



Vitamin D receptor biochemical and genetic profiling and HLA-class II genotyping among Lebanese with multiple sclerosis – A pilot study



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ABSTRACT

Background: Multiple sclerosis (MS) is an autoimmune demyelinating disease affecting mostly young adult females with multifactorial etiology. Recent studies suggested that adequate vitamin D levels may lower the risk of developing MS.

Objectives: Our aim was to explore the relationship between vitamin D receptor (VDR) polymorphism, HLA-DR locus genotype, and serum vitamins D and A levels in the Lebanese population.

Methods: Fifty MS patients were recruited for this study. The control group consisted of 48 healthy and 51 patients with other neurological disorders (non-MS). Biochemical analysis included serum 25 hydroxyvitamin D (25OHD) and vitamin A. Molecular analysis targeted VDR genotypes (*Apal*, *TaqI* and *BsmI*) and low resolution HLA typing for DRB1 locus.

Results: Healthy and non-MS groups had comparable parameters and were combined into one control group. No significant differences were found between MS and control groups for VDR genotypes. The frequency of HLA-DRB1*15 was significantly higher in MS patients (22%) compared to controls (8%) ($p = 0.018$). Odds ratio for MS in the presence of DRB1*15 allele was 3.21 ($p = 0.018$). Cosegregation with *A* (*Apal*) and *b* (*BsmI*) alleles did not influence the risk for MS. 25OHD levels were significantly higher in MS patients compared to controls ($p = 0.002$), due to more frequent oral supplementation ($p = 0.005$). Vitamin A levels were comparable between the two groups. When all parameters were included in a logistic regression model adjusted for supplementation, only HLA-DRB1*15 (OR = 3.42; $p = 0.027$) contributed significantly to MS risk.

Conclusion: There was no association between serum vitamin D or A or VDR genotypes and MS. HLA-DRB1*15 was the major factor imposing more than 3 folds greater risk for developing MS among Lebanese.

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1. Introduction

Multiple sclerosis (MS) is an autoimmune demyelinating central nervous system disorder of unknown etiology (Mehta, 2010). This inflammatory disease is among the most common neurological diseases in young adults affecting around 2000 individuals in Lebanon and 2.3 million worldwide with a predilection for females (Yamout et al., 2008). MS is characterized by demyelination, axonal loss and gliosis in the central nervous system (Milo and Kahana, 2010). According to the pattern of disease progression, MS can be either relapsing remitting or progressive (primary or secondary) (Mehta, 2010). Although the exact immunopathogenesis of MS is still unknown, susceptibility to the

disease is thought to involve a complex interplay between genetic and environmental factors (Alcina et al., 2012).

The major histocompatibility complex Human Leukocyte Antigen (HLA) exerts the largest genetic contribution to MS susceptibility (Alcina et al., 2012). This region is characterized by substantial genetic diversity thought to contribute to a vigorous immunity at the population level. HLA gene variants, not randomly distributed, tend to be linked to other variants in common haplotypes (Schmidt et al., 2007). To date, a significant association between DRB1*1501 and MS has been found in people in Palestine, Jordan and Turkey, but not in South China, Shanghai, Kuwait or Japan. These observations are strongly consistent with the idea that DRB1*15 is associated with conventional MS in areas of medium disease prevalence (Kira, 2003).

On the environmental side, vitamins D and A are thought to have a prominent role in MS pathogenesis especially during the first year of life (Agliardi et al., 2011). Previous studies provided some plausibility to the hypothesis that vitamin D could be beneficial in MS prevention and treatment. MS patients have usually lower serum 25 hydroxyvitamin

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D (25OHD) levels and this disease is mainly observed at altitudes with low sun exposure (Ascherio et al., 2010). Inert 1,25 dihydroxyvitamin D (1,25(OH)₂D) becomes active upon binding to the vitamin D receptor (VDR), which then translocates to the nucleus generating heterodimers capable of activating multiple genes (Wang et al., 2005). A recent report demonstrated the presence of a VDR element (VDRE) in the proximal promoter region of the HLA-DRB1 gene. VDREs present a multitude of sequence variations and a spectrum of binding affinities for VDR; this variability enables VDREs to respond optimally to different concentrations of the VDR/1,25(OH)₂D. The lack of variants of the VDRE sequence of the HLA-DRB1*15 allele led to the speculation that the augmented risk of MS seen in HLA-DRB1*15 carriers could be secondary to its transcription regulation through the VDR 1,25(OH)₂D complex. A recent meta-analysis showed that the *Apal* polymorphism of the VDR gene was associated with increased risk of MS (Tizaoui et al., 2015). On the other hand, vitamin A is an essential nutrient with important roles in immunological responses and in brain development. Its main metabolite is retinoic acid (RA), which is responsible for the neuroimmunological functions associated with vitamin A. In the brain, RA is known to interact with other nuclear receptor-mediated signaling pathways. RA is involved in plasticity, regeneration, cognition and behavior. In the peripheral blood, RA plays a major role both in increasing tolerance and decreasing inflammation, through balancing T-lymphocyte populations. It is likely that RA synthesis may be manipulated by complex cross-talk among cells during infection and inflammation. The role of vitamin A in multiple sclerosis (MS) could be dual: decreasing inflammation and increasing tolerance of autoimmunity and it may also help in brain protection (Fragoso et al., 2014).

To our knowledge, no previous studies tackling the association between genetic factors, vitamins A and D 25(OH), and multiple sclerosis have been performed in Lebanon or other Arab countries. Our aims in this study were to determine the frequency of VDR gene polymorphism and the interaction between genetic (HLA) and environmental (Serum 25OHD and A levels) factors in Lebanese MS patients.

2. Materials and methods

2.1. Patients and controls

Fifty unrelated patients (age: 19–71; M/F ratio: 1:2.1) with relapsing–remitting type of MS (Expanded Disability Status Scale (EDSS): 3.94 ± 2.76), previously diagnosed according to McDonald 2010 criteria (Polman et al., 2011), were selected for this study in the winter season. Control subjects consisted of 48 healthy individuals (age: 15–59; M/F ratio: 1.0:2.0) and 51 patients with neurological disorders other than MS (non-MS, age: 13–70; M/F ratio: 1.0:1.1). All patients and controls were Lebanese and were recruited at the American University of Beirut Medical Center (AUBMC). The study was approved by the Institutional Review Board (IRB) at AUBMC and a written consent form was obtained from each participant. Detailed demographic and clinical data were obtained from all patients.

2.2. Genotyping

Genomic DNA was extracted from peripheral blood collected in EDTA tubes using Invitrogen kit (Invitrogen Dynal Biotech, Bromborough, UK) following the manufacturer's recommendations, and stored at $-20\text{ }^{\circ}\text{C}$ for later VDR genotyping and HLA analysis. Specific PCR sequence specific reactions were performed to determine VDR gene polymorphism and HLA typing following previously described methods (Yavuz et al., 2011). Visualization of digested products of VDR Gene was done on 1.2% for *BsmI* and 2% agarose gel electrophoresis for *Apal* and *TaqI*. Low resolution HLA typing for HLA-DRB1 locus was performed using OneLambda's SSOP (Sequence Specific Oligonucleotide Probe) kits (OneLambda, San Francisco, USA) on the LUMINEX platform (IL-200, LUMINEX, California, USA).

2.3. Biochemical analysis

Total 25OHD in serum was analyzed using the Liaison chemiluminescence assay (DiaSorin Inc., Stillwater, Minnesota, USA). The assay had a measuring range from 4 to 150 ng/ml with an imprecision of 7.7% at mean concentration of 16.6 ng/ml and 5.2% at 55.4 ng/ml. Our laboratory participates in Vitamin D External Quality Assessment Scheme and College of American Pathologists proficiency testing programs for vitamin D. Vitamin A was referred to Cerba laboratories (France) for analysis by high performance liquid chromatography.

2.4. Statistical analysis

Data was summarized using descriptive statistics and evaluated for normality using Shapiro–Wilk test. All continuous variables (age, 25OHD and vitamin A) did not show a normal distribution and non-parametric statistics was applied. Differences among groups were assessed using Mann–Whitney U-test, while associations between various alleles and risk of MS were evaluated using the Chi-square test and odds ratio. Furthermore, to confirm predictors of MS occurrence, stepwise logistic regression analysis was performed using the following dependent variables: age, sex, 25OHD (after adjusting for vitamin D supplementation), vitamin A, HLA alleles and VDRG alleles. SPSS software (version 21.0 SPSS, Inc., California, USA) was used for analysis. A P-value <0.05 was considered significant.

3. Results

The descriptive statistics of control groups (healthy subjects and non-MS) included in this study were evaluated using Mann–Whitney U-test. All compared results for healthy ($n = 48$) and non-MS ($n = 51$) groups were not different statistically for any of the study parameters (sex, age, 25OHD, vitamin A, VDR polymorphisms, and HLA-DRB1 typing) ($p \geq 0.166$) (Table 1). Accordingly, they were combined as one control group ($n = 99$) in this study. Age was different between the MS (mean \pm SD: 42.8 ± 13.5) and control (33.8 ± 12.9) groups ($p = 0.000$), being higher in the former group.

3.1. VDR Gene genotypes and allele frequencies

The type of polymorphism in VDR gene was determined for all patients and controls. The genotypic and allelic frequencies of the VDR genes: *Apal* (AA, Aa, aa), *TaqI* (TT, Tt, tt) and *BsmI* (BB, Bb, bb) are presented in Table 2. No significant differences were found between MS patients and controls for these genotypes ($p \geq 0.465$) (Fig. 1).

3.2. HLA-DRB1*15 distribution and relationship with VDR polymorphisms

The most frequent HLA types in MS patients were HLA-DRB1*11 (46%) followed by HLA-DRB1*03 (32%) and HLA-DRB1*15 (22%), whereas HLA-DRB1*11 (61%), HLA-DRB1*04 (40%) and HLA-DRB1*13 (21%) were the most frequent in controls. The frequency of HLA-DRB1*15 was significantly higher in MS patients (22%) compared to controls (8%) ($p = 0.018$) (Fig. 2). A total of 10/11 MS patients positive for HLA-DRB1*15 were females, 3 of which were homozygous compared to 5/8 for controls and none was homozygous. The odds ratio (OR) for MS in the presence of DRB1*15 allele was 3.21 ($p = 0.016$; 95% CI = 1.2–8.59), using Chi-square test. Cosegregation of HLA-DRB1*15 and VDR gene A allele (*Apal*) did not change the risk of MS (OR = 3.40; $p = 0.022$; 95% CI = 1.14–10.19). Similarly, the combination of DRB1*15 with b allele (*BsmI*) showed an insignificant contribution to the risk of MS (OR = 4.22; $p = 0.08$; 95% CI = 0.75–23.89) (Fig. 2), while the cosegregation of HLA-DRB1*15 and t allele (*TaqI*) did not influence the risk of MS (OR = 2.52; $p = 0.105$; 95% CI = 0.80–7.96).

Table 1
Summary of study parameters in healthy and non-MS groups.

	Healthy (n = 48)	Non-MS (n = 51)	Mann Whitney U-test p value
Gender (F/M)	32/16	28/24	0.166
Age (years)	32.3 ± 12.7	35.23 ± 13.0	0.197
Mean ± SD	27.0 (18.9–58.1)	36.0 (15.0–61.0)	
Median (5th–95th percentiles)			
Vitamin A (µg/ml)	516.8 ± 158.8	503.51 ± 135.0	0.777
Mean ± SD	487.0 (241.1–826.0)	524.0 (304.0–716.0)	
Median (5th–95th percentiles)			
25OHD (µg/l)	14.0 ± 9.8	14.9 ± 11.1	0.925
Mean ± SD	11.0 (4.2–32.8)	11.1 (4.0–41.8)	
Median (5th–95th percentiles)			
HLA DRB1*15 (n/Total)	4/48	4/51	0.658
VDRG			
<i>Apal</i> (A/a ratio)	1.6	1.3	0.357
<i>BsmI</i> (B/b ratio)	1.2	1.4	0.441
<i>TaqI</i> (T/t ratio)	0.9	0.8	0.529
VDR allele frequencies			
A (%)	56.7%	61.45%	
a (%)	43.26%	38.54%	
B (%)	58.65%	54.16%	
b (%)	41.34%	45.8%	
T (%)	43.26%	42.7%	
t (%)	56.7%	57.29%	

3.3. Study parameters and disease risk

Serum levels of 25OHD were higher in MS patients compared to controls ($p = 0.002$). This was mainly attributed to higher number of MS patients taking oral vitamin (25OHD) supplementation ($p = 0.005$). When unsupplemented subjects (27 MS and 76 controls) were compared, 25OHD was not different between the two groups ($p = 0.087$). Furthermore, 12/27 (44%) and 45/76 (59%) were considered deficient for vitamin D at a cutoff of 10 ng/ml, respectively. On the other hand, vitamin A serum levels were comparable between the two groups ($p = 0.997$) (Table 3). To test for any significant trends of serum 25OHD

levels in affected subjects, logistic regression analysis (stepwise) was used after adjustment for vitamin supplementation and inclusion of the other dependent variables namely age, gender and HLA polymorphism. Neither gender ($p = 0.591$) nor serum 25OHD levels ($p = 0.299$) were found to be significant predictors of MS status in our sample. Furthermore, logistic regression model showed that age and the HLA-DRB1*15 genotype increased significantly the risk of having MS ($P = 0.002$ and 0.027 , respectively). While age was expected to appear as a significant predictor due to advanced age of MS patients, the OR for MS in the presence of HLA-DRB1*15 was determined to be 3.42 ($p = 0.027$; 95% CI: 1.15–10.13) by this model which is very close to the unadjusted OR of 3.21 ($p = 0.016$; 95% CI: 1.2–8.59) determined using Chi-square test. Logistic regression was repeated after excluding all subjects on supplementation ($n = 76$ controls and $n = 27$ MS patients), an OR of 4.82 ($p = 0.028$; 95% CI: 1.18–19.70) was determined for HLA-DRB1*15.

Table 2
Comparison of HLA and VDRG polymorphism in MS and control groups.

	MS patients n = 50	Control group n = 99	p value Mann–Whitney U-test
Genotype frequencies n (%)			
<i>Apal</i> gene			0.823
AA	19 (38%)	33 (33%)	
Aa	22 (44%)	51 (51%)	
aa	9 (18%)	15 (15%)	
Allele frequencies			0.656
A	60 (60%)	117 (59%)	
a	40 (40%)	81 (41%)	
<i>BsmI</i>			0.681
BB	10 (20%)	16 (16%)	
Bb	21 (42%)	53 (54%)	
bb	19 (38%)	30 (30%)	
Allele frequencies			0.681
B	41 (41%)	85 (43%)	
b	59 (59%)	113 (57%)	
<i>TaqI</i>			0.465
TT	19 (38%)	32 (32%)	
Tt	23 (46%)	48 (49%)	
tt	8 (16%)	19 (19%)	
Allele frequencies			0.492
T	61 (61%)	111 (56%)	
t	39 (39%)	87 (44%)	
HLA alleles (n/total)			0.018*
DRB1*15 (%)	11(22%) [^]	8 (8%)	

[^] 3 of which were homozygote females.

* Statistically significant.

4. Discussion

According to recent epidemiological studies, an increase in the prevalence of MS has been observed worldwide between 2001 and 2014 (Wade, 2014). In this study, we investigated the association between MS and VDR gene polymorphism, HLA-DR locus genotype, and 25OHD and vitamin A serum levels in the Lebanese population. We found no association between VDRG polymorphism and MS. The frequencies of the A, b and t alleles were comparable in our study between MS and controls (Table 2). However, more than fifty genomic regions have been associated in previous studies with MS, and VDR has been considered by certain authors as a contributing environmental and genetic factor in many neurodegenerative disorders including MS (Ascherio and Munger, 2007). For instance, a significantly higher prevalence of homozygote AA, or heterozygote A and b genotypes was found in MS patients as compared to controls in Japanese, British and Canadian populations (Niino et al., 2000; Ramagopalan et al., 2009; Mamutse et al., 2008). More recently, Sadeghi et al. showed that *BsmI* and *Apal* VDR polymorphisms were associated with an increased risk of MS in Iran (Sadeghi et al., 2015). The association between VDRG polymorphism and MS is however still controversial, as other studies could not document such relationship. VDRG polymorphism could be a factor in determining vulnerability to MS or its disease inflection, as shown by Fukazawa et al. in 1999 among Japanese women (Fukazawa et al., 1999; Fukazawa et al.,

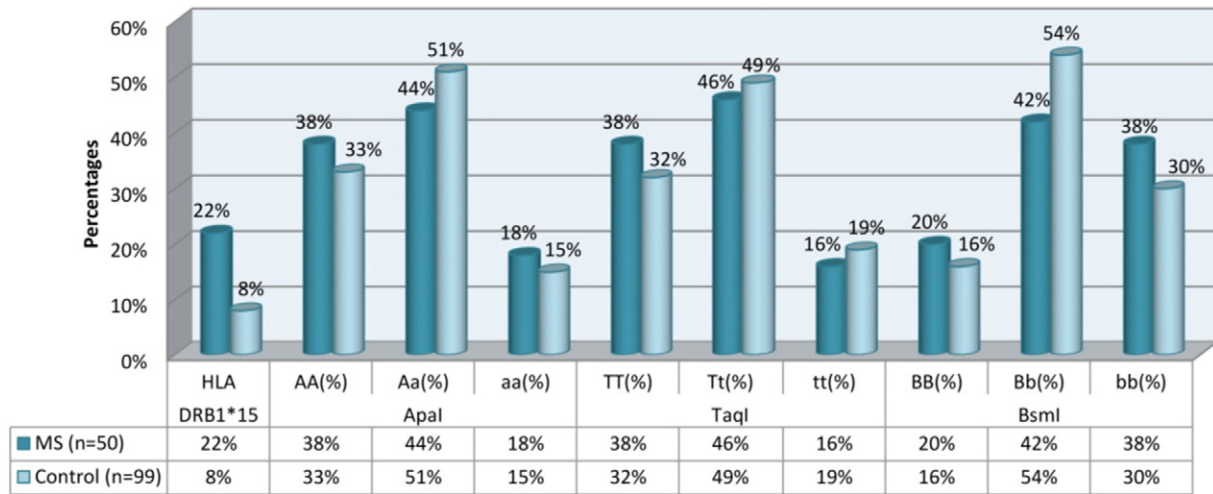


Fig. 1. Frequency of HLA-DRB1*15 ($p = 0.018$) and VDRG alleles (p value not significant) among study subjects.

2000). In another Japanese study, VDRG polymorphism showed no differences in distribution between MS patients and non-related healthy individuals (Niino et al., 2002). Similarly in a Canadian population, there was no evidence for any association between genes involved in the metabolism and function of vitamin (25OHD) and MS, including the 1-OHase gene, the Albumin D-Box Binding Protein (DBP) gene, and the VDRG (Steckley et al., 2000).

Due to funding reasons, we did not perform high resolution typing for HLA DRB1- locus specifically in search for the DRB1*15:01, DRB1*15:02, and other allelic subtypes that have been reported to be associated with MS. However, our resolution level is adequate enough to specify the corresponding Class II subtype. Thus, with respect to HLA genotyping, our study demonstrates evidence of heterogeneity in the distribution of the DRB1 locus subtypes among controls and MS patients. This is consistent with Sawcer and Compston's report in non-European countries (Sawcer and Compston, 2006). We showed that HLA-DRB1*15, present in 22% of MS patients and 8% of controls, was the allele with the strongest association with MS in the Lebanese population. This finding is consistent with most studies performed in different countries and ethnicities. The International Multiple Sclerosis Genetics Consortium in a study involving 9772 cases of European descent collected from 15 different countries, found that DRB1*15 had the strongest association with MS at an odds ratio of 3.10 which was similar to the odds ratio of 3.42 we found in our Lebanese population. Similar findings were also reported in African-American, European and Japanese populations (Kira, 2003; Oksenberg et al., 2004). More importantly, studies from our region including Tunis, Iran, Turkey and

Morocco have also shown that DRB1*15 demonstrated the strongest association with MS among HLA alleles with odds ratios of 4.34, 2.12, 2.40 and 2.70 respectively. The frequency of HLA-DRB1*15 in MS patients and controls seems to be lower in Lebanon and the region (Tunis, Turkey, Morocco) compared to frequencies reported from other countries (Table 4). An overrepresentation of the DRB1*15 was observed among females (F:M = 15:4) in our study. Previous studies from different countries examined the association between gender and HLA-DRB1*15 and found either no gender difference in Italy and Spain (Ballerini et al., 2004; Fernandez et al., 2004; Hillert et al., 1996), or higher prevalence of this haplotype in females in Japan, United Kingdom and Northern Europe (Hensiek et al., 2002; Fukazawa et al., 2000; Weatherby et al., 2001). Only one study found higher prevalence in males in Northern Ireland (McDonnell et al., 1999). Furthermore, no interaction between DRB1*15 and DRB1*08 was observed in our study; a combination usually known to increase the susceptibility to MS (Schmidt et al., 2007).

HLA-DRB1*14 was found to have a protective role towards MS in previous studies (Yeo et al., 2007) but this was not the case in our study ($p = 0.1$). Overall 15 participants exhibited DRB1*14 in their HLA-DR locus genotypes. Among them, 7 had neurological disorders, 3 were healthy participants and 5 were diagnosed with MS.

Recent studies indicated that the lack of vitamin D is a risk factor for MS and can affect disease progression, disability, and development of new enhancing lesions on MRI (Munger et al., 2006, 2014; Ascherio et al., 2014). The association between 25OHD and MS can be explained by the recent finding of the effect of VDRE on HLA-DRB1*15 that is

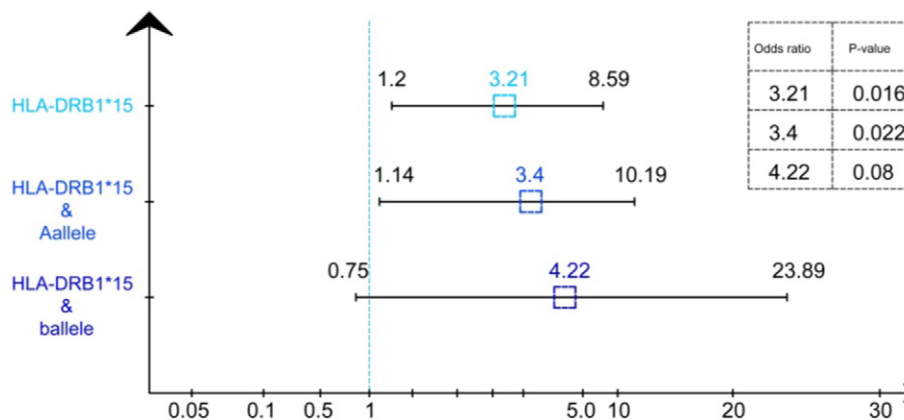


Fig. 2. Odds ratio (OR) values with 95% CI for MS conferred by HLA-DRB1*15 alone or in combination with A or b alleles.

Table 3
Comparison of vitamin A and 25OHD in MS and control groups.

	MS (n = 50)	Controls (n = 99)	Mann–Whitney U test p value
Vitamin A (µg/l)	517.9 ± 188.7	509.9 ± 146.4	0.997
Mean ± SD	523.0 (231.6–935.0)	498.0 (304.0–759.0)	
Median (5th–95th percentiles)			
25OHD (ng/ml)	21.6 ± 15.0	14.5 ± 10.4	0.002
Mean ± SD	18.7 (4.4–57.9)	11.0 (4.0–37.1)	
Median (5th–95th percentiles)			
Vitamin D supplementation n (%)	23 (46.0%)	23 (23.2%)	0.005
25OHD (ng/ml) without supplementation	13.7 ± 10.5	10.3 ± 6.0	0.087
Mean ± SD	11.1 (4.3–45.4)	8.8 (4.0–23.2)	
Median (5th–95th percentiles)			
Age (years)	42.8 ± 13.5	33.8 ± 12.9	0.000
Mean ± SD	41.0 (22.6–71.5)	31.0 (16.0–57.0)	
Median (5th–95th percentiles)			

CI: Confidence interval.

considered the major susceptibility allele. Lonergan et al. in 2011 also elaborated that insufficient HLA-DRB1*15 expression in case of low vitamin D and suboptimal binding to the VDRE with poor upregulation of HLA-DRB1*15 may increase the risk of autoimmunity in MS patients (Lonergan et al., 2011). On the other hand, the molecular role of 25OHD in MS has been highlighted by Niino et al. in 2000. In fact, 25OHD has been observed to inhibit T cell activation and an array of interleukins (IL-1, 2, 6 and 12). This immunomodulatory function consequently inhibits the development of encephalitogenic cells in Experimental Autoimmune Encephalomyelitis (EAE) animals, a useful model in MS (Niino et al., 2000).

Higher serum 25OHD levels were found in MS group compared to controls and is probably related to supplementation process (Table 3). Due to the recent data linking 25OHD to MS most of our patients at the MS center were receiving oral vitamin D supplementation. Logistic regression analysis did not reveal any contribution of low serum 25OHD levels to the risk of MS for subjects without supplementation ($p = 0.561$) or after correction for supplementation ($p = 0.299$) for all participants. Data published recently from Kuwait showed deficient vitamin D levels in both healthy control and MS groups, although supplement use was a common practice (Al-Temaimi et al., 2015). The significance found for older age ($p = 0.002$; OR = 1.05, 95% CI: 1.018–1.078) in the logistic regression analysis model can be mainly

attributed to the scarcity of completely healthy subjects at an advanced age matching MS patients recruited for this study.

An interaction between the effect of VDR polymorphism associated with circulating levels of (25OHD) or its bioavailability to target tissues and the HLA-DRB1*15 type could be connected to the recent findings of a highly conserved VDRE in the promoter region of HLA-DRB1*15. In ex vivo experiments, Ramagopalan et al. inferred that the conserved functional VDRE binds VDR at a higher affinity than other VDREs. Ramagopalan also found that cells transfected with gene constructs including the consensus HLA-DR15 and stimulated with 1,25(OH)₂D improved HLA-DR15 expression by 1.6 folds, while no modification was seen for constructs bearing sequences specific to other HLA-DR haplotypes (Ramagopalan et al., 2009). An increased expression of HLA-DR15 antigen could theoretically diminish an otherwise beneficial effect of high levels of 25OHD (Simon et al., 2011).

Results herein shed light on the interaction between the major genetic (HLA-DRB1*15) and environmental (vitamins D and A/VDR) factors associated with the pathogenesis of MS. According to our finding, HLA-DRB1*15 is associated with MS, imposing more than 3 folds greater risk for developing MS among Lebanese patients. However, there was no clear association between serum 25OHD or vitamin A levels, or VDR polymorphisms and the risk of MS. One of the greatest limitations to our study was the small sample size of the investigated groups.

Table 4
Frequencies of HLA-DRB1*15 in MS versus control subjects from different countries.

Country	Frequency of HLA-DRB1*15 in MS	Frequency of HLA-DRB1*15 in controls	Odds ratio/relative risk (p value)	Reference
Sweden	61%	31%	OR = 8.30 ($p = 0.0001$)	28
China	57.1%	13.8%	RR = 4.55 ($p = \text{Not Significant}$)	29
Australia	57%	17%	RR = 6.47 ($p = 5 \times 10^{-9}$)	30
Spain	45.6%	21.3%	OR = 3.10 ($p = 0.001$)	22
Iran	36.7%	23%	OR = 2.12 ($p = 0.062$)	31
Italy	31.6%	11.2%	OR = 3.64 ($p < 10^{-7}$)	21
Turkey	28.2%	13.9%	OR = 2.40 ($p = 0.02$)	32
Japan	27.5%	10.6%	RR = 2.50 ($p = 0.005$)	33
Tunis	14.7%	3.8%	OR = 4.34 ($p = 0.0025$)	34
Morocco	17.6%	8.4%	OR = 2.67 ($p = 0.002$)	35
Lebanon	22%	8%	OR = 3.42 ($p = 0.027$)	Current study

Accordingly, our findings need to be confirmed in a larger group of Lebanese patients. We also had only one measurement of 25OHD for each individual, which might not reflect their long term vitamin D status.

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