



DEPARTMENT OF BIOLOGY

Capstone Proposal

2011

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Title: Affect of serum 25-hydroxyvitamin D levels on the onset and progression of Multiple Sclerosis

Institution: Northeastern University

Date: 19 April 2011

Amount requested: \$334,897

Specific Aims

The long term goals of this research are to determine genetic and environmental factors that cause Multiple Sclerosis (MS) in order to develop a cure. MS is the most common cause of neurological disability in the world. MS symptoms are caused by the demyelination of nerve axons in the central nervous system (CNS) by the body's own immune system, resulting in a variety of physical manifestations. Symptoms include weakness or decreased dexterity of limbs, disturbances of vision (optic neuritis, diplopia), ataxia, Lhermitte's symptom, heat sensitivity, bladder dysfunction, fatigue, vertigo, impairment of memory, and depression. Most patients experience a progressive form of the disease.

MS has been shown to be a multifactorial and multifaceted disease. The complex interactions between genetic predisposition and exposure to environmental factors have made it difficult to determine the exact cause or a cure. An interesting and well-documented relationship has been discovered between MS and geographic distribution. The prevalence of MS cases increases dramatically with populations that are located farther from the equator. This suggests that the onset of MS is affected by ultra-violet (UV) radiation. Sunlight, or UV, exposure directly affects the natural production of vitamin D in humans. Vitamin D derivatives have been linked to calcium, neural, and immune homeostasis. Vitamin D has been shown to play a major role in the CNS and been proven efficacious in treating animal models of MS. The exact role of vitamin D metabolites in MS onset and progression is, however, still unclear.

Accelerate Cure Project (ACP) is a non-profit organization dedicated to finding a cure of MS and other related neurological diseases. ACP focuses on this goal by coordinating research and data between researchers, mainly through the creation of a biorepository of blood samples from patients and controls. This large scale, multidisciplinary repository was created to aid in research focuses on increasing the pace and impact of MS research, identifying markers to predict disease progression, and predicting treatment responses. **It is hypothesized that analysis of this repository data will lead to the identification of novel and unknown characteristics in MS patients, specifically in relation to vitamin D and MS onset and progression. It is also hypothesized that different vitamin D levels correlate with different presentations of MS.** This study will focus on early forms of MS, specifically patients within the first three years of diagnosis with relapsing-remitting or primary progressive forms of MS. Vitamin D levels in samples located in the repository have not been previously studied. The purpose of this analysis is an exploratory study of the relationship between vitamin D levels and MS using samples from the ACP repository.

Specific Aim 1: Obtain samples and corresponding patient data from ACP repository

A report will be submitted to the Repository Oversight Committee at ACP detailing the type of research and type of samples needed for experimentation. Along with serum samples from patients with relapsing-remitting MS, patients with primary progressive MS, and control subject samples, patient case report forms containing demographic, genetic, environmental, and medical information of the patients will also be requested. This information will aid in identifying specific factors that can affect vitamin D levels and analysis of results, such as: time of year samples was taken, gender, geographical location of patient, genetic lineage of patient, and other sources of vitamin D (i.e. vitamin D supplements).

Specific Aim 2: Determination of vitamin D levels: Serum 25-hydroxyvitamin D assays

Vitamin D levels from patients and control subjects will be analyzed using a radioimmunoassay measuring serum levels of 25-hydroxyvitamin D. Results will be examined using a multifactorial analysis that will take into account age, gender, race, type of MS, season sample was obtained, and latitude of residence.

Background and Significance

Multiple sclerosis (MS) is the most common cause of neurological disability in the world (Hauser et al., 2006). It is also one of the least well-known diseases, in terms of history, causes, treatments, and cures. Once thought of as a strictly autoimmune disease, MS has, more recently, proved to be much more complex, involving neuropathology, genetics, and environmental triggers. MS can progress very differently patient to patient, which has raised the question to whether MS is several diseases or one disease with multiple phenotypes (Confavreux et al., 2006). There is no cure for MS.

MS presents with a variety of clinical features

MS presents in patients very differently, causing a variety of symptoms and disability, with the majority experiencing progressive disability (Hauser et al., 2006). MS symptoms are caused by the demyelination of nerve axons in the central nervous system (CNS), either brain or spinal cord, by the body's own immune system, leading to myelin and axonal injury and changes in immunological and neurological homeostasis (Hauser et al., 2006). The peripheral nervous system is not affected (Hauser et al., 2006). The diagnosis of MS is based on a few key features: (para)clinical examination and evaluation, separation of new lesions in time and space within the CNS or spinal cord (visualized by MRI), and positive evidence of IgG bands in cerebral spinal fluid (McDonald et al., 2001; Polman et al., 2005).

The course of MS follows two general forms: relapses (presentation of demyelination) with complete or partial remission (resolving of symptoms and CNS inflammation) or relapses with progression (worsening of symptoms caused by the failure of the CNS to resolve inflammation) (Confavreux et al., 2006). Each clinical episode, or relapse, tends to present with 10 new lesions, visible by MRI (Youl et al., 1991). The onset of MS varies patient to patient and early symptoms sometimes go undetected.

Symptoms and Treatments for MS

Symptoms of neurological demyelination present as weakness or decreased dexterity of limbs, disturbances of vision (optic neuritis, diplopia), ataxia, Lhermitte's symptom, and heat sensitivity. Patient's with later stages of MS experience bladder dysfunction, fatigue, vertigo, impairment of memory, and depression (Hauser et al., 2006).

Current therapies offered to MS patients are just that; there is no cure available. Steroid treatments are usually prescribed to counter an immediate relapse by reducing inflammation and resolving symptoms if possible. Common therapies provided to patients to modulate future relapses include interferon β , glucocorticoids, and anti-TNF biologics (Hauser et al., 2006). These therapies attempt to decrease clinical relapses and slow the progression of disability, although their effects are only partial and provisional (Hauser et al., 2006).

MS presents with specific clinical symptoms

Lublin and Reingold (1996) have outlined several different presentations of MS that are currently acknowledged by clinicians: relapsing-remitting, primary progressive, secondary progressive, and progressive relapsing. Each presentation differs in age of onset, number and frequency of relapses, level of recovery or remission, degree of disability following attack, and age/gender of majority of patients.

The majority of patients who are first diagnosed present with the relapsing-remitting form of MS.

The majority of relapsing-remitting patients are young women. Neurological attacks occur one to two times per year and are followed by gradual improvements over several months with no permanent disability (Confavreux et al., 2005). **About 15% of newly diagnoses patients present with primary progressive MS.** This is a purely progressive form of the disease with patients presenting with infrequent relapses and no remission stages (Lublin et al., 1996; Hauser et al., 2006). These patients have a fairly even gender distribution and tend to be older. Secondary-progressive MS presents with an initial relapsing-remitting phase followed by a progressive course (Confavreux et al., 2005). Progressive

relapsing is characterized by distinct relapses years or decades after onset combined with the presence of a progressive course since the onset (Lublin et al., 1996; Confavreux et al., 2005).

MS is a complex, multifactorial disease

MS has proven to be a multifactorial and multifaceted disease. The factors that lead this neurological disease are unknown. MS has displayed complicated interactions between genetics and environmental factors (Cantorna, 2006). Factors that have all been linked to the onset of MS include: age, gender, family history, infections (viruses), immunizations, race, geographical location, and other autoimmune diseases.

Ebers et al. (1998) has shown a genetic clustering in MS patients, pointing to a genetic risk factor. The strongest genetic factor identified is the *HLA-DRB1* gene (Hauser et al., 2006). However, twin pair studies have shown that a genetic predisposition to the disease is not enough to develop the disease (Ebers et al., 1986; Hayes, 2000), suggesting that MS involves the polymorphic interaction of multiple genes and environmental triggers (Ebers, 1994; Compston, 1997; Noseworthy, 1999; Hauser et al., 2006).

Environmental factors that have been linked to MS include viruses, specifically Epstein Barr virus (Ascherio et al., 2001; Cepok et al., 2005; Levin et al., 2005), nutrition (VanAmerongen et al., 2004) and exposure to unknown, modern environmental factors (Hauser et al., 2006). Gender (female) and race (white) have been shown to increase risk for developing MS in predisposed patients (Hayes, 2000; Hauser et al., 2006). Clinical data has demonstrated an increased risk for patients based on geographical distribution. **Populations located farther from the equator, especially with northern European ancestry, have demonstrated a higher prevalence of, and predisposition to, MS (Hauser et al., 2006), generating a suggested link to ultraviolet radiation (UV) exposure and vitamin D levels.**

Vitamin D acquisition and production

Vitamin D is a precursor to lipid soluble active hormone 1,25-dihydroxyvitamin D. Vitamin D is acquired through two environmental sources, sunlight exposure and diet intake (De Luca, 1993). Vitamin D is produced by the dermal skin layer after sun exposure (Holick, 2003). Ultraviolet-B radiation converts provitamin D₃ to previtamin D₃ and then to the inactive vitamin D₃ (Holick, 1987; Webb and Holick, 1988; VanAmerongen et al., 2004). Vitamin D₃ is stored in fat or converted to 25-hydroxyvitamin D, the major form of vitamin D circulating in the body (Smolders et al., 2008b). 25-hydroxyvitamin D is stored in the liver or converted to its active metabolite form, 1,25-dihydroxyvitamin D (Smolders et al., 2008b). 25-hydroxyvitamin D has a serum half-life of 10 days to three weeks (VanAmerongen et al., 2004) and the concentration of 25-hydroxyvitamin D in serum is considered an accurate determination of vitamin D

levels (FNB, 1997; VanAmerongen et al., 2004), as levels of 25-hydroxyvitamin D are affected by both sunlight and dietary intake (Holick, 2004a) and has a longer half life compared to 1,25-dihydroxyvitamin D (Kumar, 1986). The fluctuation of sunlight hours between seasons has a direct effect on serum levels (Smolders et al., 2008a). 1,25-dihydroxyvitamin D induces the breakdown of 1,25-dihydroxyvitamin D and 25-hydroxyvitamin D into calcitroic acid (Esvelt, 1981). Vitamin D can be acquired through diet in two forms, via vegetables (D2, ergocalciferol) and animals (D3, cholecalciferol) (Smolders et al., 2008). Vitamin D supply from diet is considered a secondary source when enough sunlight exposure is available (Fraser, 1995; Holick, 1995; Vieth, 1999; Heaney et al., 2003; Holick, 2004b).

Vitamin D is transported to cells by vitamin D-binding protein (DBP) and albumin. 1,25-dihydroxyvitamin D enters cells by dissociating from DBP at the cell membrane, diffusing across, and binding to the vitamin D nuclear steroid receptor (VDR) (Segaert et al., 1998; Brown et al., 1999). The amount of VDR is directly affected by the serum concentration of 25-hydroxyvitamin D. VDR moves between the cytoplasm (VDR) and the nucleus (nVDR). VDR has been shown, via co-activation with Smad, to stimulate the TGF-beta signaling pathway (Yanagisawa et al., 1999). Activated nVDR, a nuclear steroid hormone, forms a heterodimer with its ligand and another nuclear receptor, retinoic acid receptor (RXR), which then binds to a vitamin D responsive element (VDRE) (Yu et al., 1991; Kliewer et al., 1992; MacDonald et al., 2001). VDRE is a promoter sequence that can induce or suppress transcription of VDRE-mediated genes (Brown et al., 1999). These genes are associated with calcium homeostasis, cell differentiation, and immune responses (Casteels et al., 1995).

Vitamin D is involved in important regulatory functions

Originally, vitamin D was linked only to the regulation of calcium homeostasis and bone formation (Cantorna, 2006). However, vitamin D is involved in many other important normal regulatory functions. VDRs have more recently been found in a variety of other cell types, including pancreatic β -cells (Casteels et al., 1995; DeLuca and Cantorna, 2001), chondrocytes, oligodendrocytes (and other CNS cells) (Garcion et al., 2002), macrophages (Koeffler et al., 1984; Veldman et al., 2000), and T-lymphocytes (Bhalla et al., 1983; Provvedini et al., 1983; Veldman et al., 2000; Adorini, 2002; Mahon et al., 2003). **As such, vitamin D is now believed to play an important role in the immune and nervous systems.** 1,25-(OH)₂D has been shown to have a direct effect on immune cells and cell cycle progression (Bouillon et al., 1995; Casteels et al., 1995; Cantorna et al., 1996, 1998, 1999; Hayes et al., 1997; Nashold et al., 2000, 2001; Gregori et al., 2001; Griffin et al., 2001; Cantorna, 2006; Smolders et al., 2008a). Smolders et al. (2008a) suggests not only that vitamin D plays a role in shifting immune cells to a more anti-inflammatory profile, but also that a majority of vitamin D action, metabolism, and

catabolism take place in the CNS. Changes in normal 25-hydroxyvitamin D serum concentration, a reflection of overall vitamin D levels, has been linked to vitamin D deficiency, chronic renal failure, sarcoidosis, tuberculosis, rickets, insulin-dependent diabetes mellitus, rheumatoid arthritis, intestinal bowel disorder, and multiple sclerosis (Garacion et al., 2002).

Current knowledge of MS and vitamin D

Although the exact relationship is still unclear, there is epidemiological, experimental, and immunological data connecting vitamin D metabolites to MS (Hayes et al., 1997; Embry et al., 2000). The main reason for studying the relationship between vitamin D levels and MS is due to the clear geographical distribution of MS cases. There is a strong increase in case prevalence further from the equator (Hayes, 2000; Smolders et al., 2008), thought to be related to decreased UV levels and exposure (Acheson et al., 1960; van der Mei et al., 2001). Munger et al. (2006) has shown that low 25-hydroxyvitamin D levels in adolescence are associated with higher MS incidence in white American populations. Vitamin D has shown a protective effect on reducing the risk of MS, although results are inconclusive (Freedman et al., 2000; van der Mei et al., 2003).

Vitamin D has also been shown to have neurological and immune effects. VDR has been shown to be expressed in CNS cells (Overbergh et al., 2000; Zehnder et al., 2001) and immune cells, such as lymphocytes, monocytes, and antigen presenting cells (Provvedini et al., 1983; Veldman et al., 2006). Treatments with 1,25-dihydroxyvitamin D have been shown to inhibit Th1-mediated immune responses (Cantorna, 2006).

Vitamin D has been shown to produce positive effects on the animal model of MS, experimental allergic encephalomyelitis (EAE). Administration of 1,25-dihydroxyvitamin D or its precursor, vitamin D₃, to EAE mouse models have prevented symptoms, halted progression, resolved inflammation, and reduced disability scores (Cantorna et al., 1996; Nataf et al., 1996; Nashold et al., 2001; Van Etten et al., 2003; Spach et al., 2005; Muthian et al., 2006).

It is unclear what role vitamin D metabolites play in MS onset and progression. Whether MS patients require higher levels of vitamin D, have genetic defects in precursor metabolism, or defects in chaperone proteins (DBP or VDR) is unknown. It is clear, however, that this relationship, specifically levels of vitamin D metabolites in MS patients of varying stages of progression, needs further investigation.

Research Design

This study intends to further research into the causes and onset of Multiple Sclerosis (MS), specifically the role of vitamin D in disease morphology. In this study, serum vitamin D levels in patients within three years of being diagnosed with low disability forms of MS will be compared with control subject samples. This specific range of patients will attempt to create results that will reflect a significant characteristic of a specific group of MS patients.

Specific Aim 1: Obtain samples and corresponding patient data from ACP repository

Patient and control subject serum samples will be acquired from Accelerated Cure Project's biorepository. This biorepository was created specifically to aid researchers studying demyelinating diseases, including MS. The repository contains a significant collection of quality samples and patient data to which researchers have access. Use of the Accelerated Cure Project (ACP) repository will allow for a larger study, with a larger sample size, to be conducted than would be possible on our own. The repository contains samples from patients across the United States from ACP's ten collection sites, resulting in a large and diverse population to examine. These large sample numbers will not only make the results more statistically significant, but will also allow for specific selection of samples/patients that fit our study criteria. All entries in the ACP repository contain not only patient blood samples, but case report forms for each patient with genetic, family, lifestyle, and disease history that will be instrumental in study design and result analysis. All results of this study will be submitted into the repository and incorporated into the database in order to further cooperation in researching a cure for MS (ACP, 2011). There has been no characterization of vitamin D serum levels in any of the repository's samples to date.

Specific Aim 2: Determination of vitamin D levels: Serum 25-hydroxyvitamin D assays

Vitamin D levels in patient and control subject samples will be determined by measuring serum levels of 25-hydroxyvitamin D, the major form of circulating vitamin D. Because 25-hydroxyvitamin D levels are affected by UV exposure and dietary intake, 25-hydroxyvitamin D is considered an accurate measure of vitamin D levels (VanAmerongen et al., 2004). Serum 25-hydroxyvitamin D will be extracted by acetonitrile and the amount of 25-hydroxyvitamin D will be measured using a ¹²⁵I labeled radioiodinated tracer, as described by Hollis et al. (1993). Results will be evaluated using a multifactorial statistical analysis, in order to take into consideration factors such as time of year samples was taken, gender, geographical location of patient or control subject, and genetic lineage of patient or control subject.

Serum vitamin D levels in the ACP biorepository have yet to be characterized. We anticipate the results will reflect a physiological difference in recently diagnosed patients with relapsing-remitting or primary

progressive forms of MS when compared with similar control subjects. Even if no statistically significant difference in serum vitamin D levels is found, the results of this study will still be incorporated into the ACP repository database to be used for future MS research.

Proposed Budget

Budget Item	Year 1	Year 2	Total
PI Salary	\$17,000	\$17,000	\$34,000
Post-doctoral Research Associate	\$37,000	\$39,000	\$76,000
Research Assistant	\$20,000	\$20,000	\$40,000
Total Salaries	\$74,000	\$76,000	\$150,000
Fringe Benefits	\$16,380	\$16,930	\$33,310
<i>Supplies & Services</i>	\$10,000	\$10,000	\$20,000
Travel	\$5,000	\$5,000	\$10,000
<i>Permanent Equipment</i>	\$0	\$0	\$0
Subtotal Direct Costs	\$105,380	\$107,930	\$213,310
<i>Other Costs</i>	\$0	\$0	\$0
Total Direct Costs	\$105,380	\$107,930	\$213,310
Indirect Costs (Overhead)	\$60,067	\$61,520	\$121,587
Indirect Cost Rate (%)	57%	57%	
Total	\$165,447	\$169,450	\$334,897

Budget Justification

Senior Personnel and Fringe Benefits. The PI estimates spending about 25% of time on the proposed work and asks for \$17,000 salary plus fringe benefits at a rate of 27.5%.

Other Personnel and Fringe Benefits. Support for a Post-doctoral Research Assistant and Research Assistant is requested to carry out the radioimmunoassays and analyze results. Annual stipends for a Post-doctoral Research Assistant are \$37,000 in Year 1 and \$39,000 in Year 2, with a 27.5% fringe benefit rate. A Research Assistant will receive an annual stipend of \$20,000 in Years 1 and 2, with a 7.65% fringe benefit rate.

Supplies & Services. \$10,000 per year is requested for laboratory maintenance and supplies for storing samples, performing the radioimmunoassays, and analyzing results.

Travel. The PI is requesting \$5,000 in travel funds to visit various Accelerated Cure Project collection sites to meet with patients and the site coordinators in Year 1. Travel funds in Year 2 will be allocated towards attending MS conferences to present results.

Permanent Equipment. No permanent equipment over \$5000 is requested.

Indirect Costs. Modified Indirect Costs at Northeastern University are 57% of the total Direct Costs.

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