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Mini Review

Genetic aspects of vitamin D receptor and metabolism in relation to the risk of multiple sclerosis

Lucia Krizova[1], Branislav Kollar[1], Daniela Jezova[2] and Peter Turcani[1]

[1] *1st Department of Neurology, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic*
[2] *Institute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovak Republic*

Abstract. Recent findings suggest that polymorphisms in vitamin D pathway genes are candidates for association with multiple sclerosis susceptibility. It has been now well demonstrated that vitamin D has immunomodulatory functions that may be favorable for reduction of multiple sclerosis risk. Current research has been focused on identification of new variants of genes involved in vitamin D pathway, namely in vitamin D receptor and enzymes of vitamin D metabolism. These variants have been intensively studied as possible genetic predictors of both vitamin D levels and the risk of multiple sclerosis. Considering the findings available up-to-date, we may recognize two groups of genetic variants. The first group of genes was found to predict vitamin D levels but not the risk of multiple sclerosis. The second group of genetic variants is represented by promising genes predicting vitamin D levels as well as the risk of multiple sclerosis. A strong association with increased risk of the disease has been observed for a rare variant in the CYP27B1 gene encoding a vitamin D-activating enzyme. Observed interaction between genetic and epidemiological findings brings the rationale for supplementation trials of vitamin D. Although promising effects of vitamin D supplementation have emerged, the results obtained so far are inconclusive and the real therapeutic significance of vitamin D supplementation remains to be elucidated.

Key words: Multiple sclerosis risk — Genetic variants — Vitamin D deficiency — Vitamin D supplementation

Introduction

Multiple sclerosis is a neurological disorder characterized by chronic inflammation and demyelinization of the central nervous system (Kipp et al. 2012). The main pathological feature is the damage to the myelin sheaths around axons due to inflammatory processes. Constant neuroinflammation as well as neurodegenerative processes in later stages of the disease lead eventually to axonal loss in the brain and spinal cord (Bartanusz et al. 2011; Franklin et al. 2012; Miller 2012; Buc 2013). Depending on the amount of damage of affected nerves, symptoms may vary widely. The most common symptoms include visual symptoms (double vision, loss of vision), motor symptoms (muscle weakness), sensory functions (changes in sensation, numbness, paresthesia, tingling), vestibular functions (vertigo, ataxia, difficulty with coordination and balance), bowel, bladder and sexual symptoms, as well as other symptoms, such as autonomic nervous system or cognitive dysfunction (Jongen et al. 2012; Nick et al. 2012; Sá 2012). Four disease courses have been identified in multiple sclerosis; namely relapsing-remitting, primary progressive, secondary progressive and progressive relapsing. Although there is still no cure for multiple sclerosis, there are many effective therapeutic strategies available (Castro-Borrero et al. 2012; Weber et al. 2012; Filippini et al. 2013).

Multiple sclerosis is generally thought to be aetiologically heterogenous disease in which both genetic and environmental factors play a significant role (Ascherio et al. 2012; Krizova et al. 2013). In the past years, major advances have been made in the genetics of multiple sclerosis. The analysis of human genome has allowed identifying a great number of risk gene loci associated with increased risk of multiple sclerosis. The genome-wide association studies (GWAS) performed up to

Correspondence to: Lucia Krizova, 1st Department of Neurology, Faculty of Medicine, Comenius University, Mickiewiczova 13, 813 69 Bratislava, Slovak Republic
E-mail: lucia.krizova@gmail.com

date resulted in the identification of susceptibility loci for multiple sclerosis, taking the total number of risk alleles to more than 50 (International Multiple Sclerosis Genetics Consortium; Wellcome Trust Case Control Consortium 2, 2011; ANZ gene 2009; Baranzini and Nickles 2012). The majority of genes associated with multiple sclerosis risk have immune-related functions. It has been suggested that vitamin D regulates a high number of genes associated with multiple sclerosis (Ramagopalan et al. 2010) including major histocompatibility complex class II „HLA-DRB1*1501" allele (Ramagopalan et al. 2009). At the interface between genes and environment, a role of vitamin D in the pathogenesis of multiple sclerosis has been proposed. Evidence for vitamin D deficiency as a risk factor tends to accumulate. Previous studies have shown that serum vitamin D levels are genetically influenced. Polymorphisms in vitamin D pathway genes are therefore candidates for association with multiple sclerosis susceptibility.

Vitamin D and its immunomodulatory effects

Bioactive vitamin D is a steroid hormone that plays an important role in a wide variety of biological processes including bone metabolism and calcium homeostasis, modulation of the immune response and regulation of cell proliferation and differentiation (Holick 2003). Vitamin D3 can be synthesized by humans in the skin upon exposure to sunlight, or it can be obtained from the diet. The precursor of vitamin D 7-dehydrocholesterol is generated in the skin. After exposure to ultraviolet-B radiation it is converted to cholecalciferol, a biologically inert substance which requires further hydroxylation. First it is metabolized to 25-hydroxyvitamin D – calcidiol (25(OH)D) in the liver and then is turned into biologically active form 1,25-dihydroxyvitamin D – calcitriol ($1,25(OH)_2D$) by the enzyme 25-hydroxyvitaminD-1-alpha-hydroxylase in the kidney (Figure 1). This enzyme is coded by *CYP27B1* (cytochrome P450 family 27 subfamily B peptide 1) on chromosome 12q13.1-3. The actions of $1,25(OH)_2D$ are mediated by the nuclear vitamin D receptor. Vitamin D receptors are present not only in the intestine and bone, but also in a wide variety of other tissues, including the brain, heart, pancreas, activated T and B lymphocytes and antigen presenting cells (Holick 2003). Recently reported immunological findings have shown that vitamin D significantly influences regulatory T lymphocyte cells (Table 1). It has

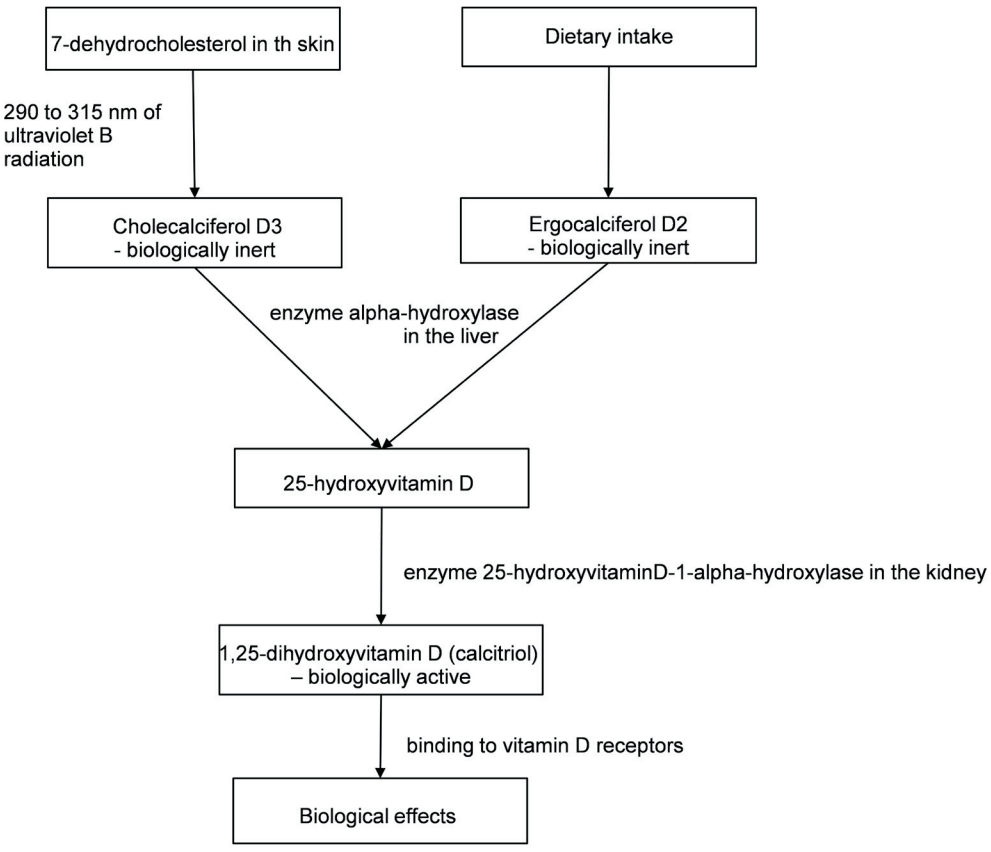

Figure 1. Vitamin D metabolism (modified according to Chhabra 2012).

Table 1. Immunomodulatory effects of vitamin D (modified according to Correale et al. 2009)

Vitamin D	– has an important role in shaping the development of T cell responses, inducing T cells with immunosuppressive properties – inhibits the proliferation of human CD4+ T cells and MBP peptide-specific T cell lines
Effects of vitamin D on cytokine secretion by CD4+ T cells	– enhances IL-10 secreting cell development – inhibits IL-6, IL-12 and IL-17 producing cell development – induces CD4+CD25+FoxP3+ regulatory T cell through IDO-mediated pathway – suppresses the production of IFN-gama by CD4+CD25- T cells
Effects of vitamin D on the expression of VDR on CD4+ T cells	– upregulates expression of VDR mRNA in both un-stimulated and activated CD4+ T cells

VDR, vitamin D receptor; IDO, indoleamine 2,3-dioxygenase.

been demonstrated that $1,25(OH)_2D$ has a suppressive role in the immune system, decreasing T cell and dendritic cell maturation, proliferation and differentiation, shifting the balance between T-helper 1 (Th1) and Th2 cells in favor of Th2 cells. Furthermore, $1,25(OH)_2D$ inhibits the proliferation of both CD4+ T cells and myelin basic protein specific T cells, enhances the development of IL-10 producing cells, and reduces the number of IL-6 and IL-17 secreting cells (Correale et al. 2009). Since T cells express alpha1-hydroxylase constitutively, they are able to metabolize 25(OH)D into biologically active $1,25(OH)_2D$. Autoreactive T cells are thus capable of metabolizing vitamin D into its active form, which results in inhibition of T cell function. Vitamin D receptor expression may be induced by $1,25(OH)_2D$ in both activated and resting T cells. $1,25(OH)_2D$ increases the expression of indoleamine 2,3-dioxygenase, mediating significant increase in the number of CD4+CD25+ T regulatory cells (Correale et al. 2009).

Vitamin D deficiency and multiple sclerosis risk

One of the environmental factors that have been implicated in multiple sclerosis is the deficiency of vitamin D. In a number of studies investigating serum levels of vitamin D in multiple sclerosis, an insufficiency was observed in the great majority of patients, including the earliest stages of the disease (Pierrot-Deseilligny et al. 2010). Patients with multiple sclerosis have lower levels of 25(OH)D in blood as well as reduced bone mass compared to healthy controls (Nieves et al. 1994). On the other hand, high circulating levels of 25(OH)D are associated with a lower risk of multiple sclerosis (Munger et al. 2006). This is consistent with findings of Salzer et al. (2012) which support the association between high 25(OH)D levels (≥75 nmol/l) and decreased risk of developing multiple sclerosis. Interesting results have been acquired by comparison of vitamin D levels in multiple sclerosis patients suffering a relapse and patients being in a remission. Vitamin D levels in patients suffering a relapse were lower than during remissions. In contrast, primary progressive patients showed similar values to controls (Correale et al. 2009). Based on these findings, an important role of vitamin D in T cell homeostasis during the course of multiple sclerosis has been proposed.

Several, though not all, studies have provided supporting data on a protective role of vitamin D intake on the risk of developing multiple sclerosis. The intake of vitamin D from supplements in adult women was found to be inversely associated with the risk of multiple sclerosis (Munger et al. 2004). Inconsistent findings were however found in cohorts of adolescents (Munger et al. 2011). Further evidence comes from a study in which the effect of gestational vitamin D on adult onset of multiple sclerosis was examined. High vitamin D intake during pregnancy was associated with a lower risk of developing multiple sclerosis in offspring daughters (Mirzaei et al. 2011). Some studies however found no association between gestational 25(OH)D levels and multiple sclerosis risk in the offspring (Salzer et al. 2012). A protective effect of vitamin D on multiple sclerosis is also supported by a reduced risk associated with frequent sun exposure. Low maternal exposure to ultraviolet radiation in the first trimester was associated with increased subsequent risk of developing multiple sclerosis in offspring (Staples et al. 2010). This is consistent with the findings of significantly fewer multiple sclerosis patients born in November and more patients born in May as observed in a cohort of Canadian and British patients (Willer et al. 2005).

Genetic predictors of vitamin D levels and their association with multiple sclerosis

Genetic predictors of high vitamin D levels and their association with multiple sclerosis risk have been intensively studied. In the human genome, there are many DNA sequence

variations referred to as "polymorphisms" which can have biological effects. Because of their high frequency in the population they may explain variation in risk of common diseases (Uitterlinden et al. 2004). Based on the findings available it was assumed that individuals carrying genetic variants that predict high 25(OH)D levels have a lower risk of multiple sclerosis (Simon et al. 2011). Recent studies have therefore tried to focus on genetic variants in the vitamin D receptor and on vitamin D metabolism-related genes (Table 2).

Genetic variants predicting vitamin D levels which failed to be confirmed as multiple sclerosis risk predictors

Several genetic variations have been identified in the vitamin D receptor gene. Polymorphisms in the vitamin D receptor gene, Apa-I, Bsm-I, Fok-I and Taq-I were reported to be associated with multiple sclerosis. A recent meta-analysis, however, found no association between these polymorphisms and risk of multiple sclerosis (Huang et al. 2012).

New genes as significant predictors of vitamin D levels have been identified: *GC* (encoding vitamin D-binding protein), *NADSYN1* (encoding nicotinamide adenine dinucleotide synthetase), *DHCR7* (encoding 7-dehydrocholesterol) and *CYP2R1* (cytochrome P450, family 2, subfamily R, polypeptide 1) (Ahn et al. 2010). The association between single nucleotide polymorphisms (SNPs) on these genes and multiple sclerosis risk was further assessed. SNPs in *GC* were predictors of 25(OH)D levels, but not risk of multiple sclerosis, in either HLA-DR15 negative or HLA-DR15 positive individuals. Cheng et al. has brought evidence that *CYP2R1* gene is a key vitamin D 25-hydroxylase. *CYP2R1* encodes enzyme that catalyzes the 25-hydroxylation step in the liver, producing the intermediate 25(OH)D, which is the major circulatory form of the vitamin D. Mutations in gene encoding *CYP2R1* enzyme lead to loss of vitamin D 25-hydroxylase enzyme activity and selective 25-hydroxyvitamin D deficiency (Cheng et al. 2004). There was a suggestion of a decreased multiple sclerosis risk associated with the 'A' allele of *CYP2R1*, which is associated with higher 25(OH)D levels. Statistically non-significant decrease of multiple sclerosis risk was observed among carriers of "A" allele in HLA-DR15 negative, but not in HLA-DR15 positive individuals. The 'C' allele of *CYP27B1* was inversely associated with multiple sclerosis risk; this association appeared stronger among HLA-DR15 negative compared to HLA-DR15 positive individuals. This study suggests that beneficial effect of vitamin D on the risk of multiple sclerosis may be attenuated in individuals carrying the HLA-DR15 risk allele (Simon et al. 2011).

Currently promising genetic variants predicting vitamin D levels associated with the risk of multiple sclerosis

A recent Spanish investigation found an increased risk of multiple sclerosis with the "G" allele of *DHCR7* gene (Alloza et al. 2012). *DHCR7* gene encodes an enzyme 7-dehydrocholesterol reductase that catalyzes the conversion of 7-dehydrocholesterol to cholesterol. Mutations in this gene cause reduction of serum cholesterol levels and elevation of 7-dehydrocholesterol levels. Although the exact role of *DHCR7* in vitamin D metabolism is not known, it is now considered to be a genetic determinant of vitamin D insufficiency as well as one of the risk genes shared between multiple sclerosis and type 1 diabetes (Cooper et al. 2011).

The *CYP27B1* genetic variant

Study of Ramagopalan et al. (2011) has revealed specific genetic variants implicated directly in the pathogenesis

Table 2. Genetic predictors of vitamin D levels and their association with multiple sclerosis risk

Polymorphisms in the VDR gene	Apa-I, Bsm-I, Fok-I, Taq-I	no association with the risk of MS
Vitamin D metabolism-related genes	*GC*	no association with the risk of MS
	NADSYN1	no association with the risk of MS
	CYP2R1	statistically non-significant association with decreased risk of MS only in HLA-DR15 negative
	DHCR7	confirmed association with increased risk of MS
	CYP27B1	confirmed association with increased risk of MS (determinant of vitamin D insufficiency)

VDR, vitamin D receptor; MS, multiple sclerosis.

of multiple sclerosis. By sequencing of all protein-coding regions of the genome in the Canadian population the evidence of association for rs118204009 (R389H) in *CYP27B1* (case carrier frequency 0.67 %) and for additional 4 very rare variants in the same gene (case carrier frequency < 0.08 %) with multiple sclerosis has been demonstrated. *CYP27B1* encodes the vitamin D-activating enzyme 1-alpha-hydroxylase, which converts 25(OH) D to $1,25(OH)_2D$ – the biologically active form of vitamin D. The *CYP27B1* genetic variant rs118204009 causes an arginine-to-histidine change at position 389 of the protein (R389H) and leads to complete loss of enzyme activity and to a significant decrease in concentrations of the active form of vitamin D. Mutations in *CYP27B1* gene are known to cause autosomal recessive vitamin D-dependent rickets type 1. Identified rare mutations in *CYP27B1* had a cumulative frequency of 0.9% in multiple sclerosis cases, whereas they were absent in controls. A significant decrease in concentrations of the active form of vitamin D as the consequence of the mutation in the gene *CYP27B1* results in an increased susceptibility to the disease. The results of this study provide evidence of association between *CYP27B1* functional variations and multiple sclerosis susceptibility (Ramagopalan et al. 2011).

The results of the above mentioned study of Ramagopalan are consistant with previously published data on the role of vitamin D deficiency as a risk factor for multiple sclerosis based on epidemiological findings related to individuals with low circulating 25(OH)D levels (Munger et al. 2006). This remarkable interaction between genetic and epidemiological findings brings the rationale for supplementation trials of vitamin D.

Two recent studies screening for mutations in *CYP27B1* in different populations attempted to confirm the association with multiple sclerosis (Ban et al. 2013; Barrizone et al. 2013). Both of them, however, failed to replicate observations of Ramagopalan and his colleagues. Barizzone et al. genotyped rs118204009 (R389H) in 2608 multiple sclerosis patients and almost 2000 healthy controls from Italy and Belgium. The frequency of this mutation among multiple sclerosis patients has been found comparable to that in controls and significantly less than what would have been expected considering the control frequency and the effect size reported by Ramagopalan (Barrizone et al. 2013).

To investigate whether *CYP27B1* mutations may contribute to multiple sclerosis risk, Ban et al. genotyped rs118204009 (R389H) in 17,073 individuals from the United Kingdom, the United States and from Norway. Mutant allele frequency in affected individuals was 0.07% compared to 0.06% in the control group. The very low frequency of mutant *CYP27B1* alleles and no differences between cases and controls do not support the suggestion that mutant *CYP27B1* alleles influence the risk of developing multiple sclerosis (Ban et. al. 2013).

Clinical trials of vitamin D supplementation

Initial small clinical studies of vitamin D supplementation in patients with multiple sclerosis have reported promising effects. Concretely, an association of high 25(OH)D levels with an improved T cells regulatory function shifting the balance between Th1 and Th2 cells towards Th2 (Smolders et al. 2009) as well as increased proportion of anti-inflammatory IL-10 cytokines and CD4+ T cells has been reported in relapsing-remitting patients after supplementation of high dose vitamin D (Smolders et al. 2010). The identification of vitamin D deficiency as a risk factor in multiple sclerosis provides an opportunity to improve current treatment strategies *via* combination with established treatments for multiple sclerosis. It has been shown that vitamin D as an add on therapy to interferon β-1b reduces MRI disease activity in multiple sclerosis as well as disability progression. There were, however, no significant differences in the annual relapse rate between patients receiving vitamin D and the control group (Soilu-Hänninen et al. 2012). An optimal dose of vitamin D has not been established as yet. Supplementation with a high-dose of vitamin D (6000 IU, targetting 25(OH)D 130–175 nm) in an Australian cohort of patients with multiple sclerosis did not show any therapeutic advantage in comparison with a low-dose vitamin D supplementation (1000 IU) (Stein et al. 2011). On the contrary, a study on a Norwegian cohort has supported therapeutic effect of a relatively higher-dose supplementation (800 IU) compared to a low-dose (400 IU). Serum levels of 25(OH)D in the upper physiological range (between 75 and 125 nmol) were associated with lower risk of relapses and magnetic resonance imaging disease activity. Supplementation with 800 IU of vitamin D and target levels of 25(OH)D of 75 to 125 nmol has been proposed by this study (Holmøy et al. 2012). Currently a multicenter, double-blind, randomized, placebo-controlled clinical trial SOLAR is underway. It will evaluate the efficacy of vitamin D as add-on therapy to interferon beta-1a in patients with relapsing-remitting multiple sclerosis (Smolders et al. 2011). More light in the detection of optimal supplementation regimes may be brought by the undergoing EVIDIMS trial - a multi-center, randomized, controlled and double-blind study which tests the hypothesis that a high-dose vitamin D supplementation of multiple sclerosis patients receiving interferon-1-beta is safe and superior to low-dose supplementation (Dörr et al. 2012). Although the results of currently powered randomized clinical trials using vitamin D supplementation have not

yet been reported, patients with multiple sclerosis who are currently in vitamin D insufficiency should be supplemented with moderate doses of the vitamin D (Pierrot-Deseilligny et al. 2013).

Conclusion

Growing evidence is accumulating on vitamin D deficiency as a key risk factor, involved in the pathogenesis of multiple sclerosis, which is at the interface between genetic and environmental factors. Variations in vitamin D metabolism and receptor related genes may predict the levels of serum vitamin D and thus predict the risk or progression of multiple sclerosis. Although promising effects of vitamin D supplementations have emerged, the real therapeutic significance of vitamin D supplementation remains to be elucidated.

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