 (never) to 10 (always).

Dietary and Supplement Intake

Participants were asked to report how often on average (rarely, occasionally, often or very often: ordinal scale 1 to 4) they ate a particular food (fish, milk and egg/cheese) in the 12 specified age-periods. Whether or not they took supplements (cod liver oil, fish oil, vitamin D/calcium and multi-vitamin) on a regular basis during the specified age periods was also reported.

Skin type

Participants indicated their skin type according to skin color, tendency to burn and ability to tan using the Fitzpatrick classification [62] (type 1: extremely fair, type 2: fair, type 3: medium, type 4: olive, type 5: brown, and type 6: black). We further re-grouped the skin types into three categories – very fair to fair (type 1 and 2), medium to olive (type 3 and 4) and brown to black (type 5 and 6).

Smoking history

From the participants' responses to several questions regarding smoking, we derived variables to address i) whether or not participants smoked prior to symptom onset, and ii) the average number of cigarettes smoked per day before MS symptoms onset, among smokers.

Other covariates

Basic demographic variables, such as age, sex, race and education as well as clinical variables including MS subtype were reported by each participant We asked the participants whether or not they had ever been diagnosed with mononucleosis and their age at diagnosis, and we used this information to generate a variable for mononucleosis diagnosis prior to symptom onset (yes/no).

Statistical analyses

The main study variable: past sun exposure was examined separately for each age period (e.g. 6-10, 11-15, 16-20, 21-25 years) to identify age periods in which sun exposure might be important to age at onset. The sample for these preliminary analyses was limited to participants who had not experienced MS symptom onset before or during the specified age period. Additionally, aggregated variables such as weighted childhood UV counts and cumulative sun exposure during age 6 to 15 years were used to examine the effect of estimated childhood residential solar radiation and sunlight exposure on age at symptom onset. Univariate and bivariate analyses were performed to respectively examine the variables' distributions and the unadjusted associations between the main study variables and outcome. T-tests and one-way ANOVA were used to determine the statistical significance of such associations. We used multivariable linear regression models to examine the relationship between estimated residential solar radiation, estimated past sun exposure, vitamin D intake, and age at MS symptoms onset, while controlling for covariates such as gender and history of smoking. The potential of some of the covariates to act as effect modifiers (e.g. interaction between residential solar radiation and past sun exposure, skin type and past sun exposure) was assessed in these

models. The sunscreen variable allowed us to examine a potential interaction between sunlight exposure and sunscreen use. Because the majority of the participants reported that they never used sunscreen during childhood, an analysis was conducted among this sub-sample to control for the potential effect of sunscreen use on the association of interest. All analyses were performed using SAS version 9.1.

Because previous studies have shown that there may be different causative mechanisms for different subtypes of MS [131], and symptom onset for the progressive type of MS could be easily overlooked due to relatively vague clinical signs, we conducted separate analyses for two groups of patients based on their MS subtype.

4.4 Results

Of the 1,328 participants, we excluded those who were born and raised outside the U.S. (n=83) and those whose age at onset of symptoms was younger than 18 years or older than 60 years (n=167). The resulting sample of 1,181 was used in this study. Overall, 46% (n = 544) of the MS Veterans were female, and the majority White (82%). Eighty percent of them reported that they had Relapsing MS (relapse-remitting (RRMS) and secondary progressive (SPMS)). Close to 70% of MS patients had smoked at some time prior to symptom onset, and about 30% reported having extremely fair or fair skin type. The mean age at symptom onset was 31 years (SD =8.9) and 36 years (SD = 10.9) among Relapsing and Progressive MS, respectively (Table 4.1). Distribution of age at symptom onset by subtype of MS is shown in Appendix B, Figure 1. Although we performed separate analyses for Relapsing and Progressive MS subtypes, in this manuscript, we focused on Relapsing MS (n = 948), subtype that was reported by the

majority of the participants. The results for Progressive MS subtype are in Appendix B, Table 1.

Relapsing MS

The self-reported average time spent outside in the sun during the specified age-periods and seasons is shown in Table 4.2. More than 50% of participants reported less than two hours of sun exposure per day during fall/winter in childhood and early adolescence, while a much higher percentage (>60%) reported being in the sun for more than 3 hours a day during spring/summer. Mean age at symptom onset by level of sun exposures in childhood and early adolescence is illustrated in Table 4.3. Although the results from the linear trend analysis using ANOVA were overall not significant in each age period, there was a pattern showing that participants with the least time (<1 hour/a day) in the sun in fall/winter, particularly during age-periods 6-10 and 11-15 years, had an earlier onset of MS ($p < 0.05$).

Associations between cumulative sun exposure in fall/winter (in quartiles) between 6-15 years of age and age at onset are depicted in Table 4.4. Patients in the lowest quartile (<16 weeks) of cumulative sun exposure in fall/winter had the youngest average age at onset of MS, although the main effect was not statistically significant ($p = 0.19$). Linear trend analysis showed a marginally significant association ($p = 0.06$) between lower cumulative fall/winter sun exposure during 6-15 years of age and younger age at onset. No significant association was observed between weighted residential solar radiation estimates during childhood and early adolescence (using three levels of weighted UV counts and latitude from birth to age 15) and age at symptom onset (Table 4.4). A significant association ($p = 0.04$) between sunscreen use during childhood and

early adolescence and younger age at onset was found. Those who reported having used sunscreen more than 50% of the times had earlier age at onset. However, close to 70% of the patients had never used sunscreen during these age-periods. Skin type was not associated with age at onset.

Table 4.5 shows that patients who took cod liver oil regularly between the ages of 6 and10 years, compared to those who did not, had significantly later onset ($p = 0.03$); also female compared to male patients had earlier onset of MS symptoms ($p < 0.0001$). There was no significant association between age at onset and either smoking history or mononucleosis diagnosis prior to the disease.

Stratified analysis was performed to examine the potential interaction effect between residential solar radiation and cumulative sun exposure between 6 and 15 years on age at onset. Figure 4.1 illustrates mean age at onset by each quartile of fall/winter childhood sun exposure and by levels of estimated childhood residential solar radiation. There was a significant effect of cumulative sun exposure on age at symptom onset ($p= 0.03$), but only among patients who resided in low to medium solar radiation areas ($n = 604$). Specifically, those who had least cumulative winter sun exposure estimates (<16 weeks) between 6-15 years of age had symptom onset on average 2.4 year earlier compared to individuals with higher sun exposure estimates ($p = 0.005$). Cumulative sun exposure in fall/winter did not affect age at onset among those who resided in high solar radiation areas during childhood and early adolescence. No interaction between cumulative sun exposure and skin type or sunscreen use was found.

A multivariable regression model was performed to examine the association between cumulative sun exposure, particularly in fall/winter and age at onset while

controlling for potential effect modifiers and covariates. We dichotomized the cumulative past sun exposure into low sun exposure (≤16 weeks) and others (>16 weeks). A significant interaction effect between sun exposure and residential solar radiation estimates was found (p =0.02). The effect of fall/winter sun exposure during age-period 6-15 years was only significant among patients who resided in low or medium solar radiation areas (p = 0.01) (see Appendix B, Table 2). As a result, a separate regression model was fitted for patients whose residential solar radiation estimates were classified as low or medium (n= 604) (Table 4.6). High sun exposure in the fall/winter was significantly associated with older age at symptoms onset (p = 0.01), after controlling for other covariates (e.g. sex, skin type). Similarly, cod liver oil intake during childhood was associated with a delay in symptoms onset by 3 years (p = 0.01). Females had earlier onset compared to males. Veterans who reported using sunscreen more than 50% of the time had earlier onset of MS symptoms. To further control for the use of sunscreen during childhood, a regression model was fitted among a sub-sample of Veterans who reported never using sunscreen between the ages of 6 and 15 years, and who resided in areas with low-medium solar radiation (n = 360). Those who reported low sun exposure during fall/winter season had symptom onset on average 3.76 years (p = 0.001) earlier compared to the rest.

4.5 Discussion

The current study provides evidence for an association of lower sun exposure during childhood and early adolescence (age-periods 6-15 years), particularly in fall/winter, with earlier age at symptom onset among Veterans with Relapsing MS. This association was only observed among patients who lived in areas with relatively low

solar radiation. Regular use of cod liver oil during childhood was associated with delayed symptom onset. These findings suggest that environmental factors, mainly related to sun exposure or vitamin D status during childhood and early adolescence, influence the age at MS symptom onset, and thus the clinical course of this disease.

Cumulative data have suggested that sun exposure or vitamin D are important protective factors against the development of MS [74, 75, 80]. The "window of susceptibility" when these protective agents might exert their beneficial effect has been suggested to occur during childhood and adolescence [47, 69-71, 120]. Several studies have shown an increased risk of MS with low sun exposure/fish consumption during childhood and adolescence [69-71]. Whether or not past sun exposure or vitamin D during this time frame is also associated with the age of disease onset has been rarely reported. To our knowledge, there is only one previous study that has directly assessed this association[69] and found none, in contrast to our current findings. The earlier study had only 134 MS patients, including both Relapsing and Progressive MS; whereas our study sample was larger and more homogeneous with 948 patients with Relapsing MS.

Our findings are consistent with the conjecture that environmental factors associated with MS risk during specific age-periods might also be related to age at MS symptom onset [161, 165]. A few studies have reported that the age at disease onset varies with the prevalence of the disease [166, 167]. That is, younger age at onset was found in areas with higher MS prevalence. Moreover, Riise et al. [165] found that the younger the age at onset the higher was the degree of clustering in adolescence. These observations suggest that some environmental risk factors (or protective factors) for MS could potentially influence the underlying disease process and thus modify the timing between disease

initiation and the first detected clinical symptom (the latent period). Our findings suggest that low sun exposure, during childhood and early adolescence, might have a role in altering the clinical course of MS by shortening the latent period. Replicating our findings in different populations would strengthen this possibility. Our study cannot exclude the possibility that sun exposure or other environmental factors later in life (after the age of 16) might also have had a role in determining age at MS onset. We observed a trend for and association between low winter sun exposure during ages 16-20 and early age at onset, but not statistically significant. And the lack of effect of low sun exposure during the age period 21-25 suggests that sun exposure, or the lack of, earlier (before age 20) is more likely the determinant.

Our finding that fall/winter sun exposure was particularly important is consistent with several previous studies in the context of childhood sun exposure and MS risk [69, 168]. A recent study found that the involvement of the vitamin D receptor gene in determining MS risk was likely to be modified by winter sun exposure during childhood [168]. These findings, in conjunction with the fact that we only observed an effect of fall/winter sun exposure among patients living in areas with relatively lower solar radiation strengthens the possibility that there might be a minimum threshold requirement for sufficient solar radiation or vitamin D to exert beneficial effects on disease susceptibility and the disease process during critical periods of development.

Skin pigmentation is an important factor in the process of vitamin D synthesis in human skin after exposure to UV radiation; increased pigmentation (darker skin tone) leads to reduction of vitamin D synthesis [135]. As a result, it would be expected that the effect of sun exposure might vary with skin type. We did not find a significant interaction

effect between skin type and sun exposure on age at onset. However, there was a trend suggesting that the effect of winter sun exposure is stronger among patients with lighter skin color, particularly very fair to fair skin type, compared to those with darker skin. Veterans with fair skin type and low childhood winter sun exposure had the youngest age at symptom onset. This finding could be potentially explained by functions of genotype associated with fair skin for MS risk or vitamin D metabolism, rather than by the differences in vitamin D synthesis in the skin. Skin type itself was not associated with age at MS onset.

Several limitations in the current study are inherent to its cross-sectional design and survey data source. The use of past sun exposure measures could be influenced by recall bias and thus result in considerable misclassification of the measurement; however, it is unlikely that our findings are due to bias or chance alone. Indeed, we observed patterns with childhood cod liver oil intake, sunscreen use, and with the analysis of different sub-samples, all of which similar to what we observed with past sun exposure; childhood and early adolescence exposure to Vitamin D surrogates seems to delay the age at MS symptom onset. Moreover, the level of solar radiation varies according to geographical locations and other climate factors. It is important to adjust for different levels of solar radiation if the study population resided in geographically varied areas, such as in the U.S. Although our study intended to control for childhood residential locations by using weighted childhood residential solar radiation estimates (taking into account both latitude and altitude) in the analysis, the variable was an estimate based on a published formula, not an observed value. Potential selection bias might have occurred in the study due to some differences in demographic characteristics between patients who

responded to the survey and those who did not. A high percentage of male, Caucasian and married individuals was found among the participants. Furthermore, the study population was a national cohort of Veterans with MS with female to male ratio about 1:1, which is lower than that of the general MS population in the US, hence generalizability could be a potential limitation. In spite of its limitations, the current study had a relatively large sample size and incorporated many MS-risk associated variables (e.g. smoking), and thus, provides the first evidence that low vitamin D status, through low sun exposure or no supplement intake (e.g. cod liver oil) during childhood and early adolescence may influence the age of onset of MS symptoms.

Table 4.1 Sociodemographic and clinical characteristics of the study sample of Veterans with relapsing and progressive MS

	Study Sample (n= 1,181)	Relapsing MS (n = 948)	Progressive MS (n=219)
Personal Characteristics			
Age (Mean ± SD)	56.0±10.4	54.9±10.3	60.9±9.6
Race, n (%)			
1.White	971 (82.2)	786 (82.9)	178 (81.3)
2. Black	143 (12.1)	103 (10.9)	34 (15.5)
Sex, n (%)			
1. Female	544 (46.1)	487 (51.4)	50 (22.8)
2. Male	637 (54.0)	461 (48.6)	169 (77.2)
Education			
Average years (SD)	14.6 ± 3.0	14.6 ± 3.0	14.4 ± 3.0
Smoking history before MS onset, n (%)			
1. Never	365 (32.3)	307 (33.8)	54 (26.0)
2. Ever	765 (67.7)	601 (66.2)	154(74.0)
Diagnosis of Mononucleosis before MS onset, n (%)			
1. Yes	185 (16.0)	151 (16.3)	33(15.4)
2. No	868 (75.0)	694 (74.7)	166 (77.6)
3. Unknown	104 (9.0)	84 (9.0)	15 (7.0)
Skin type, n (%)			
1. Extremely fair skin	61 (5.3)	52 (5.6)	9 (4.2)
2. Fair skin	306 (26.4)	256 (27.4)	46 (21.5)
3. Medium skin	510 (43.9)	405 (43.4)	101 (47.2)
4. Olive skin	150 (12.9)	117 (12.5)	32 (15.0)
5. Brown skin	48 (4.1)	39 (4.2)	8 (3.7)
6. Black skin	86 (7.4)	64 (6.9)	18 (8.4)
Clinical Characteristics			
Age (Mean ± SD) at MS			
Symptoms onset	32.0±9.5	31.1±8.9	36.1±10.9
Diagnosis	38.5±10.0	37.7±9.6	42.0±10.7

Table 4.2 Estimates of self-reported sun exposure in childhood and adolescence among Veterans with relapsing MS

Sun exposure estimate	Age 6-10	Age 11-15	Age 16-20	Age 21-25
Weekdays in fall/winter Time in sun (h/day)	N (%) Total N = 870†	N (%) Total N = 868	N (%) Total N = 784	N (%) Total N = 582
< 1	179 (20.6)	142 (16.4)	154 (19.6)	119(20.5)
1-2	300 (34.5)	287 (33.1)	209(26.7)	178(30.6)
2-3	162 (18.6)	180 (20.7)	157(20.0)	101(17.4)
3-4	102 (11.7)	126 (14.5)	120(15.3)	67(11.5)
> 4	127 (14.6)	133 (15.3)	144(18.4)	117(20.1)
Weekdays in spring/summer Time in sun (h/day)	N (%) Total N = 888	N (%) Total N =886	N (%) Total N =802	N (%) Total N =596
< 1	44(5.0)	30(3.4)	32(4.0)	44(7.4)
1-2	106 (11.9)	89(10.1)	109(13.6)	103(17.3)
2-3	172 (19.4)	167(18.9)	138(17.2)	127(21.3)
3-4	171 (19.3)	198(22.4)	165(20.6)	104(17.5)
> 4	395 (44.5)	402(45.4)	358(44.6)	218(36.6)

† The total N for each age period is not the same due to missing values and the sample restriction. For age periods 16-20 and 21-25 years, we only included patients whose age at onset older than the upper limit of the age period.

Table 4.3 Mean age at onset of disease symptoms by estimated levels of self-reported sun exposure during childhood and adolescence among Veterans with relapsing MS

Sun Exposure Estimates Weekdays in fall/winter	**Age 6-10**	**Age 11-15**	**Age 16-20**	**Age 21-25**
Time in sun (h/day)	Mean Age at MS Onset ± SD			
< 1	29.6±8.5	29.4±8.4	31.3±7.8	34.4±6.9
1-2	31.3±9.1	31.3±9.2	32.4±8.9	35.4±7.9
2-3	31.1±9.2	31.2±9.2	31.9±8.4	35.9±7.9
3-4	31.2±9.1	30.4±8.5	32.5±8.1	35.0±7.1
> 4	31.4±8.7	31.8±9.0	33.0±9.0	35.8±8.1
Linear trend†	$p = 0.14$	$p = 0.16$	$p = 0.23$	$p = 0.25$
Dichotomized (≥1 vs.<1) †	***p* = 0.02**	***p* = 0.03**	$p = 0.13$	$p = 0.16$
Sun Exposure Estimates Weekdays in spring/summer	**Age 6-10**	**Age 11-15**	**Age 16-20**	**Age 21-25**
Time in sun (h/day)	Mean Age at MS Onset ± SD			
< 1	30.1±10.1	31.1±10.5	32.3±7.7	34.8±7.6
1-2	31.0±8.2	31.1±8.9	32.1±8.4	35.6±7.7
2-3	30.9±8.5	30.2±8.5	31.5±8.2	34.7±7.9
3-4	30.5±9.3	30.6±8.8	32.6±8.5	35.3±6.7
> 4	31.2±9.0	31.5±9.1	32.3±8.7	35.5±7.8
Linear trend†	$p = 0.89$	$p = 0.55$	$p = 0.85$	$p = 0.85$

†*p* value was generated using ANOVA

Table 4.4 Mean age at onset of disease symptoms by weighted cumulative sun exposure, and residential solar radiation estimates during childhood and early adolescence, and by skin type and sunscreen use among Veterans with relapsing MS

Variables	No (%)	Mean Age onset ± SD	*p* value††
Cumulative sun exposure in fall/winter between ages 6-15 years†			
Quartile			
Quartile 1: ≤16 wks	187 (22.6)	29.6±8.5	
Quartile 2: 17-23 wks	218 (26.3)	31.1±9.2	0.19
Quartile 3: 24-36 wks	219 (26.4)	31.0±8.7	
Quartile 4: > 36 wks	205 (24.7)	31.5±9.0	
Linear Trend	----	----	**0.057**
Weighted residential solar radiation between ages 0-16 years			
UV counts in 10,000			
Low: < 118,	309 (34.7)	30.7±9.1	
Medium: 118 - <135	295 (33.2)	31.5±8.7	0.53
High: ≥135	286 (32.1)	31.0±8.9	
Latitude			
Northern: ≥ 41°	361 (40.6)	30.8±9.0	
Middle: 37- <41°	311 (34.9)	31.4±8.7	0.67
Southern: <37°	286 (32.1)	31.0±8.9	
Sunscreen use between ages 6-15 years			
Never	566 (68.8)	31.3±9.1	
10-50% of the time	185 (22.5)	31.2±8.8	**0.04**
60-100% of the time	72 (8.7)	28.5±7.3	
Skin Type			
Extremely fair to fair skin	308 (33.0)	30.4±9.0	
Medium to Olive skin	522 (56.0)	31.5±8.9	0.23
Brown to Black skin	133 (11.0)	30.7±8.8	

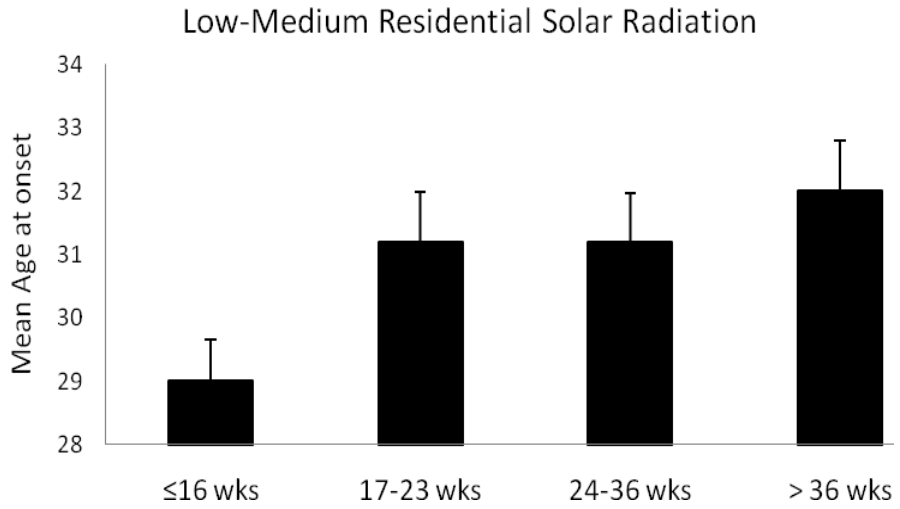
† Cumulative sun exposure estimates represent total weeks of sun exposure for the age-period of 6-15 years

†† *p* value was generated using ANOVA

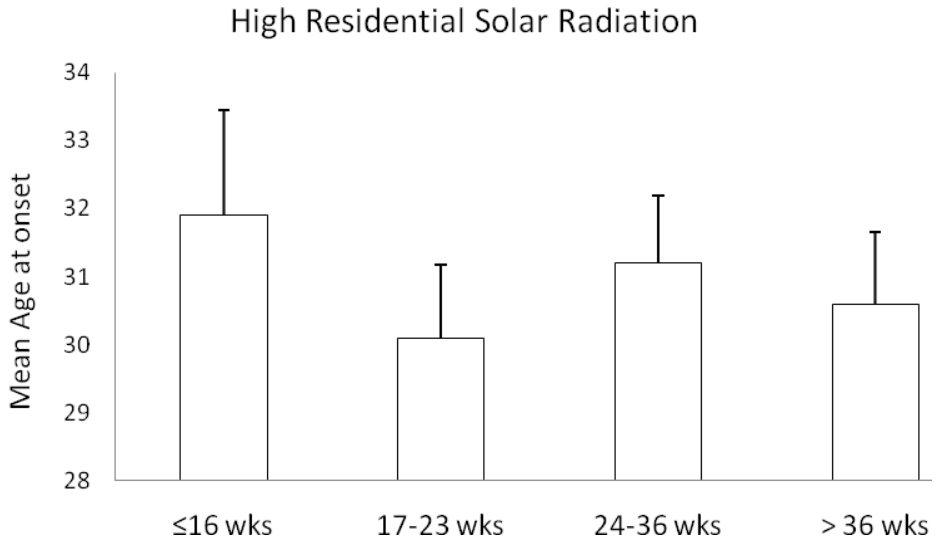
Table 4.5 Mean age at onset of disease symptoms by other important covariates among Veterans with relapsing MS

Variables	N (%)	Mean Age onset ± SD	*p* value
Cod liver oil intake between ages 6-10 years			
Yes	108 (11.6)	32.8±10.4	**0.03**
No	826 (88.4)	30.8±8.7	
Gender			
Female	487 (51.4)	29.9±8.2	**<0.0001**
Male	461 (48.6)	32.3±9.5	
Race			
White	786 (82.9)	31.1±8.9	
Black	103 (10.9)	30.4±8.4	0.42
Others	59 (6.2)	32.3±9.7	
Smoking status before MS onset			
Never	307 (33.8)	30.5±8.8	0.18
Ever	601 (66.2)	31.4±8.8	
Among smokers, # of cigarette			
1-10 cigarettes (1/2 packs)	235 (37.2)	30.5±8.6	
11-20 cigarettes (1 packs)	219 (34.7)	30.9±8.9	0.34
21-39 cigarettes (1-2 packs)	123 (19.5)	32.5±9.1	
40 or more cigarettes (> 2 packs)	54 (8.6)	31.5±8.8	
DX of Mononucleosis before MS onset			
Yes	151 (16.3)	30.6±9.3	0.27
No	694 (74.7)	31.4±9.0	
Unknown	84 (9.0)	29.9±7.5	

(a)



(b)



Cumulative sun exposure in fall/winter between ages 6-15 years

Figure 4.1 Mean age at MS onset by estimated cumulative childhood and early adolescence winter/fall sun exposure and by residential solar radiation

Table 4.6 Multivariable linear regression of age at MS symptom onset on cumulative childhood and adolescence sun exposure adjusted for important covariates among Veterans with relapsing type of MS and who resided in low-medium solar radiation areas during that exposure period.

	Relapsing MS (n = 604)		
Variables[†]	**Estimate (SE)**	**95% CI**	***p* value**
Intercept	32.67 (2.42)	27.92, 37.43	<.0001
Cumulative Childhood sun exposure in winter			
≤16 wks	0	---	---
>16 wks	2.30 (0.92)	0.50, 4.10	0.01
Percentage of sun screen use during 6-15 years			
Never	2.24 (1.42)	-0.55, 5.03	0.12
10-50%	3.22 (1.55)	0.17,6.27	0.04
60-100%	0	---	---
Cod liver intake at age 6-10 years			
No	0	---	---
Yes	3.02 (1.21)	0.64, 5.39	0.01
Sex			
Female	0	---	---
Male	1.79 (0.82)	0.17, 3.41	0.03
Skin type			
Extremely fair to fair	0	---	---
Medium	0.81 (0.88)	-0.91, 2.54	0.36
Brown to black	0.07 (1.67)	-3.21,3.35	0.97
Smoking status before MS onset			
Never	0	---	---
Ever	0.65(0.83)	-0.98, 2.29	0.35

CHAPTER V: SUN EXPOSURE, VITAMIN D INTAKE AND PROGRESSION TO DISABILITY AMONG VETERANS WITH MULTIPLE SCLEROSIS

Sun Exposure, Vitamin D intake and Progression to Disability among Veterans

with Multiple Sclerosis

Tzu-Yun McDowell[1,2], Sania Amr[2], William J. Culpepper[1,2], Patricia Langenberg[2], Walter Royal[1,3], Christopher Bever[1,3], and Douglas D. Bradham[1,4]

1. MS Center of Excellence-East, Baltimore VAMC

2. University of Maryland, Baltimore
Department of Epidemiology and Preventive Medicine

3. University of Maryland, Baltimore, School of Medicine
Department of Neurology

4. University of Kansas, School of Medicine-Wichita
Department of Preventive Medicine & Public Health

Correspondence concerning this article should be addressed to:
Tzu-Yun McDowell
MS Center of Excellence – Baltimore VAMC
100 N. Greene Street
Baltimore, MD 21210
e-mail: Tzu-Yun.Chang-McDowell@va.gov
PHONE: 410.605.7414
FAX: 410.605.7705

5.1 Abstract

Background: A few studies have shown that very early life events, presumably associated with maternal concentrations of vitamin D, could determine Multiple Sclerosis (MS) risk and potentially modulate its clinical course. However, whether or not vitamin D exposures during childhood and adolescence or later in life before disease onset could have long lasting effects on disease progression remains unknown.

Objective: To examine if sun exposure and vitamin D intake (diet and supplements) before MS symptom onset were associated with delayed disease progression to disability in a national cohort of Veterans with MS.

Methods: Patients with MS were recruited from the Veterans Health Administration (VHA) to participate in the VHA Multiple Sclerosis Surveillance Registry, a nationally representative sample of Veterans with MS. In this retrospective cohort study, participants reported their sun exposure, and vitamin D related food and supplement intake by 5-year age-periods as well as age when they progressed to disability milestones using the Patient Disease Determined Disease Steps (PDDS). Average sun exposure before symptom onset was estimated for fall/winter and spring/summer seasons. Time from onset to PDDS stages was the main outcome. Cox Proportional hazards model was used to examine the association between these variables and the time (years) to disability, controlling for known covariates.

Results: Among patients with Progressive MS, (N=151) low average sun exposure in the fall/winter before disease onset was associated with increased risk of progressing to PDDS 8 (HR: 2.13, 95% CI: 1.20-3.78). Whereas, patients who took cod liver oil

regularly during childhood and adolescence had lower risk of reaching PDDS 8(HR: 0.44, 95% CI: 0.20-0.96) compared to those who never.

Conclusions: This study provides evidence of an association of vitamin D related exposures before MS onset with disability among patients with Progressive MS. The neuro-immunomodulatory and/or neuroprotective properties of vitamin D on slowing disease- and age-related degeneration is a plausible explanation; and if proven, it would have substantial implications for intervention in MS.

Keywords: Multiple sclerosis, sun exposure, vitamin D, progression, disability

5.2 Introduction

Multiple Sclerosis (MS) is an inflammatory and degenerative disease of the central nervous system characterized by demyelination, axonal loss and eventual progressive and irreversible neurological dysfunction [2, 169]. Both genetic and environmental factors and their interactions play roles in determining MS susceptibility [27, 170].

In the past decade, cumulative evidence from immunological, ecological and epidemiological studies strongly supports a protective role of ultraviolet radiation (UVR) and vitamin D against the development of MS [50, 74-76]. High sun exposure, cod liver oil intake and fish consumption, especially during childhood and adolescence, were found to reduce the risk of MS [69, 70, 149]. These effects are believed to be due to the influence of UVR on vitamin D synthesis, and direct intake of vitamin D through diet and supplements, respectively. Indeed, a longitudinal study of American military personnel showed that low serum vitamin D levels during adolescence were associated with an increased risk of developing MS later in life [106].

While there is convincing evidence to support the protective effect of UVR and vitamin D against MS development, their role in modulating the clinical course of MS is not clear. In experimental allergic encephalomyelitis (EAE), an animal model of MS, administration of an active form of vitamin D, after immunization but before the appearance of symptoms, has been found not only to prevent the onset but to also reduce disease severity and prolong survival even after the disease occurred[80, 82, 101, 102]. These studies suggest that vitamin D level before symptom onset could potentially alter the clinical course of disease in EAE. It is also known that both UVR and vitamin D have

strong immune modulatory effects in vitro [50, 51, 76, 80]. In humans, it is not clear if vitamin D exposures that alter MS risk could also have long lasting effects on disease progression. A few studies have shown that very early exposures, probably associated with maternal vitamin D level, is related to MS risk and possibly its clinical course[124, 128, 129, 171]. Whether or not vitamin D levels before MS onset, specifically during childhood and adolescence, a time thought to be critical for MS susceptibility, are associated with disease progression remains unknown.

The aim of this study was to examine the influence of sun exposure and vitamin D intake (diet and supplements) before MS symptom onset on the long-term disease disability in a national cohort of Veterans with MS.

5.3 Methods

The University of Maryland Institutional Review Board and the VHA Research and Development Committee at Baltimore VAMC approved the present study.

Study Population

The study population was recruited from the Veterans Health Administration (VHA)-Multiple Sclerosis Surveillance Registry (MSSR). The MSSR, a population-based, self-reported registry of veterans with MS, has been described in detail elsewhere [164]. Briefly, the MSSR cohort was established in 2007 through a mail-based survey under the auspices of the VHA's Multiple Sclerosis Center of Excellence-East (MSCoE-East) in Baltimore, Maryland. From all veterans identified in the VHA system with possible MS, confirmed MS cases were selected using the statistical algorithm of Culpepper et al [139]. From this effective sampling pool, a regionally-stratified, random sample of MS cases

was then targeted for the MSSR. Female Veterans were oversampled to make the MSSR more representative of the general (non-Veteran) population. The goal of the survey that established registry membership was to obtain directly from the participants detailed MS-specific information that is not included in the routine VHA databases, such as year of onset of MS symptom and MS subtype. To date, two questionnaires have been distributed to the MS Veterans cohort to obtain detailed demographic and MS-related information for both clinical outcomes and epidemiological research purposes.

The questionnaire designed for the present study was distributed to the previous registry participants (n = 1,346) and a new cohort of veterans with MS (n = 3,000) between March and October 2009. The rationale for targeting a new cohort of veterans with MS was to expand the current size of the MSSR. As of October 2009, a total of 1,328 participants had completed the questionnaire (response rate ~31%). In the present study, we included only participants who were born and raised in the United States and whose onset of symptoms occurred between 18 and 60 years of age (n = 1,181) to limit heterogeneity due to differences in childhood onset, and very late age of MS onset.

Outcome: Time from MS symptom onset to defined stages of Disability

Participants reported retrospectively the age at which they reached disability milestones using the Patient Determined Disability Scale (PDDS). The PDDS is a simple and reproducible assessment of functional disability in MS [17, 18] that correlates well with the Expanded Disability D Status Scale (EDSS)[172, 173], the current standard measure of disability in MS. It primarily evaluates ambulation of MS patients on a scale of 1 to 9 (from normal motor function to bedridden, see Appendix A). Only the disability stages

that each participant has experienced and maintained for at least 6 months from symptom onset to the present were recorded. Ages at three PDDS stages were chosen for statistical analysis: (a) PDDS 4: Gait Disability, (b) PDDS 6: Late Cane and (c) PDDS 8: Wheelchair / Scooter. A score of 4 corresponds to limited walking ability but usually without a need for assistance. A score of 6 indicates a need for a cane or support to walk 25 feet. A score of 8 indicates that wheelchair or scooter is the main form of mobility. The disability stage that best described each participant's current condition was also reported. By definition, only stages that are equal to or are lower than the current disability stage could be reported. Time from age of MS symptom onset to each of the pre-selected stages of disability ('Time to disability') was then calculated as the study main outcome.

UVR exposure/Vitamin D intake

Participants reported their sun exposure, diet and supplement intake from childhood to the present. All the exposure variables were reported separately for 12 age-periods in 5-year increments from 6 to 65 years, and older than 65 years. Only the exposure data reported to have occurred prior to each participant's age at onset of symptoms were used in this study. For sun exposure estimates, participants reported the average length of time they usually spent in the sun (<1 hour (h) a day, 1-2 h a day, 2-3 h a day, 3-4 h a day, or >4 h a day) during each age period. Sun exposures on weekdays and weekends/holidays, as well as during fall/winter and spring/summer seasons were assessed separately. For each age period, we used the middle point of each response interval as a proxy for the average length of time the participant spent per day in the sun

(e.g. 0.5 h/day for a response of <1 h/day and 1.5 h/day for a response of 1-2 h/day). Two aggregated measurements were then constructed to indicate pre-disease-onset sun exposures: (a) Cumulative sun exposure between ages 6 and 15 years computed by summing the hours of sun exposure on weekdays and weekends for two age-periods (6-10 and 11-15 years) and converting it to a unit of total weeks of sun exposure; (b) Average sun exposure up to the age at MS symptom onset (average weeks of sun exposure per year) as calculated by summing up total hours of sun exposure from age 6 years to age at MS symptom onset and then divided by number of years from 6 years to age at symptom onset. Separate variables of these sun exposure estimates were calculated for fall/winter, spring/summer, and combined seasons.

Diet intake was obtained via questions on how often on average (rarely, occasionally, often or very often: ordinal scale 1 to 4) each participant ate a particular food (fish, milk and egg/cheese) in the specified age-periods. Whether or not each participant took supplements (cod liver oil, vitamin D/calcium and multi vitamin) on a regular basis during the specified age periods was also obtained. We further derived variables indicating frequency of each food and supplement between age 6 and15 years and up to the age at MS symptom onset.

Skin type

Participants indicated their skin type according to skin color, tendency to burn and ability to tan, using the Fitzpatrick classification [62] (type 1: extremely fair; type 2: fair; type 3: medium; type 4: olive; type 5: brown; and type 6: black skin color). Skin type was further grouped into three categories – very fair to fair (type 1 and 2), medium olive (type 3 and 4) and brown to black (type 5 and 6).

Other covariates

Basic demographic variables, such as age, sex, race and education as well as clinical variables including age at symptom onset, MS subtype, and onset symptoms (motor/coordination, sensory, vision, systematic, and bowel and bladder problems) were reported by each participant. MS subtype was reported according to the conventional classifications: 1) Relapsing MS: patients who experienced relapses/exacerbations at onset and during early course of the disease including relapse-remitting (RRMS) and secondary progressive (SPMS) MS and 2) Progressive MS: patients with progressively worsening disease from onset with or without experiencing relapses later in the disease course. Whether or not participants smoked and had mononucleosis diagnosis before onset of MS was also recorded.

Statistical analyses

The outcome measure was time from disease symptom onset to selected stages of PDDS including PDDS 4, PDDS 6 and PDDS 8. When the end-points (PDDSs) were not reported to have been reached by the participants, the time data were right-censored at the date of the survey. Kaplan-Meier analysis was used to examine the distribution and median (when appropriate[1]) of each outcome by disease subtype: Relapsing MS (both RRMS and SPMS), RRMS alone, SPMS alone, and Progressive MS. In addition, ages at each of the selected disability stages were also examined using Kaplan-Meier analysis to provide a global picture of how the disability progressed for each subtype of MS.

Considering that PDDS 4 represents a very mild stage of disability, and the

[1] Median of PDDS 8 among Veterans with relapsing-remitting MS could not be calculated due to high percentage of censoring

majority of patients with Relapsing MS did not reach the disability stage of 8 by the entry in the study, we used time to PDDS 6 as the main outcome to investigate its association with pre-onset UVR and vitamin D related exposures among patients with Relapsing MS. For patients with Progressive MS, both time to PDDS 6 and PDDS 8 were assessed. Log-rank tests were used to examine unadjusted cumulative survival rate among groups according to their sun and vitamin D-related exposure status (e.g. low and high sun exposure groups) and other covariates. Cox proportional hazards models were developed to estimate adjusted hazard ratios and 95% CIs for the associations between sun and vitamin D-related exposures and each outcome, adjusted for covariates. Covariates were assessed for potential effect modifiers in these models. Proportional hazards model assumption was tested for each model. All analyses were performed using SAS version 9.1.

5.4 Results

A total of 1,181 MS patients were included in this project (Table 5.1).. Eighty percent of participants (n = 948) reported having the relapsing form of MS. Among this group, 494 patients (52.1%) reported that they currently had reached SPMS. There were 219 subjects (18.5%) with Progressive MS at onset. Overall, the majority of the participants were White (>80%). About 50% of those with Relapsing MS were female; whereas males represented 77% of those with Progressive MS. Distributions of skin type were comparable among MS subtypes, with 30% of subjects reported very fair and fair skin type overall. Subjects with RRMS and SPMS had very similar ages at MS symptom onset, whereas patients with Progressive MS were older at disease onset. In contrast, with respect to disease duration and current disability stage, those with SPMS and Progressive

MS were similar; they had longer disease duration and greater disability compared to patients with RRMS (Table 5.1).

Table 5.2 displays Kaplan-Meier estimates of time from symptom onset to (years) and age (years) at the three PDDS stages by clinical subtype. Participants with Progressive MS reached each PDDS stage faster than those with Relapsing MS (p <0.0001). The median time to PDDS 6 was 16 (95% CI: 14-18) and 30 (95% CI: 28-31) years for Progressive and Relapsing MS, respectively. Among subjects with Relapsing MS, about 50% reached SPMS by the date of the survey, with median time to SPMS of 22 years (95% CI: 20-25). RRMS patients progressed at a much slower rate compared to SPMS. About eighty percent of RRMS patients had not reached PDDS 6 by the date of the survey. The median time to PDDS 6 was 53 (95% CI: 39-53) and 24 (95% CI: 21-27) years for RRMS and SPMS, respectively.

Estimates of the median ages at PDDS stages, on the other hand, were not substantially different among Relapsing and Progressive MS (Table 5.2). Specifically, subjects with SPMS reached disability stages at a similar age as patients with Progressive MS. The median age at PDDS 6 was 54 years (95% CI: 52-55) among Veterans with SPMS and 55 years (95% CI: 52-57) among those with progressive MS (p = 0.80).

Progressive MS

Kaplan–Meier analyses with log-rank tests were performed to examine the associations between sun exposure and vitamin D intake before MS onset (main predictors), a set of potential covariates (i.e. demographics, smoking status and diagnosis of mononucleosis before onset) and time to PDDS 6 and PDDS 8 (outcomes).

Associations at the less than 0.20 significance level and important covariates are shown in Table 5.3. The complete table with all the investigated variables is included in Appendix B, Table 3. Participants with low average sun exposure during fall/winter time before onset of MS progressed to PDDS 6 and PDDS 8 faster than those with higher average sun exposure (p = 0.006 and 0.01), respectively. The median time from disease onset to PDDS 8 was 20 years (95% CI: 16-29) for the former, compared to 29 years (95% CI: 14-42) for the latter. Among Veterans who ever took cod liver oil, 90% of them took it between 6 and 15 years of age, and reached disability stages, especially for PDDS 8, later than those who never took cod liver oil (p = 0.01). Subjects who rarely ate fish (less than once a week) seemed to progress to disability stages faster, although it was not statistically significant. Moreover, we found that subjects who reported being diagnosed with mononucleosis before symptom onset progressed to PDDS 6 and PDDS 8 more rapidly compared to those who reported never having had it (p = 0.05 and 0.005), respectively. We also found that younger age at symptom onset was associated with longer time to the disability stages (p = 0.003).

A Cox proportional hazards model was used to examine sun exposure and vitamin D intake simultaneously while controlling for other important covariates. The results for PDDS 6 and PDDS 8 were very similar and in the same direction as the Kaplan–Meier analyses, with a stronger effect observed for PDDS 8. Table 5.4 shows the factors affecting the time from symptom onset to PDDS 8 derived from the multivariate Cox model. Subjects with low fall/winter sun exposure before symptom onset had about 2.1 times (HR: 2.13, 95% CI: 1.20-3.78) the risk of reaching PDDS 8 at a given time compared to those with higher sun exposure. Diagnosis of mononucleosis before onset

was associated with a substantial increased risk of developing PDDS 8 (HR: 2.65, 95% CI: 1.33-5.27). Conversely, Veterans who reported taking cod liver oil between the ages of 6 and 15 years had lower risk than those who never took it (HR: 0.44, 95% CI: 0.20-0.96) for reaching PDDS 8. High frequency of fish consumption between age 6-15 years had a marginal protective effect ($p = 0.09$). In addition, patients who reported having sensory symptoms at onset were less likely to progress to PDDS 8. (HR: 0.56, 95% CI: 0.34-0.91). Gender was not associated with the risk of progressing to PDDS 8 ($p = 0.60$).

Relapsing MS

Unlike Progressive MS, no apparent associations were found between sun exposure and vitamin D intake before disease onset and time to PDDS 6 from the Kaplan–Meier analyses among subjects with Relapsing MS (Table 5.5). Age at symptom onset was negatively associated with the time to PDDS 6 ($p <0.0001$). Veterans with younger age at disease onset had longer time to PDDS 6. Those who subsequently developed SPMS had shorter time to PDDS 6 compared to those who remained as RRMS. We observed a small effect of vitamin D intake; those who reported never taking calcium and vitamin D seemed to have had longer time before reaching PDDS 6. However, the effect diminished after adjusting for age at MS onset in a Cox regression model. We were not able to identify any other significant factors to fit a Cox-proportional hazards model for this subgroup.

Considering that patients who remained as RRMS subtype might have much slower progression to disability, compared to those who transitioned to SPMS, we conducted the same analyses among SPMS patients alone. The results from the Kaplan-Meier analyses of this subset of Veterans were very similar to those with Relapsing MS,

with age at onset as one single factor associated with time to PDDS 6 (p <0.0001). We also examined factors associated with time to PDDS 8, a disability score reported by 36.3% (171/471) of those who progressed to SPMS. The log-rank test showed that cod liver oil intake between 6 and15 years of age was associated with a slow progression to PDDS 8 (p = 0.01). And, patients with motor symptoms at onset progressed to PDDS 8 more rapidly than those without (p = 0.02). No other variables were identified to be significantly associated with time to PDDS 8 in SPMS patients. The direction of these two effects did not change after controlling for age at symptom onset and sex in a Cox proportional hazard model (Table 5.6). The exploratory analyses/tables for Relapsing MS are included in Appendix B, Table 4.

5.5 Discussion

The present study was conducted to investigate associations between sun exposure and vitamin D-related intakes before disease onset and time to disability in a cohort of Veterans with MS. Among Veterans with Progressive MS, low average sun exposure in fall/winter before disease onset was associated with an increased risk of disability; whereas cod liver oil intake during childhood and adolescence lowered the risk. A small protective effect of cod liver oil consumption was found among those who subsequently transitioned from relapsing to SPMS.

This study is one of the first to investigate the dual influence of sun exposure and vitamin D-related intakes before MS onset on disease progression. Previous studies have found that exposures early in life, probably related to maternal levels of vitamin D, might have long lasting effects on the clinical course of the disease [124, 128, 129]. The possibility

that UVR and vitamin D exert their beneficial effects by modulating the development of nervous system and/or immune system in the fetus has been suggested. Our data extend the previous findings and suggest that exposures related to vitamin D from childhood to MS onset might also have small influences on long term disease outcomes, at least among a subset of veterans with Progressive MS.

One previous study investigated the association between disability in MS and UVR exposures during childhood (0-16 years) and adult life (17-40 years) among 448 Caucasians with MS and did not find any significant effects [138]. The discrepancy in the results between this study and ours may be in part due to methodological differences including choice of the outcomes (EDSS vs. PDDS), exposure measurements (cumulative sun exposure in specified age-periods vs. average sun exposure up to age at symptom onset) and statistical approaches (logistic regression vs. survival analysis). Moreover, the previous study did not address MS subtype and it is likely that the majority of the subjects in the study had Relapsing MS, among who we also did not find a significant association between sun exposure and disability.

The observed protective effects of sun exposure and vitamin D-related intake on disability only among subjects with Progressive MS, but not Relapsing MS are not easily explained. MS is a disease known for its variable patterns of clinical presentation and rates of disability accumulation [1, 2, 169]. Relapses (acute neurological symptoms, usually following with varying degrees of recovery-remissions), and progression (continuous worsening of symptoms and functions) represent two clinical phenomena which are predominately related to the underlying disease activities: inflammation and degeneration, respectively. Patients with Progressive MS are thought to have worse prognosis with

neurodegeneration as the dominant disease process since symptom onset. Patients with Relapsing MS experience a clinical course with variable degrees of interplay between relapse (inflammation) and progression (degeneration), with some cases never transitioning to the progressive phase. It is possible that the protective effect of sun exposure and vitamin D intake might be diluted and difficult to detect among patients with Relapsing MS, because this subset of patients represents a highly heterogeneous group with respect to prognosis. The effects are also potentially subject to other confounding factors such as duration and frequency of use of disease modifying therapy (DMT). DMT is targeted primary for patients with Relapsing MS and has demonstrated its efficacy by reducing relapse rates, relapse severity, and MRI lesions [174, 175]. In addition, the majority of Relapsing MS Veterans in the study reported not having reached the studied endpoints, which makes it difficult to investigate factors associated with long term disability. In fact, when we restricted our sample only to patients who reached SPMS, a small protective effect of cod liver oil on disability was observed.

The protective effects of vitamin D on MS susceptibility and some clinical disease activities (e.g. reducing relapse rate) are mainly linked to its potent immunomodulatory functions that suppress inflammation-initiated disease mechanisms [74, 76, 80]. On the other hand, there is evidence indicating that vitamin D has also strong neuroprotective effects that could potentially be valuable in the treatment of some neurodegenerative diseases [96, 176]. In MS, although autoimmunity and inflammation drive the development of MS and its early disease activities, neurodegenerative changes are thought to be the major causes of disease progression and disability accumulation [2]. In conjunction with the positive findings among subjects with Progressive MS, it is plausible that the neuroprotective

effect of vitamin D could potentially delay disease- or age-related degeneration in the nervous system, thus influence long term disability in MS.

The finding that Progressive MS patients with a history of mononucleosis before their disease onset had higher risk of progressing to disability is interesting. Epstein-Barr virus (EBV) has been identified as a risk factor for MS [26, 44]. Infectious mononucleosis, an indicator of late age EBV infection, was also associated with an increased risk of MS [36]. However, the role of this infectious agent in disease progression is largely unknown, although a few studies have suggested that viral or bacterial infections might trigger exacerbations among patients with RRMS [177-179]. Additional studies are required to investigate the potential role of infectious agents in long-term disability in MS.

In the present study, only a few demographic and clinical variables were found to be associated with long term disability among both Relapsing and Progressive MS patients. Indentifying reliable prognostic factors has been a challenging task. Despite considerable efforts made in the past decade to describe the natural history of MS and to investigate the potential factors affecting prognosis, very few prognostic elements have been consistently identified across studies. The finding that younger age at onset was associated with longer time to disability stages is consistent with that of previous studies [11, 180]. Younger age at onset was formally considered as a good prognostic factor for MS; however, several studies have suggested that it is not necessary the case, because individuals with younger age at onset reached disability stages at younger age [11, 180]. In addition to age at onset, the presence of sensory symptoms at onset was found in our study to be associated with better prognosis among Veterans with Progressive MS. This finding is in agreement with a recent study, in which the authors identified the existence

of sensory symptoms at onset as a main factor for favorable prognosis among patients with primary progressive MS [180].

Overall, our study findings are comparable to those of reports from other national cohorts of MS with respect to MS prognosis [7, 180]. That is, although patients with Progressive MS reached disability stages faster than those with Relapsing MS, the actual average age at disability stages was not substantially different between the two patient groups, especially not different from those who transited to SPMS. Altogether these data support the argument that progression among MS patients might be mainly age-dependent at a population level [7, 8, 11, 181].

Although we have identified some vitamin D-related exposures associated with disease progression, particularly among Veterans with Progressive MS, the results are rather preliminary and should be interpreted with caution, considering the relatively small number of patients in the final analysis. Replication by future studies is required to confirm the current findings. Many limitations are present in the study, with the major one being the potential recall bias and measurement errors related to self-reported data. Subjective reports of sun exposure and vitamin D intake could cause considerable misclassification. However, we do not think that the misclassification is differential according to severity of the disease, and is thus to bias the results towards the null. Furthermore, despite the fact that PDDS is a useful assessment for long term disability, it is highly weighted by ambulation functions, and does not consider other signs and symptoms resulting from disease progression, such as cognitive dysfunction. Additionally, our study population is more likely to consist of patients with mild to moderate disability because of the design of this study, cross-sectional and voluntary

mail-based survey. Patients with more severe disease might not be able or be willing to participate, thus were not included. As a result, our study might not represent the whole spectrum of MS patients in terms of disability.

In summary, the current study provides evidence for protective effects of vitamin D related exposures before MS onset on disability among patients with Progressive MS. The neuroprotective effect of vitamin D on slowing disease- and age-related degeneration is a plausible explanation, and if proven, it could have substantial implications for the prevention and treatment for MS.

Table 5.1 Sociodemographic and clinical characteristics of the study sample of Veterans with MS

	Relapse Remitting MS (n = 454)	Secondary Progressive MS (n = 494)	Progressive MS (n=219)
Personal Characteristics			
Age (Mean ± SD)	53.0±10.4	56.6±9.9	60.9±9.6
Race, n (%)			
1.White	381 (89.0)	405 (87.9)	178 (81.3)
2. Black	47 (11.0)	56 (12.2)	34 (15.5)
Sex, n (%)			
1. Female	273 (60.1)	214 (48.3)	50 (22.8)
2. Male	181 (39.9)	280 (56.7)	169 (77.2)
Education			
Average years (SD)	14.8 ± 2.8	14.5 ± 3.1	14.4 ± 3.0
Smoking history before MS onset, n (%)			
1. Never	154 (35.3)	153 (32.4)	54 (26.0)
2. Ever	282 (64.7)	319 (67.6)	154(74.0)
DX of Mononucleosis before MS onset, n (%)			
1. Yes	96 (21.5)	69 (9.1)	33(15.4)
2. No	311 (69.6)	372 (76.7)	166 (77.6)
3. Unknown	40 (9.0)	44 (14.2)	15 (7.0)
Skin type, n (%)			
1. Extremely fair skin	23(5.1)	29 (6.0)	9 (4.2)
2. Fair skin	136 (30.4)	120 (24.7)	46 (21.5)
3. Medium skin	194 (43.3)	211 (43.5)	101 (47.2)
4. Olive skin	49 (10.9)	68 (14.0)	32 (15.0)
5. Brown skin	16 (3.6)	23 (4.7)	8 (3.7)
6. Black skin	30 (6.7)	34 (7.0)	18 (8.4)
Clinical Characteristics			
Age (years) at MS, Mean ± SD			
Symptoms onset	31.8±8.9	30.4±8.9	36.1±10.9
Diagnosis	37.8±9.4	37.6±9.8	42.0±10.7
Disease duration (years), Mean ± SD	21.2±11.2	26.2±11.5	24.9±12.4
Current PDDS stage	4.2±2.1	6.2±1.8	6.8±1.6

Table 5.2 Kaplan-Meier estimates of time to and age at the Patient Determined Disease Stage (PDDS) among Veterans with MS

	Relapsing MS[†] (n=948)	Relapse-Remitting MS (n =454)	Secondary Progressive MS (n=494)	Progressive MS (n =219)
From onset of symptom to	**Median time (years) [95% CI] to different PDDS stages N (% censored)[††]**			
Secondary progressive MS	22 [20-25] 948 (47.9%)	---	---	---
PDDS 4	14 [13-15] 756 (26.1%)	18 [15-20] 368 (44.6%)	11 [10-13] 388 (8.5%)	8 [7-9] 156 (4.5%)
PDDS 6	30 [28-31] 844 (57.5%)	53 [39-53] 408 (79.2%)	24 [21-27] 436 (37.2%)	16 [14-18] 168 (25.0%)
PDDS 8	45[41-46] 897 (79.6%)	NA 426 (91.6%)	38 [36-42] 471 (68.8%)	27 [24-33] 204 (50.0%)
	Median age (years) [95% CI] at the time of reported PDDS			
PDDS 4	47 [45-48]	50[49-54]	44 [42-45]	47 [43-49]
PDDS 6	60 [58-61]	75[66-]	54 [52-55]	55 [52-57]
PDDS 8	74 [70-]	NA	70 [65-76]	65[61-67]

[†] Relapsing MS include both cases with relapse-remitting and secondary progressive MS

[††]n (% censored): indicates number of Veterans by subtype and by disability stage (percentage of Veterans who did not reach the end point). The differences in number of Veterans for disability stages within each subtype are due to missing values of reporting age at disability stages.

Table 5.3 Effects of sun exposure, vitamin D intake, and covariates on the estimates of time from disease onset to PDDS 6 and PDDS 8 among Veterans with progressive MS

Variable	Time to PDDS6			Time to PDDS8		
	No of patients (% censored)	Median (95% CI)	P-value†	No of patients (% censored)	Median (95% CI)	*p*-value†
Average fall/winter sun exposure before MS onset	N = 140††			N = 163††		
Low ≤ 1.6 weeks/year	33 (21.2)	11 [7-16]	0.006	36 (41.7)	20 [16-29]	0.01
High > 1.6 weeks/year	107 (28.0)	18 [14-31]		127 (55.1)	29 [24-42]	
Fish consumption between ages 6-15 years	N = 158			N = 187		
Rarely	42(26.2)	12 [9-18]	0.11	48 (41.7)	24 [16-28]	0.09
Sometimes	67 (31.3)	18 [14-26]		81 (50.6)	33 [20-43]	
Often-Very Often	49 (16.3)	16 [15-21]		58 (56.9)	30 [24-54]	
Average Fish consumption before MS onset	N= 154			N = 187		
Rarely	31 (22.6)	12 [10-20]	0.10	35 (40.0)	26 [16-29]	0.30
Sometimes	56 (33.9)	17 [14-28]		67 (46.3)	24 [19-42]	
Often-Very often	67 (19.4)	16 [14-20]		80 (57.5)	30 [23-53]	
Cod liver intake between ages 6-15 years	N = 167			N = 202		
Never	141 (24.8)	16 [14-18]	0.19	171 (49.1)	26 [21-30]	0.01
Ever	26 (26.9)	16 [14-30]		31 (58.1)	38 [31-54]	
Skin Type	N = 165			N =201		
Very fair to fair	46 (23.9)	17 [11-24]	0.88	52 (51.9)	33 [24-47]	0.47
Medium to Olive	100 (23.0)	15 [13-17]		123 (48.0)	25 [21-30]	
Brown to Black	19 (31.6)	16 [11-26]		26 (53.9)	31 [17-34]	
Gender	N = 168			N = 204		
Female	40 (35.0)	15 [10-19]	0.83	47 (59.6)	31 [17-45]	0.98
Male	128 (21.9)	16 [15-20]		157 (47.1)	27 [24-33]	

Variable	Time to PDDS6			Time to PDDS8		
	No of patients (% censored)	Median (95% CI)	P-value†	No of patients (% censored)	Median (95% CI)	*p*-value†
Smoking status before MS onset	N†† = 160			N = 193		
Never	43 (32.6)	16 [10-19]	0.36	50(58.0)	27 [20-45]	0.97
Ever	117 (22.2)	16 [15-20]		143 (49.0)	29 [24-35]	
DX of Mononucleosis before MS onset	N = 162			N = 198		
Yes	22 (22.7)	11 [7-17]	0.05	27 (44.4)	16 [12-21]	0.005
No	140 (26.4)	16 [15-20]		171 (50.9)	29 [24-33]	
Age at symptom onset	N = 168			N=204		
18-29 years	53(20.8)	23 [17-29]	0.0008	68(44.1)	34 [27-43]	0.0033
30-39 years	42 (19.1)	16 [10-20]		53 (49.1)	28 [23-37]	
40-49 years	44 (25.0)	13 [10-16]		51 (47.1)	20 [16-35]	
≥50 years	29 (41.4)	13 [6-15]		32 (68.8)	18 [14-	
Type of symptoms at onset						
Motor/Coordination	N = 168			N = 204		
Present	135(25.9)	16 [15-20]	0.46	163(52.8)	29 [24-34]	0.22
Absent	33 (21.2)	15 [11-20]		41 (21.2)	24 [17-30]	
Sensory	N = 168			N =204		
Present	114(24.6)	16 [15-20]	0.31	135(52.6)	29 [24-35]	0.12
Absent	54(25.9)	15 [10-20]		54(44.9)	24 [20-30]	

† *p* value was generated by log-rank test

†† N indicates number of Veterans who had reported the exposure and age at disability. Difference in N due to missing values in reporting particular exposure and age at the disability stage

Table 5.4 Cox Proportional Hazards Model for the factors associated with time from symptom onset to PDDS 8 among Veterans with progressive MS (n = 151) [†]

Variables	Hazard Ratio (95% CI)	*p* value
Age at symptom onset (years)	1.03 (1.0-1.06)	0.02
Gender		
Male vs. Female (reference)	1.19 (0.62-2.23)	0.60
Average fall/winter sun exposure before MS onset		
Low vs. High (reference)	2.13 (1.20-3.78)	0.01
DX of Mononucleosis before MS onset		
Yes vs. No (reference) [††]	2.65 (1.33-5.27)	0.006
Cod liver oil intake between ages 6-15 years		
Ever vs. Never (reference)	0.44 (0.20-0.96)	0.04
Fish consumption between ages 6-15 years		
Sometimes vs. rarely (reference)	0.79 (0.45-1.41)	0.43
Often-very often vs. rarely (reference)	0.58 (0.31-1.08)	0.09
Sensory symptom at onset		
Present vs. absent (reference)	0.56 (0.34-0.91)	0.02

[†]A total of 204 PPMS Veterans had data on time to PDDS 8, but only 151 were included in the model, because of some missing values.

[††]14 Veterans reported unknown for this variable. In this model, we treated unknown as No. Reassigning unknown as yes did not change the direction of findings for this variable and others in the model.

Table 5.5 Effects of sun exposure, vitamin D intake, and skin type on the estimates of the time from disease onset to PDDS 6 among Veterans with Relapsing MS

Variable	Time to PDDS6		
	No of patients (% censored)	Median (95% CI)	*p*-value[†]
Average fall/winter sun exposure before MS onset	N = 737		
Low ≤ 1.6 weeks/year	183 (60.1)	31[28-38]	0.33
High > 1.6 weeks/year	554 (57.9)	28[27-31]	
Cod liver intake between ages 6-15 years	N = 836		
Never	737 (58.3)	29 [27-31]	0.37
Ever	99 (52.5)	33 [26-38]	
Calcium/ Vitamin D intake before MS onset	N=835		
Never	717(58.0)	30 [28-33]	
Ever-less than half of time	69(63.8)	23 [20-36]	0.05
Ever-more than half of time	49 (44.9)	25 [17-31]	
Skin Type	N = 832		
Very fair to fair	276 (54.4)	28 [26-31]	
Medium to Olive	467 (59.3)	31 [28-35]	0.46
Brown to Black	89 (58.4)	30 [20-36]	
Gender	N = 844		
Female	439 (53.1)	29 [26-33]	0.54
Male	405 (51.4)	30 [27-32]	
Age at symptom onset	N = 844		
<25 years	211 (56.4)	35 [31-39]	
25-34 years	363 (54.6)	28 [25-32]	<0.0001
35-44years	188 (63.3)	25 [20-28]	
≥ 45 years	82 (59.8)	15 [12-14]	
MS subtype	N = 844		
SPMS	436 (37.2)	24 [21-27]	<0.0001
RRMS	408 (79.2)	53 [39-53]	
Sensory symptom at onset	N = 844		
Present	568 (56.7)	30 [28-34]	0.24
Absent	276 (59.1)	29 [25-31]	

[†]p values generated from log-rank test

Table 5.6 Cox Proportional Hazards Model for the factors associated with time from symptom onset to PDDS 8 among SPMS patients (n = 466) [†]

Variables	Hazard Ratio (95% CI)	*p* value
Age at symptom onset (years)	1.05 (1.03-1.07)	<.0001
Gender		
Male vs. Female (reference)	1.03 (0.72-1.47)	0.85
Cod liver oil intake between ages 6-15 years		
Ever vs. Never (reference)	0.54 (0.31-0.94)	0.03
Motor symptom at onset		
Present vs. absent (reference)	1.61(1.06-2.43)	0.02

[†]A total of 471 SPMS patients had data on time to PDDS 8, but only 466 were included in the model, because of some missing values.

CHAPTER VI: DISCUSSION AND CONCLUSIONS

Multiple Sclerosis (MS), an immune-mediated inflammatory and neurodegenerative disease, is the most common neurological disorder among young adults. The clinical course of MS is variable and its progression can lead to considerable disability, and thus significantly impacts an individual's health and quality of life[1-3, 15, 182]. The underlying factors that cause MS are as yet largely unknown. Both genetic and environmental factors, likely with complex interactions between them, are involved in the etiology of MS[120, 121, 183, 184]. The role of the environment is most evident from studies of the disease geographical distribution,[31, 45-47] migration studies,[123, 158-160]and more recently the month of birth effects on MS risk[122, 124, 126, 185].

Among the environmental factors reported to be associated with MS, vitamin D emerges as one of the most studied and convincing candidates; it also has the greatest potential in terms of clinical implications for disease prevention and intervention[74-76, 80, 186]. The protective role of vitamin D is based on mounting evidence from multiple fields of research. In immunological and experimental studies, vitamin D has potent anti-inflammatory and immunomodulatory effects[76, 80, 89, 101-103], the benefit of which has been postulated as the underlying mechanism for decreasing MS risk and potentially modulating its course. In addition, observations from epidemiological research including geographical distribution of the disease, effects of past sun exposure, vitamin D intake, and serum vitamin D levels on MS risk have further strengthened the protective role of vitamin D in MS susceptibility[31, 45-47, 69-71, 83, 106]. In particular, the timing when environmental factors including vitamin D could presumably influence MS risk has been suggested. Very early in

life (in utero or right after birth) as well as childhood and early adolescence are critical periods where vitamin D might be involved in MS pathogenesis[69-71, 120, 124].

Few studies have suggested that environmental factors associated with MS risk during specific age-periods might also impact the course of MS clinical manifestations[128, 161, 165]. Month of birth, presumably a proxy for an early life event that could be related to maternal concentrations of vitamin D levels, had been suggested, not only to influence MS susceptibility, but also its clinical course[124, 128, 129]. However, it is unknown if this early life event could influence other MS related outcomes such as age at onset. In addition, whether or not vitamin D related exposures after birth, specifically during childhood and early adolescence, could have some bearing on the course of the disease remains unclear.

The overall objective of this dissertation was to examine if vitamin D related exposures before MS onset, particularly early in life and during childhood and adolescence, were associated with its clinical course, including age at symptom onset and time to long term disability. We conducted a cross-sectional study, using a questionnaire designed to assess the different parameters that contribute to UVR exposure and vitamin D synthesis, in a cohort of Veterans with MS. Our study is the first to examine the dual influences of (1) timing and geographical location of birth and (2) sun exposure and vitamin D-related intakes before MS onset on the clinical course of this disease. The investigation is also the first to assess the proposed associations by the disease subtype (Relapsing vs. Progressive MS).

6.1 UVR Exposure, Vitamin D Intake and Clinical Manifestation of MS

In the original MSSR cohort of 731 Veterans with Relapsing MS, we found that those who were born in winter and whose birthplace was in low solar radiation areas, had disease

symptom onset on an average 2.8 years earlier than those born in other than winter season and in medium/high solar radiation areas. Furthermore, in the current cohort of 948 Veterans with Relapsing MS, low sun exposure during childhood and early adolescence (age-periods 6-15 years), particularly in fall/winter, was associated with earlier age at symptom onset. This association was only observed among those who resided in areas with low to medium solar radiation from birth to adolescence. Sunscreen use from ages 6 to 15 years was also associated with younger age at symptom onset, though the majority of participants (68.8%) reported never using sunscreen during that period. Conversely, the regular use of cod liver oil during childhood was associated with later onset of MS symptoms. Altogether these findings support the notion that sun exposure and vitamin D intake have an impact on the clinical course of Relapsing MS, particularly age of symptom onset.

To further investigate whether or not vitamin D related exposures before MS onset also has long lasting effects on disease progression, we examined the time to different disability stages, using Patients Determined Disease Steps (PDDS) as outcomes. We were not able to identify any association between the environmental factors studied and time to disability among subjects with Relapsing MS. However, among participants with Progressive MS (n=151), those with low average fall/winter sun exposure before symptom onset had about 2.1 times (HR: 2.13, 95% CI: 1.20-3.78) the risk of reaching PDDS 8 at a given time pat onset compared to those with higher sun exposure; whereas regular intake of cod liver oil during childhood and adolescence decreased the risk. Interestingly among Veterans with this subtype of MS, we did not find any associations between the same variables and age at symptom onset.

Overall, our findings expand the current literature and suggest that environmental factors before MS onset, mainly related to sun exposure or vitamin D status, not only influence MS susceptibility but also have some impact on its clinical course. Specifically, exposures early in life as well as during childhood and early adolescence seem more critical for the time when the disease occurred among participants with Relapsing MS; whereas exposures later in adulthood could still have some influence on disease progression among subjects with Progressive MS.

For vitamin D-related environmental factors to exert their beneficial effects on MS susceptibility, exposure timing has been suggested to occur during prenatal periods or near birth as well as during childhood and adolescence[69-71, 120, 124]. Recently, month of birth, was found to have an effect on MS risk in northern countries, with the highest risk for May births and the lowest risk for November births[124, 126]. Remarkably, the observed effect was entirely derived from patients with relapsing-remitting MS but not progressive MS[128]. This finding strongly indicates that the presumed early environmental life event(s) are associated with MS phenotype, and potentially with disease progression. Indeed, association of the latter with the month of birth has been reported, but the observations are scarce and controversial[129, 130]. Although the nature of the environmental factors that contribute to these findings has yet to be determined, maternal vitamin D levels, which potentially influence the development of the central nervous system and/or immune system, is a plausible candidate[124]. Our results support the role of early life environmental events in MS pathogenesis. Specifically, exposure early in life to geographical and seasonal factors possibly related to the protective effect of sunlight, and thus vitamin D, is associated with delay in MS symptom onset. Whether our findings

relate to maternal or perinatal vitamin D levels or some other seasonally varied environmental factors in early life remains to be determined.

Low sun exposure and fish consumption, thus presumably low vitamin D levels, during childhood and adolescence have also been suggested to increase MS risk[69-71]. We found that low sun exposure in fall/winter during this period was associated with earlier age at symptom onset, and therefore one would assume that sun exposure and vitamin D, as protective factors for MS, influence the underlying disease process by modifying the latent period between disease initiation and its first clinical manifestation. The actual mechanisms for how vitamin D is involved in MS pathogenesis during childhood and adolescence are still unclear. However, it is suspected that interactions between MS-associated genes and vitamin D status as well as related epigenetic effects might take place during this critical period, and subsequently contribute to MS pathogenesis[113, 168, 184].

Our study is consistent with several previous ones, in which fall/winter sun exposure was particularly important in the context of childhood sun exposure and MS risk [69, 168]. In addition, we only observed an effect of fall/winter sun exposure among subjects residing in areas with relatively lower solar radiation. In a recent study, winter sun exposure during childhood and adolescence was found to modify the association between vitamin D receptor gene and MS risk [168]. Taken together, these findings imply that a minimum threshold requirement might exist for solar radiation and vitamin D to exert their effects on disease susceptibility and process during critical periods of development. It is possible that any genetic effects or gene-environment interactions during childhood and adolescence are most obvious under the conditions in which UVR exposure is inadequate or vitamin D status is insufficient.

As for disease progression, we found that low average sun exposure in fall/winter from childhood to age at onset was associated with high risk of MS progression to disability among participants with Progressive MS. Cumulative sun exposure during childhood and adolescence was not associated with disease progression. These findings suggest that chronic deficiencies of winter sun exposure and/or vitamin D status before onset might also have some influences on the clinical course of the disease such as disability.

The beneficial effects of vitamin D on MS are most likely related to its potent anti-inflammatory and immunomodulatory properties, which have been proposed to reduce MS risk and modulate early clinical disease activities (reduce relapse rate)[74, 76, 80]. MS is generally regarded as an immune-mediated disease with inflammation and demyelination causing reversible functional deficits, in the early relapsing phase of the disease. However, it is becoming clearer that the degree of neurological disability in MS is predominantly related to the extent of neurodegeneration, the central pathology underlying the chronic progressive stage of MS.[1] As a result, there is an emerging perspective of broadening therapeutic repertoire for MS to include neuroprotective and neurodegeneration treatment strategies[187-189]. It is recognized that vitamin D, a steroid hormone, has a variety of neuroendocrine functions[190, 191]. It is also involved in multiple functions in the brain and nervous system including neuroprotection [96, 176 190, 191]. Numerous vitamin D and vitamin D receptor-mediated neuroprotection effects have been reported and suggested to be beneficial in treating neurodegenerative disorders such as Alzheimer's as well as serving as a neuroprotective therapy in the elderly in general[191, 192]. These data, in conjunction with our findings that vitamin D-related exposures decreased the risk of MS progression to disability

among subjects with Progressive MS, provide support for vitamin D being neuroprotective, in addition to being an immunomodulator.

Skin pigmentation is an important factor in the process of vitamin D synthesis in humans after exposure to sun light; increased pigmentation (darker skin tone) acts as a screen for UVR and leads to reduction in vitamin D synthesis [135]. Therefore, one might expect the effect of sun exposure to be modified by skin type. We did not find a significant interaction effect between skin type and sun exposure on either age at onset or time to disability. Skin type itself was also not associated with either outcome. Considering that only 10% of our study participants reported having brown or black skin, it is possible that we did not have sufficient power to examine such an interaction. For age at symptom onset, we did observe a trend suggesting that the effect of winter sun exposure is stronger among patients with lighter skin color, particularly very fair to fair skin type, compared to those with darker skin. Patients with fair skin type and low childhood winter sun exposure had the youngest age at symptom onset. This finding, which did not support our hypothesis, could partly be explained by functions of genotype associated with fair skin for MS risk, or vitamin D metabolism rather than the differences in vitamin D synthesis in the skin.

6.2 Subtype Difference, Disease Onset and Progression

In our project, we found that (1) season of birth and solar radiation level of birthplace as well as (2) sun exposure and cod liver oil intake during childhood and adolescence influence age at symptom onset among veterans with Relapsing MS but not Progressive MS. On the other hand, protective effects of vitamin D related exposures on disease progression were found among participants with Progressive MS but not Relapsing MS. A variety of

factors might contribute to such differences in the effect of UVR and vitamin D intake on clinical course of MS. Age at onset, an endpoint likely related to susceptibility and triggering of the disease, peaks at around 20 to 30 years of age in MS. Patients with Progressive MS are known to have older age at onset compared to those with Relapsing MS. Pugliatti et al. [131] suggested that MS clinical heterogeneity might depend on different causative mechanisms. Consistent with our results, studies have found that the month of birth influences susceptibility only among patients with Relapsing MS but not Progressive MS[128]. It is possible that exposures to other environmental factors later in life are critical for risk and occurrence of the Progressive MS type.

MS is a heterogeneous disease known for its variable patterns of clinical presentation and rates of disability accumulation [1, 2, 169]. The patho-physiology of MS is complex, with demyelination and neuronal-loss contributing to what is essentially an inflammatory neurodegenerative disease. Relapse-remitting MS (RRMS) represents episodic focal demyelination and remyelination, which usually occur at early stages of the disease and are thought to be primarily related to inflammation-driven disease activities. About half of patients with RRMS enter into the progressive stage of the disease with neurodegeneration as the main underlying disease process, resulting in constant worsening of function and irreversible disability. Hence, compared to patients with Progressive MS, those with Relapsing MS experience a diverse clinical course with varying degrees of interplay between relapse (inflammation) and progression (degeneration), with some cases never transiting to the progressive phase.

As patients with Relapsing MS represent a highly heterogeneous group with respect to prognosis, it is possible that the protective effect of sun exposure and vitamin D intake

might be diluted and not easy to detect among this subset of patients. In addition, the multifarious interactions between two main disease mechanisms, inflammation and neurodegeneration, might introduce further challenges to the assessment of prognostic factors for long term disability among this group. Some clinical observations suggest that inflammation can be neuroprotective in part through the production of neurotrophic factors by immune cells[193, 194]. This new argument adds another level of complexity in the patho-physiology and prognosis of Relapsing MS. Future studies are required to clarify the role of inflammation in the long term disability of MS. Moreover, the current available disease-modifying therapies (DMT) are primarily indicated for patients with Relapsing MS as immuno-modulators to suppress inflammation, thus reducing relapse rates. Although the long term effects of DMT on MS progression are yet to be known, a few studies have indicated their effect on the onset of progressive stage of the disease (onset of SPMS)[195, 196]; an effect that could potentially confound the studied associations in our project. Lastly, fewer than half of Relapsing MS patients in the study have reached PDDS 6 and only close to 20 % reached PDDS 8, which makes it difficult to investigate factors associated with long term disability.

6.3 Other Prognostic Factors for MS Progression

In our study, younger age at onset was associated with longer time to disability stages among both Relapsing and Progressive MS. This finding is consistent with previous studies [11, 180]. Whereas younger age at onset was previously viewed as a good prognostic factor for MS[1], a number of studies have challenged this viewpoint[7, 8, 11]. These studies evaluated the natural history of MS in cohorts of patients and found that individuals with younger age at

onset reached disability stages at younger age even if it took them a longer time to progress to disability milestones [11, 180]. Our finding of similar results (Appendix B, Table 5) provides further support for the notion that younger age at MS onset does not necessarily indicate a better disease outcome.

In addition to age at symptom onset, we were unable to identify other significant prognostic factors among participants with Relapsing MS. As for subjects with Progressive MS, presence of sensory symptoms at onset was found to be associated with better prognosis, a finding that is consistent with a recent study of patients with primary progressive MS [180]. We also observed that among the same subtype of MS, having had mononucleosis infection before onset increased the risk of progressing to disability. The evidence for Epstein-Barr virus (EBV) involvement in MS pathogenesis is compelling [26, 44]. In addition, EBV infection during adulthood (over 25 years) exhibited the greatest risk for MS, compared to infection at younger age [44]. Infectious mononucleosis, a strong indicator of late age EBV infection, was also found to be a robust risk factor for MS in a meta-analysis [36]. A few studies have suggested that viral or bacterial infections might trigger exacerbations among patients with RRMS [177-179]. However, whether or not the EBV infection before onset has long impacts on the disease progression is unknown. Future studies are needed to investigate the potential role of infectious agents (chronic infection, and/or recurrent infection) in long-term disability in MS.

Despite substantial efforts to investigate potentially reliable prognostic factors for MS, very few have been consistently recognized across studies, most likely because of the heterogeneous nature of the disease within and among individuals. In our study, we found only a few demographic and clinical variables associated with long term disability. Recent

studies of the natural history of MS have claimed that progression among MS patients might be mainly age-dependent at a population level regardless of the clinical features at onset [7, 8, 11, 181]. In these studies, they found that once the disease reached a threshold of irreversible disability, the time course of disability accumulation beyond this point is essentially similar regardless of prior clinical history of the disease, including MS subtype. Moreover, the age at disability landmarks was in fact alike between Relapsing and Progressive MS. Our results indicate that the time from PDDS 4 to PDDS 6 or PDDS 8 were nearly identical between Relapsing and Progressive MS (see Appendix B, Table 6) and the median age at the disability stages was not substantially different between the two groups, especially those who transited to SPMS. Overall, our findings are comparable to reports from other national cohorts of MS regarding history of MS prognosis [7, 180] and support the notion that progression among MS patients might be mainly an age dependent process[7, 8, 11, 181].

6.4 Study Strengths and Limitations

There are some limitations inherent to the nature of the study design: a cross-sectional, mail- based survey. First, we did not have data for actual UVR exposure and serum vitamin D levels in the study population to objectively examine their effects on disease occurrence and progression. Even though we attempted to measure variables retrospectively over time in order to better quantify temporal relationships between exposures and outcomes, causal relationships cannot be determined without a well-designed prospective study. However, it is extremely difficult to conduct a prospective cohort study to examine if UVR/vitamin D exposure affects the age at onset of the disease, because MS is not highly prevalent in the population. In addition, a prospective study or a randomized trial to examine the association

between UVR/vitamin D and long term outcomes of MS would be very costly and time consuming.

Another limitation of this study is that all the major exposure and outcome variables were collected through a mail-based survey. Any self-response survey data are subject to measurement errors and recall bias. There is inherent variability and unreliability in the study participant responses. Self-report of past sun exposure and vitamin D intake could result in considerable misclassification of the measurement. It has been challenging to investigate environmental risk factors thought to be acquired early in life in an adult onset disease, as is suggested for MS. At present, we do not have the means to assess the reliability of these data. However, it is unlikely that our results are due to bias or chance alone given that we consistently observed the protective effects of several vitamin D related variables (e.g. sun exposure, cod liver oil intake) on MS clinical course.

We also relied on participants' reports of important disease characteristics (e.g. MS subtype) and disability. In the MSSR, MS subtype, when classified as Relapsing versus Progressive MS as used in our study, has been previously shown to have 100% agreement with physician determined MS subtype in 41 participants for whom the clinical data were available[164]. In addition, the distribution of MS subtype in this study was consistent with what have been reported in the literature[1, 7]. Therefore, the participant-reported MS subtype provides a relatively reliable assessment of MS subtype. As for disability, participants reported age at disability stages using the PDDS. Recall of past events has varying degrees of accuracy depending on the significance of the event or the time at which the event occurred. To some extent, recall of major life events is less prone to recall bias. PDDS 6, the main outcome in the study, indicates a need for a cane or support to walk, which represents a key

disability milestone. Although patients may have less difficulty recalling when they started requiring assistance to ambulate, we currently do not have ways to verify this outcome and thus cannot exclude potential misclassification of the outcome due to poor recall by subjects.

Missing responses on essential exposure variables, especially sun exposure measurements (12.6% and 20.1% of the subjects with Relapsing and Progressive MS, respectively) might also introduce a potential bias in our study. To address this limitation, we compared the demographic and clinical characteristics (e.g. disease duration) of those with known and unknown sun exposure status, and found no differences. The same analyses were performed to assess the effects of missing values for the main outcome: age at disability stages. Again, there were no differences found with respect to demographic and clinical characteristics between those who did and those who did not report this major outcome. Thus, the possible bias due to missing information in our study was likely small and probably not of any great concern.

In this study, the overall response rate (30.6%) was lower than what we expected, though common for mail based surveys[197]. When we compared demographic characteristics between survey-responders and non-responders, slightly higher proportions of Caucasian, male and married individuals were among the responders. Despite these observed differences, our survey responders were nearly identical to the target population, all the veterans with MS in the VHA system, with respect to age, race and marital status. The difference in gender distribution was expected since we oversampled females to compensate for their low representation in the VHA population, compared to the general MS population. Overall, our study population appears to be representative of the VHA MS population. Additionally, the preliminary results from the MSSR baseline survey indicate that there is no clinical

difference in demographic and clinical characteristics when comparing the MSSR cohort with other large cohorts of MS patients from different countries and populations[140]. These results suggest that the MSSR veteran population can represent the general MS population and hence serve as an important and unique cohort for future MS research.

A large number of Veterans with MS, as well as detailed information on their demographic and clinical characteristics, were included in this study. All data collection was centralized to ensure the quality and validity of the survey. The project used a comprehensive approach to include numbers of environmental and individual factors associated with UVR/vitamin D exposure and target important endpoints along the course of disease. Therefore, this project provided a unique perspective to examine the effect of UVR/vitamin D on the clinical course of MS in a relatively efficient manner, and could provide support for more costly designs in the future.

6.5 Conclusions and Implications

Our study provided evidence for associations between vitamin D related exposures before MS symptom onset and the disease clinical outcomes. Specifically, we found that (1) winter birth and low solar radiation level of birthplace and (2) low fall/winter sun exposure during childhood and adolescence as well as never use of cod liver oil in childhood are associated with earlier onset of the disease among Veterans with Relapsing MS. High risk of progressing to disability was found among subjects with Progressive MS who had low average fall/winter sun exposure prior to symptom onset and who had never took cod liver oil during childhood and early adolescence. Overall, our study expands the current literature and suggests that UVR and/or vitamin D status before symptom onset, especially early in life

and during the critical period of childhood and early adolescence, affect MS clinical course. Future studies are needed to investigate vitamin D in conjunction with the MS-associated genes and assess how and when they could interact with each other to impact disease susceptibility and activities. Continuous research efforts in the context of gene-environment contributions to MS causation can greatly increase our understanding of the disease pathogenesis and ultimately lead to comprehensive prevention strategies.

Our study suggests that chronic deficiencies of vitamin D before symptom onset might increase risk of MS progressing to disability. Although this finding was observed only among a small subset of participants, those with Progressive MS, it offers hope for future intervention. Vitamin D, by virtue of its immuno-modulatory and neuroprotective effects, could potentially be used to treat an inflammatory and neurodegenerative disease like MS. Along with the current view on MS prognosis, in which disease progression to disability is mainly age-related and brought upon by underlying neuro-degeneration, the development of therapeutic interventions with neuroprotective impact seems of great importance.

Although a well-designed clinical trial is a gold standard to examine effectiveness of vitamin D on disease progression, the safety concerns of vitamin D treatment and more than decades of long-term follow-up are likely to put off the anticipated progress. A cross-sectional study design is valuable to provide supportive evidence in a timely manner but will need to incorporate methodological and analytical strategies to deal with several vitamin D-related exposures, disease-associated behavioral changes and possible confounding, such as duration and frequency of DMT use among patients with Relapsing MS. In parallel, research efforts should also focus on searching and developing novel neuroprotective agents in MS,

including vitamin D-related compounds that could potentially pair with the current immuno-modulaory therapy in reducing disability in what can be a devastating disease.

APPENDIX A: MSSR SURVEY

VHA MS Surveillance Registry

Study ID#: 00011

Please answer the following questions by completely darkening the circle to the left of the appropriate answer like this ● or by entering names or numbers in the square.

Who completed this questionnaire? ○ patient ○ spouse, or child ○ care giver ○ other

MS Diagnosis

1a. At what age were you diagnosed with MS? ☐☐ **years**

1b. Were you diagnosed while on active duty? ○ Yes ○ No

1c. Did you receive service-connected disability for MS? ○ Yes ○ No

2. At what age did you first experienced symptom(s) from MS? ☐☐ **years**

3. There are two main types of MS (Relapsing or Progressive).
a. Please select which type of MS you had. Within each type of MS, you might or might not have changed from one subtype to another.
b. Please write down your age when you first experienced the subtype(s) that best describes the status of your MS at that time. For example, if your MS began as relapsing-remitting and then changed to the secondary progressive, please write down your age at diagnosis for the relapsing-remitting MS and your age when you changed to the secondary progressive MS.

○ **Relapsing MS**

Age

☐☐ You may have had attacks (relapses, exacerbations) followed by periods of partial or total remissions (remission means that disease activity quiets down). **(Relapsing- remitting MS)**

☐☐ Your MS started off as relapsing-remitting. Then, your symptoms got progressively worse with or without attacks (relapses, exacerbations). During remissions, your symptoms may have improved but did not entirely disappear. **(Secondary Progressive MS)**

OR

○ **Progressive MS**

Age

☐☐ Your disease has gotten progressively worse over time from the onset of your symptoms. You never had any attacks and remissions. **(Primary Progressive MS)**

☐☐ Your disease started off getting progressively worse over time and then you begin to have relapses without having any recovery of symptoms. **(Relapsing- progressive MS)**

VHA MS Surveillance Registry

Study ID#: 00011

Relapse Experience A relapse, sometimes called an attack or an exacerbation, is the occurrence and worsening of MS symptoms that last more than 24 hours and is followed by partial or complete recovery. Fatigue alone or fever-related worsening of symptoms are not considered as relapses. **If you have not experienced relapses since the onset of your MS, please disregard this section, and go to question 7.**

4. As best as you can recall, please enter

a. How many relapses did you have in the first three years after you were diagnosed with MS? ☐☐

b What is the interval between your first relapse and second relapse? ☐☐ **months**

5. How many relapses did you have in the past three years? ☐☐

6. Are you currently experiencing an MS attack? O Yes O No

If no, how long ago was your last MS attack? ☐☐ **years** ☐☐ **months**

Demographics

7. What is your current body weight ? ☐☐☐ **lbs**.

8. Marital Status: O Never Married O Married O Separated O Divorced O Widowed

9. With whom do you currently live? (Check all that apply)

O Live alone O Spouse/ Partner O Sibling O Children O Parent
O Other relative O Friend/companion O Nursing home O Health-care worker

10. Does one of the persons you live with regularly provide assistance and care for you?

O Yes O No

11a. What is your current employment status? (Check only one)

O Employed full-time O Employed part-time O Volunteer
O Unemployed, looking for work O Unemployed, not looking for work O Retired

11b. Do you currently receive Social Security Disability Income? O Yes O No

12. What is your total household income per year?

O < $15,000 O $15,000-30,000 O $30,000 to 50,000
O $50,000 to 100,000 O >$100,000 O Decline to answer

VHA MS Surveillance Registry

Study ID#: 00011

13. It's important that we understand the places you have lived.
Think back carefully and list the places along with your age when you moved to each new location from birth to your current age. Please write your answers with capital letters.

Age (years) From	To	City	State	Country (if not United States)
00				

VHA MS Surveillance Registry

Study ID#: 00011

Smoking History/Status This section asks for information about your cigarette-smoking history, whether you are a current smoker or if you smoked in the past.

14. Have you smoked at least 100 cigarettes in your whole life? (1 pack = 20 cigarettes)

O Yes O No O Don't know **If no, then skip questions 15-23, go to 24**

15. How old were you when you began smoking? ☐☐ **years** O Don't know

16. Do you smoke cigarettes now? O Yes O No

17. Have you ever quit smoking?

O Yes, **if yes, how old were you when you most recently quit smoking?** ☐☐ **years**
O Have tried to quit but did not succeed (skip questions 18-19)
O Have never tried to quit (skip questions 18-20)
O Don't know (skip questions 18-20)

18. How many times have you quit smoking for more than 3 months? ☐☐ **times**

19. How long have you been smoke free? ☐☐ **years** ☐☐ **months**

20. What method did you use to quit? (Please check all the apply)

O Patch O Gum O Medication O Self Will-Power Alone

Alternative Assistance such as:

O Acupuncture O Hypnosis O Homeopathic Treatments O Other

21. How many cigarettes did/do you usually smoke *per day*?

O 1-10 cigarettes (1/2 pack) O 11-20 cigarettes (1 pack)
O 21-39 cigarettes (1-2 packs) O 40 or more (>2 packs)

22. Not including naps, how soon after you wake up did/do you have your first cigarette?

O Less than 10 minutes O From10 to 30 minutes O More than 30 minutes

23. If you wake up during the night, did/do you have a cigarette before going back to bed?

O Yes O No

VHA MS Surveillance Registry

Study ID#: 00011

24. Did/do you use other types of tobacco? (select all that apply)
Please write down how long (number of years) you have used the selected product.

O Pipe , ☐☐ **years** O Cigars, ☐☐ **years** O Kretek (clove cigarettes), ☐☐ **years**

O Other tobacco (chewing tobacco, snuff), ☐☐ **years**

25. Did/do you frequently (more than 3 days a week) expose yourself to second hand smoke from your family members, peers or colleagues during the listed age periods?

a. 6-10 years	O Yes	O No	**b. 11-15 years**	O Yes	O No
c. 16- 25 years	O Yes	O No	**d. 26-35 years**	O Yes	O No
e. 36- 50 years	O Yes	O No	**f. > 50 years**	O Yes	O No

Health Services

26. Where do you receive the majority (> 50% of visits) of your MS medical care?

O VA facility O Civilian hospital O Doctors office / Outpatient clinic
O DOD facility

27. Where do you get the majority (> 50% of prescriptions) of your MS medications?

O VA O Civilian hospital O Doctors office / Outpatient clinic
O DOD facility

28. Who prescribes your MS medications?

O Physician (MD, DO) O Nurse Practitioner O Physician's Assistant
O Don't know O Other : _____

29. What type of healthcare coverage do you have? (Check all that apply)

O VA O Medicare / Medicaid
O Champus / TriCare O Private insurance (Aetna, Kaiser, BCBS)
O Department of Defense (Army, Navy, Air Force or Medical Facility)
O Other: _____ O None

Skin Type

30. Please indicate your skin type (select only one)

O Extremely fair skin, always burns, never tans
O Fair skin, usually burns, tans slowly and with difficulty
O Medium skin, sometimes mild burns, gradually tans
O Olive skin (typical Mediterranean Caucasian skin) , rarely burns, tans with ease
O Moderately pigmented brown skin (mid-eastern skin types), never burns, tans very easily
O Markedly pigmented black skin, never burns, always tans

Study ID#: 0001

Time In Sun Please try to remember how much time you spent in the sun during different age periods and seasons, including work activities, gardening and playing sports.

31. During weekdays or workdays, how much time, on average, did/do you spend in the sun in the following time periods and seasons? (NOTE: Weekends and holidays are in next question)

	Spring/Summer (hour/per day)					Fall/Winter (hour/per day)				
Age Period	< 1 hour	1-2 hours	2-3 hours	3-4 hours	> 4 hours	< 1 hour	1-2 hours	2-3 hours	3-4 hours	> 4 hours
6-10 years	○	○	○	○	○	○	○	○	○	○
11-15 years	○	○	○	○	○	○	○	○	○	○
16-20 years	○	○	○	○	○	○	○	○	○	○
21-25 years	○	○	○	○	○	○	○	○	○	○
26-30 years	○	○	○	○	○	○	○	○	○	○
31-35 years	○	○	○	○	○	○	○	○	○	○
36-40 years	○	○	○	○	○	○	○	○	○	○
41-45 years	○	○	○	○	○	○	○	○	○	○
46-50 years	○	○	○	○	○	○	○	○	○	○
51-55 years	○	○	○	○	○	○	○	○	○	○
56-60 years	○	○	○	○	○	○	○	○	○	○
61-65 years	○	○	○	○	○	○	○	○	○	○
>65 years	○	○	○	○	○	○	○	○	○	○

Study ID#: 0001

32. During <u>weekends and holidays</u>, how much time, on average, did/do you spend in the sun in the following time periods and seasons?

	Spring/Summer (hour/per day)					Fall/Winter (hour/per day)				
Age Period	< 1 hour	1-2 hours	2-3 hours	3-4 hours	> 4 hours	< 1 hour	1-2 hours	2-3 hours	3-4 hours	> 4 hours
6-10 years	○	○	○	○	○	○	○	○	○	○
11-15 years	○	○	○	○	○	○	○	○	○	○
16-20 years	○	○	○	○	○	○	○	○	○	○
21-25 years	○	○	○	○	○	○	○	○	○	○
26-30 years	○	○	○	○	○	○	○	○	○	○
31-35 years	○	○	○	○	○	○	○	○	○	○
36-40 years	○	○	○	○	○	○	○	○	○	○
41-45 years	○	○	○	○	○	○	○	○	○	○
46-50 years	○	○	○	○	○	○	○	○	○	○
51-55 years	○	○	○	○	○	○	○	○	○	○
56-60 years	○	○	○	○	○	○	○	○	○	○
61-65 years	○	○	○	○	○	○	○	○	○	○
>65 years	○	○	○	○	○	○	○	○	○	○

Study ID#: 0001

Sun Exposure Behaviors Please answer **question 33** according to the frequency, **N: Never; R: Rarely; Oc: Occasionally; or O: Often. For question 34**, please select **percent of time** you use or used sunscreen **during all outside activities** : **0 indicates you never and 100 indicates that you always use or used sunscreen.**

Age Period	33. How often did/do you sunbathe?				34. How frequently did/do you use sunscreen when you were outside?										
	N	R	Oc	O	*0*	*10*	*20*	*30*	*40*	*50*	*60*	*70*	*80*	*90*	*100*
6-10 years	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
11-15 years	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
16-20 years	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
21-25 years	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
26-30 years	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
31-35 years	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
36-40 years	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
41-45 years	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
46-50 years	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
51-55 years	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
56-60 years	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
61-65 years	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
>65 years	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○

Study ID#: 0001 1

Diet and Supplement Intake

35. Please answer how often on average did/do you have one of the dishes listed below.
Please answer each question according to the frequency, R: Rarely (<1 time a month) ;
Oc: Occasionally (1-3 times a month); O: Often (~1 time a week) or VO: Very Often (>3 times a week)

Age Period	A fish dish (any kind of fish)				A fish dish like salmon, tuna, herring, sardines or mackerel				Milk (drink or with cereal), including chocolate and other flavored milks				Egg/Cheese			
	R	Oc	O	VO	R	Oc	O	VO	R	Oc	O	VO	R	Oc	O	VO
6-10 years	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
11-15 years	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
16-20 years	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
21-25 years	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
26-30 years	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
31-35 years	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
36-40 years	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
41-45 years	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
46-50 years	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
51-55 years	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
56-60 years	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
61-65 years	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
>65 years	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○

Study ID#: 0001

36. This section is about your intake of different dietary supplements. Please mark the supplement that you have taken daily for at least one year during the listed age periods

Age Period	a. Cod-liver oil supplement	b. Fish Oil supplement (omega 3 or omega 6)	c. Calcium/Vitamin D	d. Multi-vitamin
6-10 years	○	○	○	○
11-15 years	○	○	○	○
16-20 years	○	○	○	○
21-25 years	○	○	○	○
26-30 years	○	○	○	○
31-35 years	○	○	○	○
36-40 years	○	○	○	○
41-45 years	○	○	○	○
46-50 years	○	○	○	○
51-55 years	○	○	○	○
56-60 years	○	○	○	○
61-65 years	○	○	○	○
>65 years	○	○	○	○

VHA MS Surveillance Registry

Study ID#: 00011

History about Mononucleosis (kissing disease, glandular fever)

37. Have you ever had Mononucleosis? ○Yes ○No ○Unknown

38. If yes, at what age did you have the illness? ☐☐ **years**

39. If yes, did you miss school or work, or were you hospitalized? ○Yes ○No ○Unknown

Disease Modifying Agents (Medication for MS)

This section asks about the disease-modifying agents (DMA) you have taken for your MS during the past year. Current DMA include: Copaxone, Avonex, Rebif, Betaseron, Tysabri and Mitoxantrone. If you have taken more than one of these medications in the last year, please complete the following questions for each of the therapies in the order in which you took them.

40. If you have never used any DMA, please indicate reason(s) why? (Select all that apply)

- ○ DMA was not offered by your physicians
- ○ You were informed by your doctor that DMA wasn't appropriate for you
- ○ You decided not to take DMA because of side effect profile
- ○ You decided not to take DMA because of injection
- ○ Cost too much
- ○ DMA is not available at local pharmacy

41. Which DMAs have you taken in the past year (select all that apply):

- ○ Copaxone (Glatiramer Acetate)
- ○ Avonex (Interferon beta-1a)
- ○ Rebif (Interferon beta-1a)
- ○ Betaseron (interferon beta-1b)
- ○ Tysabri (Natalizumab)
- ○ Mitoxantrone
- ○ None **(go to item # 44)**

42a. Which DMA did you use first? Starting Date (MM / YYYY): ☐☐/☐☐☐☐

- ○ Copaxone (Glatiramer Acetate)
- ○ Avonex (Interferon beta-1a)
- ○ Rebif (Interferon beta-1a)
- ○ Betaseron (interferon beta-1b)
- ○ Tysabri (Natalizumab)
- ○ Mitoxantrone

42b. Where did you obtain this DMA (choose one)? ○VA ○DOD ○Community

42c. How closely did you follow the prescribed injection schedule with this DMA?

- ○ Rarely missed/skipped an injection (always followed injection scheduled)
- ○ Occasionally missed/skipped an injection (no more than twice a month)
- ○ Sometimes missed/skipped an injection (3 or 4 times a month)
- ○ Frequently missed/skipped an injection (more than 4 times a month)

VHA MS Surveillance Registry

Study ID#: 00011

42d. Did you switch to another MS DMA?

- O Yes, switched to Copaxone
- O Yes, switched to Avonex
- O Yes, switched to Rebif
- O Yes, switched to Betaseron
- O Yes, switched to Tysabri
- O Yes, switched to Mitoxantrone
- O No, still taking the DMA **(go to item #44)**
- O No, stopped and did not switch to another DMA,

Stopping date **(MM / YYYY)**: ☐☐ / ☐☐☐☐ **(go to item #44)**

42e. Why did you stop taking this DMA? (Please check all that apply)

- O Too frequent injections
- O Injection site reaction(s)
- O Flu-like symptoms
- O Developed progressive MS
- O Was not effective
- O Impaired liver function tests
- O Developed interferon antibodies
- O High cost or lack of health insurance
- O No longer available at my Facility
- O Other: _____

43a. What was the second DMA you used? Starting Date (MM / YYYY): ☐☐ / ☐☐☐☐

- O Copaxone (Glatiramer Acetate)
- O Avonex (Interferon beta-1a)
- O Rebif (Interferon beta-1a)
- O Betaseron (interferon beta-1b)
- O Tysabri (Natalizumab)
- O Mitoxantrone

43b. Where did you obtain this DMA? (Choose one) O VA O DOD O Community

43c. How closely did you follow the prescribed injection schedule with this DMA?

- O Rarely missed/skipped an injection (always followed injection scheduled)
- O Occasionally missed/skipped an injection (no more than twice a month)
- O Sometimes missed/skipped an injection (3 or 4 times a month)
- O Frequently missed/skipped an injection (more than 4 times a month)

43d. Did you switch to another MS DMA?

- O Yes, switched to Copaxone
- O Yes, switched to Avonex
- O Yes, switched to Rebif
- O Yes, switched to Betaseron
- O Yes, switched to Tysabri
- O Yes, switched to Mitoxantrone
- O No, still taking the DMA **(go to item #44)**
- O No, stopped and did not switch to another DMA,

Stopping Date **(MM / YYYY)**: ☐☐ / ☐☐☐☐ **(go to item #44)**

VHA MS Surveillance Registry

Study ID#: 00011

43e. Why did you stop taking this DMA? (Please check all that apply)

- O Too frequent injections
- O Flu-like symptoms
- O Was not effective
- O Developed interferon antibodies
- O No longer available at my Facility
- O Injection site reaction(s)
- O Developed progressive MS
- O Impaired liver function tests
- O High cost or lack of health insurance
- O Other: _____

MS Symptoms and Outcomes

44. ACTIVITIES REQUIRING ASSISTANCE. Please indicate how frequently you have needed help from others to perform the following activities over the last 4 weeks.

ACTIVITIES	Never	Almost Never	Occasionally	Usually	Almost Always	Always
Cut your food	O	O	O	O	O	O
Get in & out of tub or shower	O	O	O	O	O	O
Turn from side to side while in a lying position	O	O	O	O	O	O
Work buttons / zippers / laces	O	O	O	O	O	O
Walk inside the house	O	O	O	O	O	O
Walk up or down a ramp	O	O	O	O	O	O
Get to & from present method of travel (e.g., car, bus)	O	O	O	O	O	O
Read printed material	O	O	O	O	O	O
Use a telephone	O	O	O	O	O	O
Write clearly	O	O	O	O	O	O
Participate in social activities outside the home	O	O	O	O	O	O
Participate in recreational activities outside the home	O	O	O	O	O	O

45. What symptoms did you experience at the onset of MS? (Please check all that apply)

O	Fatigue	O	Blurred vision	O	Double vision	O	Tremors
O	Arm weakness	O	Leg weakness	O	Forgetfulness	O	Spasms
O	Difficulty sleeping	O	Difficulty swallowing	O	Burning sensation	O	Pain
O	Balance problems	O	Heat sensitivity	O	Numbness	O	Falling
O	Pins and needles	O	Knee locking or collapsing	O	Bowel/Bladder incontinence		
O	Other, specify						

VHA MS Surveillance Registry

Study ID#: 00011

46. MS SYMPTOMS. Please indicate how frequently you have experienced the following symptoms over the last 4 weeks.

SYMPTOMS		Never	Almost Never	Occasionally	Usually	Almost Always	Always
Fatigue		○	○	○	○	○	○
Heat intolerance		○	○	○	○	○	○
Arm weakness		○	○	○	○	○	○
Leg weakness		○	○	○	○	○	○
Spasms		○	○	○	○	○	○
Tremors		○	○	○	○	○	○
Knee locking or collapsing		○	○	○	○	○	○
Balance problems		○	○	○	○	○	○
Falling		○	○	○	○	○	○
Double vision		○	○	○	○	○	○
Blurred vision		○	○	○	○	○	○
Difficulty swallowing		○	○	○	○	○	○
Forgetfulness		○	○	○	○	○	○
Difficulty sleeping		○	○	○	○	○	○
Loneliness		○	○	○	○	○	○
Depression		○	○	○	○	○	○
Anxiety		○	○	○	○	○	○
Pain		○	○	○	○	○	○
Burning sensation		○	○	○	○	○	○
Numbness		○	○	○	○	○	○
Pins and needles		○	○	○	○	○	○
Increased urinary frequency - DAY	Catheter ○	○	○	○	○	○	○
Increased urinary frequency - NIGHT	○	○	○	○	○	○	○
Trouble making toilet - DAY	○	○	○	○	○	○	○
Trouble making toilet - NIGHT	○	○	○	○	○	○	○
Difficulty starting to urinate	○	○	○	○	○	○	○
Urinary infection or burning	○	○	○	○	○	○	○

VHA MS Surveillance Registry

Study ID#: 00011

47. Patient-Determined Disease Steps (PDDS)

This scale focuses mainly on how well you walk. You may not have experienced all stages since you have had MS. Not everyone will find a description that describes their situation exactly. **1. In column one, please indicate the age at which you entered each stage**. Please start with the age when you first experienced MS symptoms and complete all the disease stages you have experienced to the present **(each situation should last at least *6 months*)**. **2. In column two, please mark one category that best describes or that is closest to your current situation.**

1. Age	2. Current Stage		
☐☐	○	**Normal**	I might have had some mild symptoms, mostly sensory due to MS but they did not limit my activity. If I had an attack (exacerbation), I returned to normal when the attack has passed.
☐☐	○	**Mild Disability**	I have had some noticeable symptoms from my MS but they were minor and had only a small affect on my lifestyle.
☐☐	○	**Moderate Disability**	I didn't have any limitations in my walking ability. However, I did have significant problems due to MS that limit my daily activities in other ways.
☐☐	○	**Gait Disability**	MS did interfere with my activities, especially when walking. I could work a full day, but athletic or physically demanding activities were more difficult than they used to be. I usually did not need a cane or other assistance to walk, but I might need some assistance during an attack (exacerbation).
☐☐	○	**Early Cane**	I used a cane or a single crutch or some other form of support (such touching a wall or leaning on someone's arm) for walking all the time or part of the time, especially when walking outside. I think I could walk 25 feet in 20 seconds without a cane or crutch. I always needed some assistance (cane or crutch) if I wanted to walk as far as 3 blocks.
☐☐	○	**Late Cane**	To be able to walk 25 feet, I had to have a cane or crutch or someone to hold onto. I could get around the house or other buildings by holding onto furniture or touching walls for support. I might use a scooter or wheel chair if I wanted to go greater distances.
☐☐	○	**Bilateral Support**	To be able to walk as far as 25 feet I must have 2 canes or crutches or a walker. I might use a scooter or wheelchair for longer distances.
☐☐	○	**Wheelchair / Scooter**	My main form of mobility was a wheelchair or scooter. I might be able to stand and/or take one or two steps, but I could not walk 25 feet, even with crutches or a walker.
☐☐	○	**Bedridden**	Unable to sit in a wheelchair or scooter for more than one hour.

VHA MS Surveillance Registry

Study ID#: 00011

48. Multiple Sclerosis Impact Scale (MSIS-29)

The following questions ask for your views about the impact of MS on your day-to-day life **during the past two weeks**. For each statement, please select **the best description about your current situation**. **Please answer ALL questions.**

In the past 2 weeks, how much has your MS limited your ability to…	**Not at all**	**A little**	**Moderately**	**Quite a bit**	**Extremely**
Do physically demanding tasks?	○	○	○	○	○
Grip things tightly (e.g., turning on faucets)?	○	○	○	○	○
Carry things?	○	○	○	○	○
In the past 2 weeks, how much have you been bothered by…	**Not at all**	**A little**	**Moderately**	**Quite a bit**	**Extremely**
Problems with your balance?	○	○	○	○	○
Difficulty moving about indoors?	○	○	○	○	○
Being clumsy?	○	○	○	○	○
Stiffness?	○	○	○	○	○
Heavy arms and/or legs?	○	○	○	○	○
Tremor of your arms or legs?	○	○	○	○	○
Spasms in your limbs?	○	○	○	○	○
Your body not doing what you want it to?	○	○	○	○	○
Having to depend on others to do things for you?	○	○	○	○	○
Limitations in your social and leisure activities at home?	○	○	○	○	○
Being stuck at home more than you would like to be?	○	○	○	○	○
Difficulties using your hands in everyday tasks?	○	○	○	○	○
Having to cut down the amount of time you spent on work or other daily activities?	○	○	○	○	○
Problems using transport (e.g., car, bus, train, taxi, etc.)?	○	○	○	○	○
Taking longer to do things?	○	○	○	○	○

VHA MS Surveillance Registry

Study ID#: 00011

48. Multiple Sclerosis Impact Scale (cont'd)

In the past 2 weeks, how much have you been bothered by…	Not at all	A little	Moderately	Quite a bit	Extremely
Difficulty doing things spontaneously (e.g., going out on the spur of the moment)?	○	○	○	○	○
Needing to go to the toilet urgently?	○	○	○	○	○
Feeling unwell?	○	○	○	○	○
Problems sleeping?	○	○	○	○	○
Feeling mentally fatigued?	○	○	○	○	○
Worries related to your MS?	○	○	○	○	○
Feeling anxious or tense?	○	○	○	○	○
Feeling irritable, impatient, or short tempered?	○	○	○	○	○
Problems concentrating?	○	○	○	○	○
Lack of confidence?	○	○	○	○	○
Feeling depressed?	○	○	○	○	○

49. Would you be willing to complete a future questionnaire about finances? ○Yes ○No

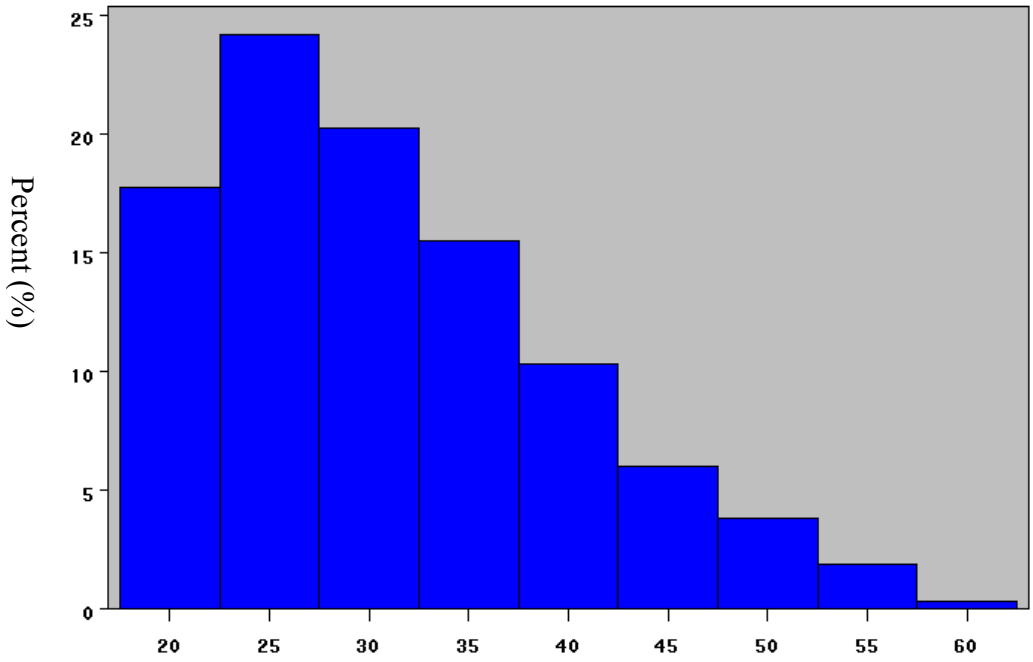
THANK YOU

Please return this survey in the stamped, self-addressed envelope.

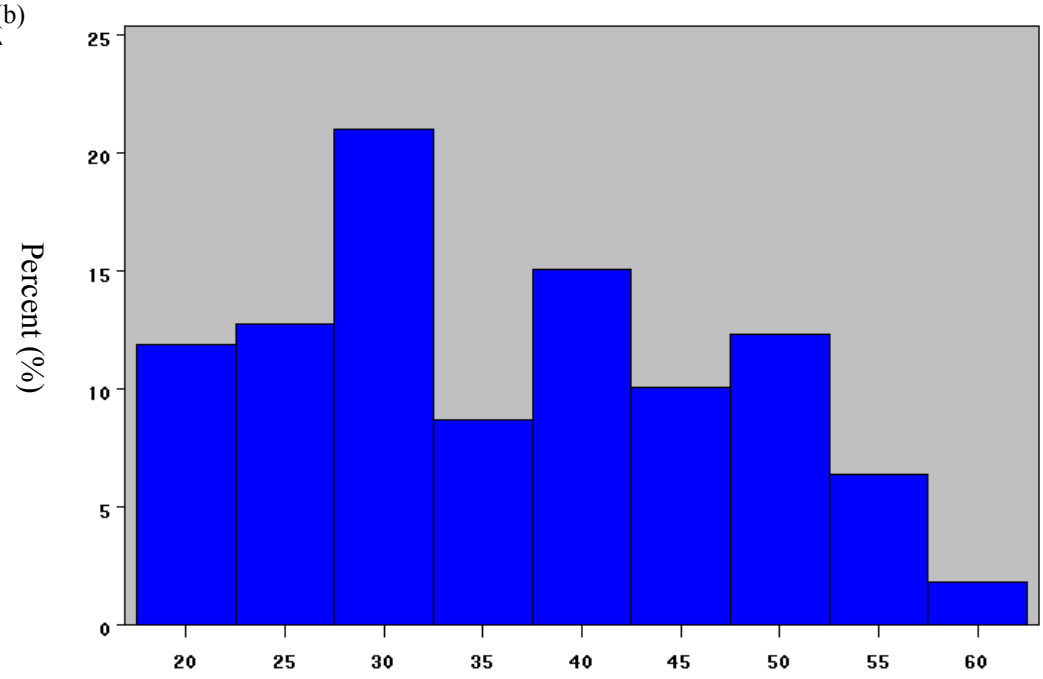
Please remember to sign the consent form and HIPPA form and include them in the return envelope as well.

APPENDIX B: FIGURES & TABLES

(a)



(b)



Age at Symptom onset (years)

Figure A.1 Distribution of Age at Symptom onset among Subjects with (a) Relapsing MS and (b) Progressive MS

Table A.1 Mean age at onset of disease symptoms by skin type, and by levels of sun exposure and residential solar radiation estimates during childhood and early adolescence among Veterans with Progressive MS[2]

Cumulative sun exposure between ages 6-15 y	No (%)	Mean (SD)	*p* value
In fall/winter (wks)			
Quartile			
Quartile 1: ≤16	39 (22.0)	37.3±10.1	0.33
Quartile 2: 17-23	43 (24.3)	37.1±11.6	
Quartile 3: 24-36	48 (27.1)	36.9±10.8	
Quartile 4: > 36	47 (26.7)	33.9±10.5	
In spring/summer (wks)			
Quartile			
Quartile 1: ≤29.5	41 (21.8)	37.4±11.5	0.15
Quartile 2: >29.5-41	43 (22.9)	38.8±10.6	
Quartile 3: >41-49	36 (19.2)	35.9±10.9	
Quartile 4: > 49	68 (36.2)	34.2±10.4	
In four seasons (wks)			
Quartile			
Quartile 1: ≤46.5	33 (18.8)	37.2±10.8	0.31
Quartile 2: >46.5 – 65	56 (31.8)	37.9±10.8	
Quartile 3: >65-80	39 (22.2)	36.4±11.3	
Quartile 4: > 80	48 (27.3)	34.0±10.3	
Weighted residential UV counts between ages 0-15 y in 10,000			
Low: < 118	78 (39.2)	38.0±11.5	0.09
Medium: 118 - <135	63 (31.7)	36.0±10.8	
High: ≥135	58 (29.2)	33.7±9.7	
Weighted residential latitude between ages 0-15 y			
Northern: ≥ 41°	96(48.2)	37.8±11.5	0.08
Middle: 37- <41°	61 (30.7)	35.1±10.5	
Southern: <37°	46 (21.1)	33.6±9.4	
Skin Type			
Very fair-fair (type 1 &2)	55(25.7)	35.8±11.6	0.94
Medium (type 3)	101 (47.2)	36.4±10.8	
Olive to Black (type 4, 5 & 6)	58 (27.1)	36.1±10.3	

[2] We did not observe any significant association between vitamin D intake (from diet and supplement) and age at symptom onset. After controlling for the important covariates, there was no significant association between all the studied variables and age at symptom onset.

Table A.2 Multivariable linear regression of age at MS symptom onset on cumulative childhood and early adolescence sun exposure among Veterans with relapsing type of MS

	Relapsing MS (n = 948)		
Variables	**Estimate (SE)**	**95% CI**	***p* value**
Intercept	31.3 (1.98)	27.46, 35.24	<.0001
Low-Medium residential solar radiation			
Winter sun exposure >16 wks vs. ≤16 wks	2.32 (0.91)	0.53, 4.11	0.01
High residential solar radiation			
Winter sun exposure >16 wks vs. ≤16 wks	-1.84 (1.58)	-4.95, 1.27	0.25
Sun screen use between ages 6-15 years			
Never	1.98 (1.20)	-0.39, 4.36	0.10
10-50% of the time	2.92 (1.32)	0.33, 5.50	0.03
60-100% of the time (reference)	0	---	---
Cod liver oil intake between ages 6-10 years			
No	0	---	---
Yes	2.64 (1.03)	0.62, 4.67	0.01
Sex			
Female (reference)	0	---	---
Male	1.55 (0.69)	0.20, 2.91	0.02
Skin type			
Extremely fair to fair (reference)	0	---	---
Medium	0.74 (0.73)	-0.70, 2.18	0.31
Brown to black	-0.53 (1.22)	-2.94,1.87	0.66
Smoking history			
Never (reference)	0	---	---
Ever	1.22(0.71)	-0.17, 2.60	0.09

Table A.3 Effects of sun exposure, vitamin D intake, and covariates on the estimates of time from disease onset to PDDS 6 and 8 among Veterans with Progressive MS

Variable	Time to PDDS6			Time to PDDS8		
	No of patients (% censored)	Median (95% CI)	*p*-value†	No of patients (% censored)	Median (95% CI)	*p*-value†
Average fall/winter sun exposure before onset of MS	N = 140††			N = 163††		
Low ≤ 1.6 weeks/year	33 (21.2)	11 [7-16]	0.006	36 (41.7)	20 [16-29]	0.01
High > 1.6 weeks/year	107 (28.0)	18 [14-31]		127 (55.1)	29 [24-42]	
Cumulative sun exposure between ages 6-15 years	N= 143			N = 168		
Quartile 1	28 (35.7)	20 [10-24]	0.94	28 (35.7)	35 [24-45]	0.75
Quartile 2	46 (26.1)	16 [14-20]		53 (52.8)	26 [19-]	
Quartile 3	29 (24.1)	13 [10-17]		36 (50.0)	27 [20-54]	
Quartile 4	40 (22.5)	16 [11-20]		48 (45.8)	25 [21-42]	
Fish consumption between ages 6-15 years	N = 158			N = 187		
Rarely	42(26.2)	12 [9-18]	0.11	48 (41.7)	24 [16-28]	0.09
Sometimes	67 (31.3)	18 [14-26]		81 (50.6)	33 [20-43]	
Often-Very Often	49 (16.3)	16 [15-21]		58 (56.9)	30 [24-54]	
Average Fish consumption before MS onset	N= 154			N = 187		
Rarely	31 (22.6)	12 [10-20]	0.10	35 (40.0)	26 [16-29]	0.30
Sometimes	56 (33.9)	17 [14-28]		67 (46.3)	24 [19-42]	
Often-Very often	67 (19.4)	16 [14-20]		80 (57.5)	30 [23-53]	
Cod-liver intake between ages 6-15 years	N = 167			N = 202		
Never	141 (24.8)	16 [14-18]	0.19	171 (49.1)	26 [21-30]	0.01
Ever	26 (26.9)	16 [14-30]		31 (58.1)	38 [31-54]	

Variable	Time to PDDS6			Time to PDDS8		
	No of patients (% censored)	Median (95% CI)	*p*-value†	No of patients (% censored)	Median (95% CI)	*p*-value†
Calcium/ Vitamin D intake before MS onset	N=167			N = 202		
Never	140 (25.0)	16 [14-20]	0.86	167 (48.5)	27 [24-33]	0.80
Ever	27(26.0)	16 [10-20]		35 (60.0)	38 [21-40]	
Multi-vitamin intake before MS onset	N= 167			N= 202		
Never	110 (56.6)	16 [15-20]	0.73	130 (46.2)	28 [24-30]	0.45
Ever	57 (60.9)	15 [11-19]		72 (58.3)	29 [20-40]	
Skin Type	N = 165			N =201		
Very fair to fair	46 (23.9)	17 [11-24]	0.88	52 (51.9)	33 [24-47]	0.47
Medium to Olive	100 (23.0)	15 [13-17]		123 (48.0)	25 [21-30]	
Brown to Black	19 (31.6)	16 [11-26]		26 (53.9)	31 [17-34]	
Gender	N = 168			N = 204		
Female	40 (35.0)	15 [10-19]	0.83	47 (59.6)	31 [17-45]	0.98
Male	128 (21.9)	16 [15-20]		157 (47.1)	27 [24-33]	
Smoking status before MS onset	N = 160			N = 193		
Never	43 (32.6)	16 [10-19]	0.36	50(58.0)	27 [20-45]	0.97
Ever	117 (22.2)	16 [15-20]		143 (49.0)	29 [24-35]	
DX of Mononucleosis before MS onset	N = 162			N = 198		
Yes	22 (22.7)	11 [7-17]	0.05	27 (44.4)	16 [12-21]	0.005
No	140 (26.4)	16 [15-20]		171 (50.9)	29 [24-33]	

Variable	Time to PDDS6			Time to PDDS8		
	No of patients (% censored)	Median (95% CI)	*p*-value[†]	No of patients (% censored)	Median (95% CI)	*p*-value[†]
Age at symptom onset	N = 168			N=204		
18-29 years	53(20.8)	23 [17-29]	0.0008	68(44.1)	34 [27-43]	0.0033
30-39 years	42 (19.1)	16 [10-20]		53 (49.1)	28 [23-37]	
40-49 years	44 (25.0)	13 [10-16]		51 (47.1)	20 [16-35]	
≥50 years	29 (41.4)	13 [6-15]		32 (68.8)	18 [14-	
Type of symptoms at MS onset						
Motor/Coordination	N = 168			N = 204		
Present	135(25.9)	16 [15-20]	0.46	163(52.8)	29 [24-34]	0.22
Absent	33 (21.2)	15 [11-20]		41 (21.2)	24 [17-30]	
Sensory	N = 168			N =204		
Present	114(24.6)	16 [15-20]	0.31	135(52.6)	29 [24-35]	0.12
Absent	54(25.9)	15 [10-20]		54(44.9)	24 [20-30]	
Vision	N = 168			N = 204		
Yes	74(23.0)	16 [12-21]	0.92	92(50.0)	27 [21-33]	0.91
No	94 (26.6)	16 [14-20]		112 (50.0)	29 [23-34]	

[†] *p* value was generated by log-rank test

[††] N indicates number of patients who had reported the exposure and age at disability. Differences in N are due to missing values of particular exposure and age at the disability stage

Table A.4 Effects of sun exposure, vitamin D intake, and skin type on the estimates of time from disease onset to PDDS 6 among Veterans with Relapsing MS

		Time to PDDS6	
Variable	No of patients (% censored)	Median (95% CI)	*p*-value†
Average fall/winter sun exposure before MS onset*	N = 737		
Low ≤ 1.6 weeks/year	183 (60.1)	31[28-38]	0.33
High > 1.6 weeks/year	554 (57.9)	28[27-31]	
Cumulative sun exposure between ages 6-15 years	N= 751		
Low: (Q1 & Q2)**	372 (57.8)	30 [28-35]	0.83
High: (Q3 & Q4)	379 (58.8)	30 [27-32]	
Residential solar radiation estimates: Weighted UV counts between ages 0-15 years	N = 797		
Low	278 (57.6)	31 [27-33]	
Medium	260 (60.4)	31 [28-35]	0.23
High	259 (57.1)	28 [25-32]	
Fish consumption between ages 6-15 y	N = 789		
Rarely	195 (60.0)	30 [27-35]	
Sometimes	380 (57.6)	28 [26-31]	0.79
Often-Very Often	214 (57.9)	31 [28-34]	
Cod-liver intake between ages 6-15 y	N = 836		
Never	737 (58.3)	29 [27-31]	0.37
Ever	99 (52.5)	33 [26-38]	
Calcium/Vitamin D intake before MS onset	N=835		
Never	717(58.0)	30 [28-33]	
Ever-<50% of the time	69(63.8)	23 [20-36]	0.05
Ever->50% of the time	49 (44.9)	25 [17-31]	
Multi-vitamin intake before MS onset	N= 836		
Never	515 (55.7)	30 [28-33]	
Ever-<50% of the time	137 (65.0)	28 [20-]	0.23
Ever->50% of the time	184 (57.6)	30[26-35]	
Skin Type	N = 832		
Very fair to fair	276 (54.4)	28 [26-31]	
Medium to Olive	467 (59.3)	31 [28-35]	0.46
Brown to Black	89 (58.4)	30 [20-36]	

Gender	N = 844		
Female	439 (53.1)	29 [26-33]	0.54
Male	405 (51.4)	30 [27-32]	
Age at symptom onset	N = 844		
<25 years	211 (56.4)	35 [31-39]	<0.0001
25-34 years	363 (54.6)	28 [25-32]	
35-44years	188 (63.3)	25 [20-28]	
≥ 45 years	82 (59.8)	15 [12-14]	
MS subtype	N = 844		
SPMS*	436 (37.2)	24 [21-27]	<0.0001
RRMS	408 (79.2)	53 [39-53]	
Smoking status before MS onset	N = 810		
Never	282 (60.2)	29 [24-35]	0.74
Ever	528 (56.1)	30 [28-32]	
DX of Mononucleosis before MS onset	N = 832		
Yes	115 (68.7)	35 [30-]	0.27
No	717 (56.1)	29 [27-31]	
Types of symptoms at MS onset			
Motor	N = 844		
Yes	587(55.5)	29 [27-32]	0.53
No	257 (61.9)	30 [27-33]	
Vision	N = 1023		
Yes	431(55.0)	30 [28-32]	0.67
No	413 (60.1)	29 [27-34]	
Sensory	N = 844		
Present	568 (56.7)	30 [28-34]	0.24
Absent	276 (59.1)	29 [25-31]	

Table A.5 Effects of age at symptom onset on the estimates of time (years) from onset to PDDS 6 among Veterans with Relapsing and Progressive MS [3]

	Age at PDDS 6					
Variables	**Relapsing MS**			**Progressive MS**		
	No of patients (% censored)	Median (95% CI) CI)	*p*-value†	No of patients (% censored)	Median (95% CI)	*p*-value†
Age at symptom onset	N = 844			N=128		
18-29 years	424 (53.5)	55 [54-59]	<0.0001	43 (2.3)	43 [40-49]	<0.0001
30-39 years	259 (61.8)	61 [58-65]		34 (0.0)	45.5 [43-50]	
40-49 years	121 (62.8)	65 [59-73]		34 (2.9)	54.5 [52-57]	
≥50 years	40 (55.0)	65 [62-]		17 (0.0)	60 [58-64]	

[3] Veterans who had younger age at symptom onset reached PDDS 6 at younger age

Table A.6 Kaplan-Meier estimates of time from Patient Determined Disease Stage (PDDS) to more advanced stage among Veterans with different MS subtypes

	Relapsing MS† (n=948)	Relapse-Remitting MS (n =454)	Secondary Progressive MS (n=494)	Progressive MS (n =219)
From PDDS 4 to disability stage of				
PDDS 6	5 [4-5]*	4 [3-5]	5 [4-6]	4 [3-5]
PDDS 8	9 [8-10]	7 [5-12]	9 [8-10]	9 [7-12]
From PDDS 6 to disability stage of				
PDDS 8	4[3-5]	4[3-6]	4[3-5]	4 [3-6]

***median time in years and [95% CI]**

REFERENCES

1. Lublin FD. Clinical features and diagnosis of multiple sclerosis. Neurol Clin. 2005;23:1-15, v

2. Hauser SL, Oksenberg JR. The neurobiology of multiple sclerosis: genes, inflammation, and neurodegeneration. Neuron. 2006;52:61-76

3. Whitaker JNM, G.W. Clinical features of multiple sclerosis. In: McFarland HFT, W.W., ed. Multiple sclerosis. Clinical features and pathogenetic basis. London: Chaoman and Hall Medical, 1997:3-19

4. All about Multiple Sclerosis. 2 ed: Multiple Sclerosis Association of American (MSAA2), 2006

5. Kantarci OH, Weinshenker BG. Natural history of multiple sclerosis. Neurol Clin. 2005;23:17-38, v

6. Langer-Gould A, Popat RA, Huang SM et al. Clinical and demographic predictors of long-term disability in patients with relapsing-remitting multiple sclerosis: a systematic review. Arch Neurol. 2006;63:1686-1691

7. Confavreux C, Vukusic S. Natural history of multiple sclerosis: a unifying concept. Brain. 2006;129:606-616

8. Confavreux C, Vukusic S. Age at disability milestones in multiple sclerosis. Brain. 2006;129:595-605

9. Confavreux CC, A. The natural history of multiple sclerosis. In: Compston A, ed. McAlpine's Multiple sclerosis. 4th ed. London: Churchill Livingstone Elsevier, 2006:183-272

10. Trojano M, Liguori M, Bosco Zimatore G et al. Age-related disability in multiple sclerosis. Ann Neurol. 2002;51:475-480

11. Vukusic S, Confavreux C. Natural history of multiple sclerosis: risk factors and prognostic indicators. Curr Opin Neurol. 2007;20:269-274

12. Ebers GC, Koopman WJ, Hader W et al. The natural history of multiple sclerosis: a geographically based study: 8: familial multiple sclerosis. Brain. 2000;123 Pt 3:641-649

13. Kremenchutzky M, Cottrell D, Rice G et al. The natural history of multiple sclerosis: a geographically based study. 7. Progressive-relapsing and relapsing-progressive multiple sclerosis: a re-evaluation. Brain. 1999;122 (Pt 10):1941-1950

14. Rodriguez M, Siva A, Ward J et al. Impairment, disability, and handicap in multiple sclerosis: a population-based study in Olmsted County, Minnesota. Neurology. 1994;44:28-33

15. Weinshenker BG. The natural history of multiple sclerosis. Neurol Clin. 1995;13:119-146

16. Weinshenker BG, Bass B, Rice GP et al. The natural history of multiple sclerosis: a geographically based study. 2. Predictive value of the early clinical course. Brain. 1989;112 (Pt 6):1419-1428

17. Marrie RA, Cutter G, Tyry T et al. Validation of the NARCOMS Registry: pain assessment. Mult Scler. 2005;11:338-342

18. Hohol MJ, Orav EJ, Weiner HL. Disease steps in multiple sclerosis: a simple approach to evaluate disease progression. Neurology. 1995;45:251-255

19. Orton SM, Herrera BM, Yee IM et al. Sex ratio of multiple sclerosis in Canada: a longitudinal study. Lancet Neurol. 2006;5:932-936

20. Pittock SJ, Mayr WT, McClelland RL et al. Quality of life is favorable for most patients with multiple sclerosis: a population-based cohort study. Arch Neurol. 2004;61:679-686

21. Koch-Henriksen N. The Danish Multiple Sclerosis Registry: a 50-year follow-up. Mult Scler. 1999;5:293-296

22. Koch-Henriksen N, Hyllested K. Epidemiology of multiple sclerosis: incidence and prevalence rates in Denmark 1948-64 based on the Danish Multiple Sclerosis Registry. Acta Neurol Scand. 1988;78:369-380

23. Minden SLM, W.D.; Harrold, L.N.;Dor, A. Multiple Sclerosis-A statistical Portrait: A Compendium of Data of Demographic, Disability, and Health Services Utilization in the United States. Cambridge: Mass: Abt Associated Inc., 1993

24. Kurtzke JF. Epidemiology of multiple sclerosis. Does this really point toward an etiology? Lectio Doctoralis. Neurol Sci. 2000;21:383-403

25. Ascherio A, Munger KL. Environmental risk factors for multiple sclerosis. Part II: Noninfectious factors. Ann Neurol. 2007;61:504-513

26. Ascherio A, Munger KL. Environmental risk factors for multiple sclerosis. Part I: the role of infection. Ann Neurol. 2007;61:288-299

27. Kantarci O, Wingerchuk D. Epidemiology and natural history of multiple sclerosis: new insights. Curr Opin Neurol. 2006;19:248-254

28. Compston DA, Batchelor JR, McDonald WI. B-lymphocyte alloantigens associated with multiple sclerosis. Lancet. 1976;2:1261-1265

29. Jersild C, Svejgaard A, Fog T. HL-A antigens and multiple sclerosis. Lancet. 1972;1:1240-1241

30. Jersild C, Dupont B, Fog T et al. Histocompatibility determinants in multiple sclerosis. Transplant Rev. 1975;22:148-163

31. Kurtzke JF, Beebe GW, Norman JE, Jr. Epidemiology of multiple sclerosis in U.S. veterans: 1. Race, sex, and geographic distribution. Neurology. 1979;29:1228-1235

32. Ebers GC, Sadovnick AD. The role of genetic factors in multiple sclerosis susceptibility. J Neuroimmunol. 1994;54:1-17

33. Compston A, Coles A. Multiple sclerosis. Lancet. 2002;359:1221-1231

34. Ascherio A, Munger K. Epidemiology of multiple sclerosis: from risk factors to prevention. Semin Neurol. 2008;28:17-28

35. Wucherpfennig KW, Strominger JL. Molecular mimicry in T cell-mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein. Cell. 1995;80:695-705

36. Thacker EL, Mirzaei F, Ascherio A. Infectious mononucleosis and risk for multiple sclerosis: a meta-analysis. Ann Neurol. 2006;59:499-503

37. Hernan MA, Zhang SM, Lipworth L et al. Multiple sclerosis and age at infection with common viruses. Epidemiology. 2001;12:301-306

38. Fleming J, Fabry Z. The hygiene hypothesis and multiple sclerosis. Ann Neurol. 2007;61:85-89

39. Levin LI, Munger KL, Rubertone MV et al. Multiple sclerosis and Epstein-Barr virus. Jama. 2003;289:1533-1536

40. Serafini B, Rosicarelli B, Franciotta D et al. Dysregulated Epstein-Barr virus infection in the multiple sclerosis brain. J Exp Med. 2007;204:2899-2912

41. Larsen PD, Bloomer LC, Bray PF. Epstein-Barr nuclear antigen and viral capsid antigen antibody titers in multiple sclerosis. Neurology. 1985;35:435-438

42. Bray PF, Bloomer LC, Salmon VC et al. Epstein-Barr virus infection and antibody synthesis in patients with multiple sclerosis. Arch Neurol. 1983;40:406-408

43. Kinnunen E, Valle M, Piirainen L et al. Viral antibodies in multiple sclerosis. A nationwide co-twin study. Arch Neurol. 1990;47:743-746

44. Levin LI, Munger KL, Rubertone MV et al. Temporal relationship between elevation of epstein-barr virus antibody titers and initial onset of neurological symptoms in multiple sclerosis. Jama. 2005;293:2496-2500

45. Hammond SR, de Wytt C, Maxwell IC et al. The epidemiology of multiple sclerosis in Queensland, Australia. J Neurol Sci. 1987;80:185-204

46. Hammond SR, McLeod JG, Millingen KS et al. The epidemiology of multiple sclerosis in three Australian cities: Perth, Newcastle and Hobart. Brain. 1988;111 (Pt 1):1-25

47. Wallin MT, Page WF, Kurtzke JF. Multiple sclerosis in US veterans of the Vietnam era and later military service: race, sex, and geography. Ann Neurol. 2004;55:65-71

48. Berwick M, Kesler D. Ultraviolet radiation exposure, vitamin D, and cancer. Photochem Photobiol. 2005;81:1261-1266

49. Bodiwala D, Luscombe CJ, Liu S et al. Prostate cancer risk and exposure to ultraviolet radiation: further support for the protective effect of sunlight. Cancer Lett. 2003;192:145-149

50. Ponsonby AL, Lucas RM, van der Mei IA. UVR, vitamin D and three autoimmune diseases--multiple sclerosis, type 1 diabetes, rheumatoid arthritis. Photochem Photobiol. 2005;81:1267-1275

51. Ponsonby AL, McMichael A, van der Mei I. Ultraviolet radiation and autoimmune disease: insights from epidemiological research. Toxicology. 2002;181-182:71-78

52. McMichael AJ, Hall AJ. Does immunosuppressive ultraviolet radiation explain the latitude gradient for multiple sclerosis? Epidemiology. 1997;8:642-645

53. Halliday GM, Byrne SN, Kuchel JM et al. The suppression of immunity by ultraviolet radiation: UVA, nitric oxide and DNA damage. Photochem Photobiol Sci. 2004;3:736-740

54. Duthie MS, Kimber I, Norval M. The effects of ultraviolet radiation on the human immune system. Br J Dermatol. 1999;140:995-1009

55. Lucas RMM, A.J.; Armstrong, B.K., Smith, W.T. The Global Burden of Disease due to Ultraviolet Radiation. Geneva: World Health Organization, 2006

56. Diffey BL. Sources and measurement of ultraviolet radiation. Methods. 2002;28:4-13

57. Roy CR, Gies HP, Lugg DJ et al. The measurement of solar ultraviolet radiation. Mutat Res. 1998;422:7-14

58. Clydesdale GJ, Dandie GW, Muller HK. Ultraviolet light induced injury: immunological and inflammatory effects. Immunol Cell Biol. 2001;79:547-568

59. IARC Working Group on the Evaluation of Carcinogenic risks to Humans: Solar and Ultraviolet Radiation. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 55. Lyon: IARC, 1992

60. Scotto JF, T.R.; Gori, G.B. Measurements from ultraviolet meters at ten locations in the United States during 1974 and comparisons with recent skin cancer data. Bethesda, MD: National Cancer Institute, 1974

61. Gies P, Roy C, Toomey S, Tomlinson D. Ambient solar UVR, personal exposure and protection. J Epidemiol. 1999;9:S115-122

62. Fitzpatrick TB. The validity and practicality of sun-reactive skin types I through VI. Arch Dermatol. 1988;124:869-871

63. Lucas RM, Ponsonby AL. Considering the potential benefits as well as adverse effects of sun exposure: can all the potential benefits be provided by oral vitamin D supplementation? Prog Biophys Mol Biol. 2006;92:140-149

64. Acheson ED, Bachrach CA, Wright FM. Some comments on the relationship of the distribution of multiple sclerosis to latitude, solar radiation, and other variables. Acta Psychiatr Scand Suppl. 1960;35:132-147

65. van der Mei IA, Ponsonby AL, Blizzard L, Dwyer T. Regional variation in multiple sclerosis prevalence in Australia and its association with ambient ultraviolet radiation. Neuroepidemiology. 2001;20:168-174

66. Norman JE, Jr., Kurtzke JF, Beebe GW. Epidemiology of multiple sclerosis in U.S. veterans: 2. Latitude, climate and the risk of multiple sclerosis. J Chronic Dis. 1983;36:551-559

67. Kurtzke JF. On the fine structure of the distribution of multiple sclerosis. Acta Neurol Scand. 1967;43:257-282

68. Freedman DM, Dosemeci M, Alavanja MC. Mortality from multiple sclerosis and exposure to residential and occupational solar radiation: a case-control study based on death certificates. Occup Environ Med. 2000;57:418-421

69. van der Mei IA, Ponsonby AL, Dwyer T et al. Past exposure to sun, skin phenotype, and risk of multiple sclerosis: case-control study. Bmj. 2003;327:316

70. Kampman MT, Wilsgaard T, Mellgren SI. Outdoor activities and diet in childhood and adolescence relate to MS risk above the Arctic Circle. J Neurol. 2007;254:471-477

71. Islam T, Gauderman WJ, Cozen W, Mack TM. Childhood sun exposure influences risk of multiple sclerosis in monozygotic twins. Neurology. 2007;69:381-388

72. Swank RL, Lerstad O, Strom A, Backer J. Multiple sclerosis in rural Norway its geographic and occupational incidence in relation to nutrition. N Engl J Med. 1952;246:722-728

73. Agranoff BW, Goldberg D. Diet and the geographical distribution of multiple sclerosis. Lancet. 1974;2:1061-1066

74. Hayes CE. Vitamin D: a natural inhibitor of multiple sclerosis. Proc Nutr Soc. 2000;59:531-535

75. Hayes CE, Cantorna MT, DeLuca HF. Vitamin D and multiple sclerosis. Proc Soc Exp Biol Med. 1997;216:21-27

76. VanAmerongen BM, Dijkstra CD, Lips P, Polman CH. Multiple sclerosis and vitamin D: an update. Eur J Clin Nutr. 2004;58:1095-1109

77. van der Mei IA, Ponsonby AL, Dwyer T et al. Vitamin D levels in people with multiple sclerosis and community controls in Tasmania, Australia. J Neurol. 2007;254:581-590

78. Wingerchuk DM, Lesaux J, Rice GP et al. A pilot study of oral calcitriol (1,25-dihydroxyvitamin D3) for relapsing-remitting multiple sclerosis. J Neurol Neurosurg Psychiatry. 2005;76:1294-1296

79. Spach KM, Hayes CE. Vitamin D3 confers protection from autoimmune encephalomyelitis only in female mice. J Immunol. 2005;175:4119-4126

80. Smolders J, Damoiseaux J, Menheere P, Hupperts R. Vitamin D as an immune modulator in multiple sclerosis, a review. J Neuroimmunol. 2008;194:7-17

81. Rapuri PB, Gallagher JC. Effect of Vitamin D supplement use on serum concentrations of total 25OHD levels in elderly women. J Steroid Biochem Mol Biol. 2004;89-90:601-604

82. Nashold FE, Hoag KA, Goverman J, Hayes CE. Rag-1-dependent cells are necessary for 1,25-dihydroxyvitamin D(3) prevention of experimental autoimmune encephalomyelitis. J Neuroimmunol. 2001;119:16-29

83. Munger KL, Zhang SM, O'Reilly E et al. Vitamin D intake and incidence of multiple sclerosis. Neurology. 2004;62:60-65

84. Mahon BD, Gordon SA, Cruz J et al. Cytokine profile in patients with multiple sclerosis following vitamin D supplementation. J Neuroimmunol. 2003;134:128-132

85. Lips P. Vitamin D physiology. Prog Biophys Mol Biol. 2006;92:4-8

86. Alpert PT, Shaikh U. The effects of vitamin D deficiency and insufficiency on the endocrine and paracrine systems. Biol Res Nurs. 2007;9:117-129

87. Burgaz A, Akesson A, Oster A et al. Associations of diet, supplement use, and ultraviolet B radiation exposure with vitamin D status in Swedish women during winter. Am J Clin Nutr. 2007;86:1399-1404

88. Nashold FE, Miller DJ, Hayes CE. 1,25-dihydroxyvitamin D3 treatment decreases macrophage accumulation in the CNS of mice with experimental autoimmune encephalomyelitis. J Neuroimmunol. 2000;103:171-179

89. Cantorna MT, Hayes CE, DeLuca HF. 1,25-Dihydroxyvitamin D3 reversibly blocks the progression of relapsing encephalomyelitis, a model of multiple sclerosis. Proc Natl Acad Sci U S A. 1996;93:7861-7864

90. Cantorna MT, Humpal-Winter J, DeLuca HF. Dietary calcium is a major factor in 1,25-dihydroxycholecalciferol suppression of experimental autoimmune encephalomyelitis in mice. J Nutr. 1999;129:1966-1971

91. Cantorna MT, Woodward WD, Hayes CE, DeLuca HF. 1,25-dihydroxyvitamin D3 is a positive regulator for the two anti-encephalitogenic cytokines TGF-beta 1 and IL-4. J Immunol. 1998;160:5314-5319

92. Moon SJ, Fryer AA, Strange RC. Ultraviolet radiation, vitamin D and risk of prostate cancer and other diseases. Photochem Photobiol. 2005;81:1252-1260

93. Mullin GE, Dobs A. Vitamin d and its role in cancer and immunity: a prescription for sunlight. Nutr Clin Pract. 2007;22:305-322

94. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357:266-281

95. Brown AJ, Dusso A, Slatopolsky E. Vitamin D. Am J Physiol. 1999;277:F157-175

96. Garcion E, Wion-Barbot N, Montero-Menei CN et al. New clues about vitamin D functions in the nervous system. Trends Endocrinol Metab. 2002;13:100-105

97. Baas D, Prufer K, Ittel ME et al. Rat oligodendrocytes express the vitamin D(3) receptor and respond to 1,25-dihydroxyvitamin D(3). Glia. 2000;31:59-68

98. Deluca HF, Cantorna MT. Vitamin D: its role and uses in immunology. Faseb J. 2001;15:2579-2585

99. Provvedini DM, Rulot CM, Sobol RE et al. 1 alpha,25-Dihydroxyvitamin D3 receptors in human thymic and tonsillar lymphocytes. J Bone Miner Res. 1987;2:239-247

100. Bhalla AK, Amento EP, Clemens TL et al. Specific high-affinity receptors for 1,25-dihydroxyvitamin D3 in human peripheral blood mononuclear cells: presence in monocytes and induction in T lymphocytes following activation. J Clin Endocrinol Metab. 1983;57:1308-1310

101. Lemire JM, Archer DC. 1,25-dihydroxyvitamin D3 prevents the in vivo induction of murine experimental autoimmune encephalomyelitis. J Clin Invest. 1991;87:1103-1107

102. Muthian G, Raikwar HP, Rajasingh J, Bright JJ. 1,25 Dihydroxyvitamin-D3 modulates JAK-STAT pathway in IL-12/IFNgamma axis leading to Th1 response in experimental allergic encephalomyelitis. J Neurosci Res. 2006;83:1299-1309

103. Van Etten E, Branisteanu DD, Overbergh L et al. Combination of a 1,25-dihydroxyvitamin D3 analog and a bisphosphonate prevents experimental autoimmune encephalomyelitis and preserves bone. Bone. 2003;32:397-404

104. Garcion E, Sindji L, Nataf S et al. Treatment of experimental autoimmune encephalomyelitis in rat by 1,25-dihydroxyvitamin D3 leads to early effects within the central nervous system. Acta Neuropathol. 2003;105:438-448

105. Feskanich D, Willett WC, Colditz GA. Calcium, vitamin D, milk consumption, and hip fractures: a prospective study among postmenopausal women. Am J Clin Nutr. 2003;77:504-511

106. Munger KL, Levin LI, Hollis BW et al. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. Jama. 2006;296:2832-2838

107. Niino M, Fukazawa T, Yabe I et al. Vitamin D receptor gene polymorphism in multiple sclerosis and the association with HLA class II alleles. J Neurol Sci. 2000;177:65-71

108. Fukazawa T, Yabe I, Kikuchi S et al. Association of vitamin D receptor gene polymorphism with multiple sclerosis in Japanese. J Neurol Sci. 1999;166:47-52

109. Tajouri L, Ovcaric M, Curtain R et al. Variation in the vitamin D receptor gene is associated with multiple sclerosis in an Australian population. J Neurogenet. 2005;19:25-38

110. Steckley JL, Dyment DA, Sadovnick AD et al. Genetic analysis of vitamin D related genes in Canadian multiple sclerosis patients. Canadian Collaborative Study Group. Neurology. 2000;54:729-732

111. Partridge JM, Weatherby SJ, Woolmore JA et al. Susceptibility and outcome in MS: associations with polymorphisms in pigmentation-related genes. Neurology. 2004;62:2323-2325

112. Yeo TW, Maranian M, Singlehurst S et al. Four single nucleotide polymorphisms from the vitamin D receptor gene in UK multiple sclerosis. J Neurol. 2004;251:753-754

113. Ramagopalan SV, Maugeri NJ, Handunnetthi L et al. Expression of the multiple sclerosis-associated MHC class II Allele HLA-DRB1*1501 is regulated by vitamin D. PLoS Genet. 2009;5:e1000369

114. Goldberg P, Fleming MC, Picard EH. Multiple sclerosis: decreased relapse rate through dietary supplementation with calcium, magnesium and vitamin D. Med Hypotheses. 1986;21:193-200

115. Nordvik I, Myhr KM, Nyland H, Bjerve KS. Effect of dietary advice and n-3 supplementation in newly diagnosed MS patients. Acta Neurol Scand. 2000;102:143-149

116. Kimball SM, Ursell MR, O'Connor P, Vieth R. Safety of vitamin D3 in adults with multiple sclerosis. Am J Clin Nutr. 2007;86:645-651

117. Soilu-Hanninen M, Airas L, Mononen I et al. 25-Hydroxyvitamin D levels in serum at the onset of multiple sclerosis. Mult Scler. 2005;11:266-271

118. Soilu-Hanninen M, Laaksonen M, Laitinen I et al. A longitudinal study of serum 25-hydroxyvitamin D and intact parathyroid hormone levels indicate the importance of vitamin D and calcium homeostasis regulation in multiple sclerosis. J Neurol Neurosurg Psychiatry. 2008;79:152-157

119. Smolders J, Menheere P, Kessels A et al. Association of vitamin D metabolite levels with relapse rate and disability in multiple sclerosis. Mult Scler. 2008

120. Goodin DS. The causal cascade to multiple sclerosis: a model for MS pathogenesis. PLoS One. 2009;4:e4565

121. Ebers GC. Environmental factors and multiple sclerosis. Lancet Neurol. 2008;7:268-277

122. Sadovnick AD, Yee IM. Season of birth in multiple sclerosis. Acta Neurol Scand. 1994;89:190-191

123. Dean G, Kurtzke JF. A critical age for the acquisition of multiple sclerosis. Trans Am Neurol Assoc. 1970;95:232-233

124. Willer CJ, Dyment DA, Sadovnick AD et al. Timing of birth and risk of multiple sclerosis: population based study. Bmj. 2005;330:120

125. Wiberg MT, DI. Season of birth in Multiple Sclerosis in Sweden: replication of Denmark findings. J Orthomol Med. 1994;9:71-74

126. Salzer J, Svenningsson A, Sundstrom P. Season of birth and multiple sclerosis in Sweden. Acta Neurol Scand. 2010;121:20-23

127. Ramagopalan SV, Link J, Byrnes JK et al. HLA-DRB1 and month of birth in multiple sclerosis. Neurology. 2009;73:2107-2111

128. Sadovnick AD, Duquette P, Herrera B et al. A timing-of-birth effect on multiple sclerosis clinical phenotype. Neurology. 2007;69:60-62

129. Tremlett HL, Devonshire VA. Does the season or month of birth influence disease progression in multiple sclerosis? Neuroepidemiology. 2006;26:195-198

130. Koch M, De Keyser J, Tremlett H. Timing of birth and disease progression in multiple sclerosis. Mult Scler. 2008;14:793-798

131. Pugliatti M, Riise T, Sotgiu MA et al. Evidence of early childhood as the susceptibility period in multiple sclerosis: space-time cluster analysis in a Sardinian population. Am J Epidemiol. 2006;164:326-333

132. Dean G. Annual incidence, prevalence, and mortality of multiple sclerosis in white South-African-born and in white immigrants to South Africa. Br Med J. 1967;2:724-730

133. Kurtzke JF, Beebe GW, Norman JE, Jr. Epidemiology of multiple sclerosis in US veterans: III. Migration and the risk of MS. Neurology. 1985;35:672-678

134. Pugliatti M, Rosati G, Carton H et al. The epidemiology of multiple sclerosis in Europe. Eur J Neurol. 2006;13:700-722

135. Clemens TL, Adams JS, Henderson SL, Holick MF. Increased skin pigment reduces the capacity of skin to synthesise vitamin D3. Lancet. 1982;1:74-76

136. Marrie RA, Cutter G, Tyry T et al. Does multiple sclerosis-associated disability differ between races? Neurology. 2006;66:1235-1240

137. Naismith RT, Trinkaus K, Cross AH. Phenotype and prognosis in African-Americans with multiple sclerosis: a retrospective chart review. Mult Scler. 2006;12:775-781

138. Woolmore JA, Stone M, Pye EM et al. Studies of associations between disability in multiple sclerosis, skin type, gender and ultraviolet radiation. Mult Scler. 2007;13:369-375

139. Culpepper WJ, II, Ehrmantraut M, Wallin MT et al. Veterans Health Administration multiple sclerosis surveillance registry: The problem of case-finding from administrative databases. J Rehabil Res Dev. 2006;43:17-24

140. Culpepper WJ, II. Effectiveness of Practice Guidelines for Disease Modifying Therapy in Multiple Sclerosis within the Veteran's Heath Administration. Department of Epidemiology and Preventive Medicine. Baltimore: University of Maryland, Baltimore, 2008

141. Dillman D. The Tailored Design Method. New York: John Wiley & Sons, 2000

142. Edwards P, Roberts I, Clarke M et al. Increasing response rates to postal questionnaires: systematic review. Bmj. 2002;324:1183

143. United States Department of Commerce: Maps pf Annual Mean Daily Solar Radiation for the United States. Washington, DC: US Govement Printing Office, 1974

144. Hanson RL. National Water Summary 1988-89: Hydrologic Events and Floods and Droughts. In: Paulson RWC, E.B.; Roberts, R.S.; Moody, D.W., ed: U.S. Geological Survey **http://geochange.er.usgs.gov/sw/changes/natural/et/**, 1991:99-104

145. National Oceqanic and Atmospheric Administration. Climatological data, national summary, annual summary. Vol. 26. Asheville (NC): Environmental Data Service, National Climatic Data Center, 1975

146. Mo T, Green AE. A climatology of solar erythema dose. Photochem Photobiol. 1974;20:483-496

147. Gluckman PD, Hanson MA. Living with the past: evolution, development, and patterns of disease. Science. 2004;305:1733-1736

148. McLeod JG, Hammond SR, Hallpike JF. Epidemiology of multiple sclerosis in Australia. With NSW and SA survey results. Med J Aust. 1994;160:117-122

149. Islam T, Gauderman WJ, Cozen W et al. Differential twin concordance for multiple sclerosis by latitude of birthplace. Ann Neurol. 2006;60:56-64

150. Hammond SR, English DR, McLeod JG. The age-range of risk of developing multiple sclerosis: evidence from a migrant population in Australia. Brain. 2000;123 (Pt 5):968-974

151. Ebers GC, Sadovnick AD, Dyment DA et al. Parent-of-origin effect in multiple sclerosis: observations in half-siblings. Lancet. 2004;363:1773-1774

152. Jacobs LD, Wende KE, Brownscheidle CM et al. A profile of multiple sclerosis: the New York State Multiple Sclerosis Consortium. Mult Scler. 1999;5:369-376

153. Minden SL, Frankel D, Hadden L et al. The Sonya Slifka Longitudinal Multiple Sclerosis Study: methods and sample characteristics. Mult Scler. 2006;12:24-38

154. U.S. Geological Survey: Department of the Interior/USGS

155. Garland CF, Garland FC. Do sunlight and vitamin D reduce the likelihood of colon cancer? Int J Epidemiol. 1980;9:227-231

156. Kurtzke JF, Page WF, Murphy FM, Norman JE, Jr. Epidemiology of multiple sclerosis in US veterans. 4. Age at onset. Neuroepidemiology. 1992;11:226-235

157. Marrie RA, Horwitz R, Cutter G et al. Comorbidity delays diagnosis and increases disability at diagnosis in MS. Neurology. 2009;72:117-124

158. Cabre P, Signate A, Olindo S et al. Role of return migration in the emergence of multiple sclerosis in the French West Indies. Brain. 2005;128:2899-2910

159. Dean G, Kurtzke JF. On the risk of multiple sclerosis according to age at immigration to South Africa. Br Med J. 1971;3:725-729

160. Elian M, Nightingale S, Dean G. Multiple sclerosis among United Kingdom-born children of immigrants from the Indian subcontinent, Africa and the West Indies. J Neurol Neurosurg Psychiatry. 1990;53:906-911

161. Pugliatti M, Cossu P, Sotgiu S et al. Clustering of multiple sclerosis, age of onset and gender in Sardinia. J Neurol Sci. 2009;286:6-13

162. Riise T, Gronning M, Klauber MR et al. Clustering of residence of multiple sclerosis patients at age 13 to 20 years in Hordaland, Norway. Am J Epidemiol. 1991;133:932-939

163. Raghuwanshi A, Joshi SS, Christakos S. Vitamin D and multiple sclerosis. J Cell Biochem. 2008

164. Culpepper WJ, II. Effectiveness of Practice Guidelines for Disease Modifying Therapy in Multiple Sclerosis within the Veteran's Heath Administration. Department of Epidemiology and Preventive Medicine. Baltimore: University of Maryland, Baltimore, 2009

165. Riise T, Klauber MR. Relationship between the degree of individual space-time clustering and age at onset of disease among multiple sclerosis patients. Int J Epidemiol. 1992;21:528-532

166. Westlund KB, Kurland LT. Studies on multiple sclerosis in Winnepeg, Manitoba, and New Orleans, Louisiana. I. Prevalence; comparison between the patient groups in Winnipeg and New Orleans. Am J Hyg. 1953;57:380-396

167. Alter M. Multiple sclerosis in the Negro. Arch Neurol. 1962;7:83-91

168. Dickinson JL, Perera DI, van der Mei AF et al. Past environmental sun exposure and risk of multiple sclerosis: a role for the Cdx-2 Vitamin D receptor variant in this interaction. Mult Scler. 2009;15:563-570

169. Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG. Multiple sclerosis. N Engl J Med. 2000;343:938-952

170. Compston A, Coles A. Multiple sclerosis. Lancet. 2008;372:1502-1517

171. McDowell TA, S.; Langenberg, P; Royal, W; Bever, C.; Culpepper WJ; Bradham, DD. Timing of Birth, Residential Solar Radiation and Age at Onset of Multiple Sclerosis. Neuroepidemiology. 2010;34:238-244

172. Schwartz CE, Vollmer T, Lee H. Reliability and validity of two self-report measures of impairment and disability for MS. North American Research Consortium on Multiple Sclerosis Outcomes Study Group. Neurology. 1999;52:63-70

173. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology. 1983;33:1444-1452

174. Gauthier SA, Buckle GJ, Weiner HL. Immunosuppressive therapy for multiple sclerosis. Neurol Clin. 2005;23:247-272, viii-ix

175. Kappos L. Interferons in multiple sclerosis. Neurol Clin. 2005;23:189-214, vii-viii

176. Kalueff AV, Eremin KO, Tuohimaa P. Mechanisms of neuroprotective action of vitamin D(3). Biochemistry (Mosc). 2004;69:738-741

177. Edwards S, Zvartau M, Clarke H et al. Clinical relapses and disease activity on magnetic resonance imaging associated with viral upper respiratory tract infections in multiple sclerosis. J Neurol Neurosurg Psychiatry. 1998;64:736-741

178. Sibley WA, Bamford CR, Clark K. Clinical viral infections and multiple sclerosis. Lancet. 1985;1:1313-1315

179. Casetta I, Granieri E. Clinical infections and multiple sclerosis: contribution from analytical epidemiology. J Neurovirol. 2000;6 Suppl 2:S147-151

180. Koch M, Kingwell E, Rieckmann P, Tremlett H. The natural history of primary progressive multiple sclerosis. Neurology. 2009;73:1996-2002

181. Koch M, Mostert J, Heersema D, De Keyser J. Progression in multiple sclerosis: further evidence of an age dependent process. J Neurol Sci. 2007;255:35-41

182. Weinshenker BG, Bass B, Rice GP et al. The natural history of multiple sclerosis: a geographically based study. I. Clinical course and disability. Brain. 1989;112 (Pt 1):133-146

183. Chao MJ, Ramagopalan SV, Herrera BM et al. Epigenetics in multiple sclerosis susceptibility: difference in transgenerational risk localizes to the major histocompatibility complex. Hum Mol Genet. 2009;18:261-266

184. Lincoln JA, Cook SD. An overview of gene-epigenetic-environmental contributions to MS causation. J Neurol Sci. 2009;286:54-57

185. Sotgiu S, Pugliatti M, Sotgiu MA et al. Seasonal fluctuation of multiple sclerosis births in Sardinia. J Neurol. 2006;253:38-44

186. Myhr KM. Vitamin D treatment in multiple sclerosis. J Neurol Sci. 2009;286:104-108

187. Aktas O, Kieseier B, Hartung HP. Neuroprotection, regeneration and immunomodulation: broadening the therapeutic repertoire in multiple sclerosis. Trends Neurosci. 2010 (in press)

188. Stangel M. Neuroprotection and neuroregeneration in multiple sclerosis. J Neurol. 2008;255 Suppl 6:77-81

189. Van der Walt A, Butzkueven H, Kolbe S et al. Neuroprotection in multiple sclerosis: A therapeutic challenge for the next decade. Pharmacol Ther. 2010 (In press)

190. Kalueff AV, Minasyan A, Keisala T et al. The vitamin D neuroendocrine system as a target for novel neurotropic drugs. CNS Neurol Disord Drug Targets. 2006;5:363-371

191. Kalueff AV, Tuohimaa P. Neurosteroid hormone vitamin D and its utility in clinical nutrition. Curr Opin Clin Nutr Metab Care. 2007;10:12-19

192. Gezen-Ak D, Dursun E, Ertan T et al. Association between vitamin D receptor gene polymorphism and Alzheimer's disease. Tohoku J Exp Med. 2007;212:275-282

193. Hohlfeld R, Kerschensteiner M, Stadelmann C et al. The neuroprotective effect of inflammation: implications for the therapy of multiple sclerosis. J Neuroimmunol. 2000;107:161-166

194. Palace J. Inflammation versus neurodegeneration: consequences for treatment. J Neurol Sci. 2007;259:46-49

195. Trojano M, Pellegrini F, Fuiani A et al. New natural history of interferon-beta-treated relapsing multiple sclerosis. Ann Neurol. 2007;61:300-306

196. Koch M, Uyttenboogaart M, van Harten A, De Keyser J. Factors associated with the risk of secondary progression in multiple sclerosis. Mult Scler. 2008;14:799-803

197. Dillman D. Mail and Telephone Surveys, the Total Design Methods. New York: John Wiley and Sons, 1978