CASE REPORT

Quantifying Environmental Risk Factors for Multiple Sclerosis in Discordant Monozygotic Twins: A Case Report

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ABSTRACT

Relative contribution of genetic and environmental risk factors in complex disorders is widely explored through discordant identical twins. Multiple sclerosis is a demyelinating disease of the central nervous system in which the interplay of genetic and environmental risk factors define the disease pathogenicity. Robust epidemiological studies in different populations suggested that active levels of serum vitamin D and viral load implicate in MS pathogenicity and severity. In order to refine non-shared components of susceptibility factors in MS, we investigated the role of serum 25-hydroxyvitamin D and viral infection in a pair of identical twins remained discordant for MS during the course of 5 years follow up. Here we report serological finding regarding the viral load and serum 25-hydroxyvitamin D level in a pair of discordant monozygotic twins. Based on our observation, lower levels of serum 25-hydroxyvitamin D and higher anti-viral IgG titre was consistent with the disease statues in the affected sib.

Keywords: Multiple Sclerosis, Identical Twins, Vitamin D, Viral Infection

INTRODUCTION

The relative contribution of genes and the environment in human common complex disorders is widely explored through identical twins. Multiple sclerosis is a complex neurodegenerative disease in which the interplay between inflammatory and neurodegenerative processes results in demyelination of neurons in the central nervous system. The interplay of nurture (environmental risk factors) and nature (genetic background) in the context of multiple sclerosis has long been debated. Substantially increased risk of recurrence in relatives of affected individuals implicates a role for genetic factors.[1,2] Linkage studies have shown that Major Histocompatibility Complex (MHC) region on chromosome 6p21 plays a major role in susceptibility to the disease.[3-16] In addition, genetic association studies have identified more than 50 additional risk loci each with a modest effect on risk.[17-22] While genetic factors pose a clear -and relatively strong- effect in susceptibility to MS, epidemiological studies underpin the critical role of environment in the development of MS.[23-25] A growing body of work suggests that the level of serum 25-hydroxyvitamin D (25-OH-D)[26-30] and Epstein-Barr virus (EBV) infection[31-38] collectively contribute to the pathogenesis of the disease. In order to control for genetic risk factors and uniquely examine the role of vitamin D and infectious agents on the onset of disease we investigated the status of serum 25-hydroxyvitamin D (25-OH-D) and antibody titres against EBV, CMV and HHV-6 in a pair of discordant monozygotic twins.

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METHOD

A pair of discordant MZ twins were recruited from our registry of MS patients at the Kashani Hospital. The family pedigree and clinical features of the sib are provided in the Figure 1 and Table 1. For the affected individual McDonald's criteria of MS diagnosis was carefully fulfilled. Absence of MS complications in the health sib was also investigated through clinical evaluation and confirmed by MRI and immunologic assay. The subjects were fully informed about the study procedure and written consent was obtained.

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5 mL of whole blood was collected from each subject and samples were immediately transferred to laboratory for immunologic and biochemical assays.

The level of serum 25-hydroxyvitamin D (25-OH-D) was measured using commercially available IDS-iSYS 25-hydroxyvitamin D kit (immunodiagnostic systems Ltd., UK.) The level of serum vitamin D was accordingly labelled into four categories:

(1) toxicity level (>100ng/mL)
(2) vitamin D sufficiency (30-100ng/mL)
(3) moderate vitamin D deficiency (10-30ng/Ml), and
(4) severe vitamin D deficiency (<10ng/mL). In order to robustly control for additional confounding factors during the course of study a descriptive questionnaire was prepared and used to eliminate bias concerning the use of nutritional supplements, tanning beds and sunshine vacations.

Presence of anti EBNA-1 and anti-CMV IgG antibodies was investigated using commercially available ELISA technique by Vircell MICROBIOLOGISTS Co. (Granada, Spain). Immune response to the EBV-encoded nuclear antigen-1 considered significant if the anti EBNA-1 titre was greater than 20U/ml. Cytomegalovirus (CMV) infection was explored through the same procedure and titres greater than 0.6 reported positive.

RESULT

Elevated serum levels of 25-hydroxyvitamin D (25-OH-D) was observed in the healthy sib, whereas in the affected patient, 25-OH-D level was consistently lower compared to that of unaffected pair (11ng/ml versus 39ng/ml). According to the quantifying categories the serum 25-OH-D level in the affected patient falls into the moderate deficiency level while vitamin D concentration in the healthy sib represents the sufficiency spectrum.

Intriguingly, a selective increase of EBNA-1/CMV-specific immune response was also observed in the affected sib. While anti EBNA-1 IgG titre in the healthy individual was 44.8 U/mL, a value of 109 U/mL was recorded for the affected sib. The immune response against CMV was also reported positive in the affected subject. A summary of serological results is provided in the Table 2.

DISCUSSION

Monozygotic twins are of particular importance in respect to variability of phenotypic expression, pathogenic mechanism and epigenetic differences. There is compelling evidence that both genes and environment implicate in the pathogenesis of multiple sclerosis. Discordant monozygotic twins significantly

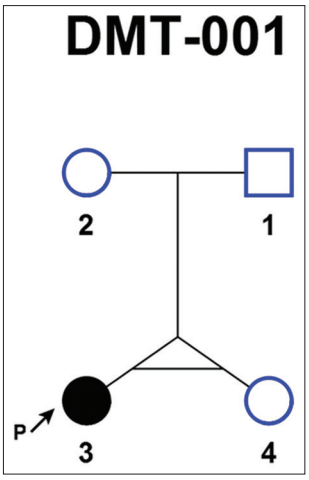

Figure 1: Pedigree of subject family

Table 1: Clinical features of MZ twin

Variables	Affected Sib	Normal Sib
Age	19	19
Sex	Female	Female
EDSS	1.5	-
Disease type	RR	-

EDSS: Expanded disability scale score; RR: Relapsing- remitting

Table 2: Assay results from the laboratory report

Test	MZ twin	
	Affected	Unaffected
Vitamin D (25 OH)	11 ng/mL	39 (ng/mL)
Anti EBNA-1 (IgG)	109 (U/mL)	44.8 (U/mL)
C.M.V Ab (IgG)	1.4 (IU/mL)	<0.2 (IU/mL)
HHV-6	Negative	Negative

contribute to our understanding of disease pathogenesis and variability of disease phenotype. Association of major histocompatibility complex human leukocyte antigen- 1-β-D-ribofuranosyl-benzimidazole 1 (HLA-DRB1) locus has been consistently replicated in genetic studies.[39-41] In addition, more than 50 additional non-MHC loci has been also identified that contribute to the disease risk,[42] among them vitamin D receptor and 1-α-hydroxylase (*CYP27B1)* with functional significance in vitamin D synthesis. The causative variant in CYP27B1 substitute arginine to histidine at position 389 (R389H) and thereby impedes the 25- hydroxyvitamin D conversion to its biologically active form (1,25 dihydroxyvitamin D).[43] It has also been suggested that the active form of vitamin D (calcitriol) implicates in the regulation of more than 80% of MS associated genes including HLA-DRB1. In a 2010 study by Baranzini *et al.* genetic, epigenetic and transcriptomic differences were investigated in 3 pairs of discordant monozygotic twins. According to this study no consistent differences in the sequence of genomic DNA from $CD4^+$ cells or gene expression were found. DNA methylation analysis also failed to demonstrate a significant difference between the subject pairs. In order to refine non-shared components

that influence the susceptibility to MS, we studied the role of environmental risk factors in a pair of MZ twins. Critical association of low serum concentration of 25- OH-D with prevalence[44-46] and fluctuations of disease activity[47,48] has been well documented in clinical observations. In view of this correlation, we measured the 25-OH-D scrum concentration at the relapse stage and in a follow up course of 2 years after disease onset. In order to minimize the inter-individual bias and experimental error in vitamin D measurements, assays conducted in the beginning of each season for follow up course of two years. Chronic low concentration of vitamin D was consistent with the disease status in the affected sib. Interestingly, increased titer of anti-EBNA1 was evident in the affected individual. EBNA-1 is a dimeric protein with Gly-Ala repeats that actively contribute to the virus latency by impairing antigen processing and MHC class 1 restricted antigen presentation.[49] Association of EBV with the disease aetiology and/or activity has long been debated. About 99% of MS patients are seropositive for EBV[23] and It has been suggested that individuals with a history of infectious mononucleosis (IM) are more susceptible to MS. Given that no history of IM was recorded for the twins, we believe higher anti-EBNA1 titer in the affected sib can be the result of impaired immunity due to chronic vitamin D deficiency. However speculations on the role EBV infection as a causative or bystander agent should be interpreted with caution.

EBV-specific immune response usually keeps EBV infection under tight control by eliminating proliferating and lytically infected B-Cells. $CD8^+$ T-cells critically implicate in this pathway.[50] In a recent study by Van der Windt *et.al*, it has been suggested that $CD8^+$ memory T Cells maintain a substantial spare respiratory capacity (SRC) in their mitochondria. This extra mitochondrial capacity assist $CD8^+$ memory T Cells to persist longer and provide a robust immune response upon antigen re-encounter. Unlike $CD8^+$ resting and $CD8^+$ effector T cells that primarily employ, $CD8^+$ memories T Cells represent an extra mitochondrial capacity for energy production. It should be noted that this study has not intended to draw any conclusions about either the sole effect(s) of environmental factors or genetics on disease causality, rather, it indicates that the role of environmental must be scrutinized in future studies.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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