

ASSOCIATION OF VARIOUS GENES WITH SUSCEPTIBILITY TO MULTIPLE SCLEROSIS IN LEBANESE POPULATION OF BEKAA REGION: A PRELIMINARY STUDY

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ABSTRACT. Multiple sclerosis (MS) is a multifactorial, polygenic, neurodegenerative autoimmune disease. Interleukin 7–receptor alpha (IL7-R α), Human Leukocyte Antigen - DRB1*1501 (HLA-DRB1*1501), Tumor protein p53 (Tp53) and Synapsin III (SynIII) genes play a crucial role in this disease. This study aims at investigating specific genetic variants with MS occurrence in the Lebanese population of Bekaa province. MS patients (n=28) and controls (n=28) living in the Bekaa region in Lebanon participated in the study. DNA was purified from the collected blood samples. PCR-RFLP and sequencing of amplified PCR products from the targeted genes were performed along with convenient statistical tests. Genotype and allele frequencies of the studied genes were not statistically significant between MS patients and controls. No significance was noticed in rs1494558 and rs1494555 of IL7-R α where T/T genotype was lower in patients (P=0.106) and A allele was higher in patients (P=0.108), respectively. Haplotypes stratification for rs1494555 and rs6897932 of IL7-R α shows an increase in AT haplotype (P=0.248) and a decrease in GC haplotype (P=0.251) in MS patients and this was independent with HLA - DRB1*1501. Also, no association was shown neither with smoking (P=0.105) nor with gender (P=0.788). Although no association was shown between the studied SNPs and MS in the Lebanese population living in the Bekaa region, this research is considered of high interest since it is one of the first studies done in Lebanon that permits a better comprehension of the genetic implication in the disease.

Keywords: gene, multiple sclerosis, polymorphism.

INTRODUCTION

Multiple sclerosis (MS) is a complex neurodegenerative disorder of the central nervous system occurring mainly in young adults (COOPER and STROEHLA, 2003). It is characterized by neuronal demyelination, degeneration and progressive axonal loss.

Studies reveal that MS is triggered by the peripheral activation of Th-1-cells (SUAREZ-LUIS, 2003; DOMINGUES *et al.*, 2010; HOLMAN *et al.*, 2011), upregulating the very late antigen-4 (VLA-4) on their surfaces to bind to vascular cell adhesion molecule-1 (VCAM-1). This interaction allows them to cross the blood brain barrier and enter the central nervous system to mediate inflammatory responses that lead to myelin and axonal loss (FRISCHER *et al.*, 2009). Activated B cells can also migrate to the central nervous system (CNS) where they differentiate to plasma cells and secrete antibodies leading to myelin injury (NIKBIN *et al.*, 2007). Toxic mediators such as the CD8 cytotoxic T lymphocytes (CD8 CTLs) cause damage to the oligodendrocytes, producers of myelin sheath in the central nervous system.

The disease may progress from relapsing-remitting form to much more complicated courses (MILO and KAHANA, 2010; MEHTA, 2010). The main clinical features of the disease are cerebellar symptoms, sensory and motor perturbations, visual and eye movement abnormalities, as well as bowel and bladder troubles, spasticity and numbness (YAMOUT *et al.*, 2008).

Although its pathogenesis is poorly understood, various studies showed that MS results from a combination of genetic and environmental factors such as high latitude, low vitamin D levels due to insufficient sun exposure and Epstein-Barr Virus (EBV) infection (ALCINA *et al.*, 2012; GIANFRANCESCO *et al.*, 2014; STERNBERG, 2016; OLSSON *et al.*, 2017). According to Multiple Sclerosis News Today statistics, around 2.5 million MS cases are known worldwide, of which 2000 cases were registered in Lebanon in 2008 (YAMOUT *et al.*, 2008) and the number still on the rise in the region due to the lack of highly effective cure.

To date, several genetic factors are linked to the susceptibility to MS and are divided mainly into two categories: Human Leukocyte Antigen (HLA) and non-HLA related genes. Most studies on MS shed the light about the contribution of Major Histocompatibility Complex (MHC) class II, especially Human Leukocyte Antigen-DRB1 (HLA-DRB1), in the development of the disease (XIAO *et al.*, 2015; NAKAMURA *et al.*, 2016; MOSCA *et al.*, 2017; HEDSTROM *et al.*, 2017). On the other hand, many non-HLA genes have been reported to have a strong impact in the development of MS including Interleukin 7-receptor alpha (IL7-R α) (HAJ *et al.*, 2015; ZHUANG *et al.*, 2015), Kinesin family member 1B (KIF1B) (KUDRYAVTSEVA *et al.*, 2011; KOUTSIS *et al.*, 2011), Interleukin 2-receptor alpha (IL2-R α) (FIELD *et al.*, 2017), Cluster of Differentiation 58 (CD58) molecule (LIU *et al.*, 2016), and Synapsin III (SynIII) (LIGUORI *et al.*, 2004; OTAEGUI *et al.*, 2009).

The contribution of genetic factors to MS in the Middle East region is not tackled yet. MS occurrence in the last few years is on the rise. The aim of this study is to investigate the association of HLA-DRB1*1501, IL7-R α , SynIII and TP53 polymorphisms with MS susceptibility among Lebanese MS patients living in the Bekaa region along the highly polluted Litani River (SAADEH *et al.*, 2012).

MATERIALS AND METHODS

MS patients and control subjects

This preliminary study comprised 56 participants (28 controls and 28 MS patients) from the Bekaa region in Lebanon during the year 2017 with a mean age of 39.1 ± 14.6 . The patients were diagnosed by their physicians according to McDonald's criteria (POLMAN *et al.*, 2011). All patients were in the relapsing-remitting course of the disease. Informed consent was obtained from all participants before blood sampling. Relevant clinical data including age, sunlight exposure extent, family neurological history, medical history, treatment and clinical complications were collected using a well-designed questionnaire. This work was

approved by the Institutional Review Board at Beirut Arab University (BAU) and from Al-Abdallah hospital in Bekaa, Lebanon.

DNA extraction and PCR amplification

DNA was extracted from whole blood collected in EDTA tubes using a DNA extraction kit (Sigma-Aldrich). The absorbance of extracted DNA was measured at 260 and 280 nm to assess its concentration and purity. DNA integrity was confirmed by agarose electrophoresis. DNA primers were synthesized by Macrogen-Korea. Sequences of primer were obtained from different studies and reconfirmed using BLAST (NCBI). The primers used for amplification are presented in Table 1.

Table 1. Forward and reverse primers sequences of studied genes.

Gene	PCR primers 5'→3' *	Reference
IL7-R α (Exon 2)	F:ATGTCTGCCACAGAGTCTGCT R:GCCTTTGGGAATTCCTTAGATG	
(Exon 4)	F: TATTCCTTGGCTGCCCTTAG R:GAAATGCACTACTAGGCCAACA	(TEUTSCH <i>et al.</i> , 2003)
(Exon 6)	F:CCAGGGGAGATGGATCCTATCTTACTAA R: GATAGATAGGGATACTGGGCAC	
TP53 (Exon 4)	F:CTAGCAGAGACCTGTGGGAAG R:CCTTTGGCTTCCTGTGAGTGTGTTT	(NAJJAR SADEGHI <i>et al.</i> , 2013)
SynIII (Promoter)	F:TCCTTTCCAGAAGGATGTCC R:AAGCCAACAAATACATAAGTGGA	(LIGUORI, <i>et al.</i> , 2004)
HLA- DRB1*1501 (Exon 2)	F:CCTTTCCCGGTAAAATATCTGAATG R:CCATGCATTCTGAGATCCATACCTT	(GORIS <i>et al.</i> , 2008)

* F: Forward; R: Reverse.

Amplified fragments were purified using a PCR purification kit (Sigma-Aldrich) and sent for sequencing at Macrogen, Korea.

PCR reaction was done in a total volume of 25 μ l containing 1 μ l template DNA (10 ng), 1 μ l of forward and reverse primers (5 μ M), 12.5 μ l of 2x PCR Master Mixture (Sigma-Aldrich) and nuclease free water to make up to volume. PCR condition consisted of initial denaturation at 95°C/5 minutes, followed by 35 cycles at 95°C for 30 seconds, annealing temperatures were ranging from 55°C to 58°C and 72°C for 30 seconds. The termination of the cycle was performed at 72°C for 5 minutes. The size of the obtained PCR products was confirmed by 3 % agarose electrophoresis and visualized using UV (BIO-RAD, California, USA ChemiDoc-It@2 515 Imager P/N 95-0441-04).

TP53 variants were confirmed by RFLP. PCR products were digested with BstU1 (ThermoFisher). Reactions were performed in a total volume of 15 μ l and consisted of 5 μ l of the amplified PCR product, 1 μ l of BstU1 enzyme (10 U.I), 2 μ l of R- buffer supplied with an enzyme (1x) and nuclease free water to make up to volume.

Statistical analysis

All statistical analyses were performed using SPSS version 20. Chi-square and Fischer's exact tests were used to assess SNPs. Odd's ratio (OR) and 95% confidence interval (CI) were applied to assess risk factors. P-value of ≤ 0.05 was considered significant.

RESULTS

Demographic characteristics

The characteristics of both MS patients and controls are shown in Table 2. Thirteen MS patients were male and 15 were female. As for the controls, 9 were male and 19 were female. The average age of MS patients was 40.6 years versus 37.8 years for controls. All participants were from Bekaa region-Lebanon. All MS patients were in the relapsing-remitting MS (RRMS) disease course with an Expanded Disability Status Scale (EDSS) mean of 4.6 meaning a moderate activity in the functional systems of the central nervous system.

Table 2. Characteristics of patients and controls.

	Cases n=28 (mean age \pm SD)	Controls n=28 (mean age \pm SD)
Mean \pm SDE age	40.6 \pm 12.27	37.8 \pm 16.55
Male	13 (41.64 \pm 12.09)	9 (48.33 \pm 19.75)
Female	15 (39.46 \pm 12.82)	19 (32.79 \pm 12.47)
Disease course	Relapsing-remitting	-----
EDSS mean	4.6	-----

Clinical profile of MS patients

Bladder dysfunction was noticed in approximately 50% of the MS patients. Constipation was a frequent trait that may be due to spinal cord impairment, reduced motility and diet composition and has been registered in 50% of the study population. Low physical disability was reported in 46.4% of patients and 28.6% of the patients were ambulant, self-dependent and capable of performing daily activities. This is correlated with the partially preserved cognitive functions of the patients in the current study. Optic nerve involvement was also noticed in 21.4% of the patients of our study.

*SNPs of IL7-R α , HLA-DRB1*1501, SynIII and TP53 genes*

Seven SNPs were analyzed in the amplified regions of exons 2, 4 and 6 of IL7-R α of all participants in the study; three in exon 2, two in the intronic region of exon 2, one in exon 4 and one in exon 6. In exon 2, rs35967524, rs11567704 and rs1494558 show respectively an amino acid change from Leu to Gln or Arg at position 33, from Ser to Arg at position 44 and from Ile to Thr at position 66. The intronic rs11567705 and rs969128 SNPs represent a variation from C to G and from A to G alleles respectively. In exon 4, rs1494555 results in a Val to Ile variation at position 138 and rs6897932 of exon 6 results in a change from Thr to Ile at position 244.

The rs3135388 of HLA-DRB1*1501 is represented by C to T variation. In SynIII, rs133945 in the promoter region shows a variation from G to A at position -196. As for TP53 exon 4, rs1042522 leads to an amino acid change from Pro to Arg at position 72.

Allelic and genotypic frequency of IL7-R α

The allelic and genotypic frequency analyses of IL7-R α in MS patients and controls participants are shown in Table 3.

In exon 2, for rs35967524 and rs11567704, all participants were homozygous for T and C wild type alleles, respectively. For rs1494558, no significant association was registered

in terms of genotypic frequencies ($P=0.160$) as 28.6% of MS patients and 32.1% of controls were homozygous for C, 67.9% of MS patients and 53.6% of controls were heterozygous and 3.5% of MS patients and 14.3% of controls were homozygous for T allele. However, 46.4% of MS patients and 53.6% of controls carried the C mutant allele while 53.6% of MS patients and 46.4% of controls carried the wild type allele T.

Table 3. Allele and genotype frequencies of IL7-R α SNPs in MS patients and controls.

Exon	SNP/ Risk allele	Genotypes and alleles	MS patients	Controls	Risk vs normal genotype or allele	P-value	Odds ratio (95% CI)
2	rs35967524/A	AA	0 (0%)	0 (0%)		N/A	N/A
		AT	0 (0%)	0 (0%)			
		TT	28 (100%)	28 (100%)			
		A	0 (0%)	0 (0%)			
		T	28 (100%)	28 (100%)			
	rs11567704/T	TT	0 (0%)	0 (0%)		N/A	N/A
		TC	0 (0%)	0 (0%)			
		CC	28 (100%)	28 (100%)			
		T	0 (0%)	0 (0%)			
		C	28 (100%)	28 (100%)			
rs1494558/C	CC	8 (28.6%)	9 (32.1%)	(CC+CT) vs. TT	0.160	1.125 (0.952-1.330)	
	CT	19 (67.9%)	15 (53.6%)				
	TT	1 (3.5%)	4 (14.3%)	C vs. T	0.593	0.867 (0.512-1.467)	
	C	13 (46.4%)	15 (53.6%)				
	T	15 (53.6%)	13 (46.4%)				
rs11567705/G	GG	2 (7.1%)	0 (0%)	(GG+GC) vs. CC	1.000	1.000 (0.521-1.918)	
	CG	9 (32.1%)	11 (39.2%)				
	CC	17 (60.8%)	17 (60.8%)	G vs. C	1.000	1.000 (0.220-4.536)	
	G	3 (10.7%)	3 (10.7%)				
	C	25 (89.3%)	25 (89.3%)				
rs969128/G	GG	0 (0%)	0 (0%)	(GG+GA) vs. AA	0.752	1.167 (0.448-3.036)	
	GA	7 (25%)	6 (21.4%)				
	AA	21 (75%)	22 (78.6%)		N/A	N/A	
	G	0 (0%)	0 (0%)				
	A	28 (100%)	28 (100%)				
4	rs1494555/A	AA	13 (46.4%)	9 (32.1%)	(AA+AG) vs. GG	1.000	1.042 (0.854-1.270)
		AG	12 (42.9%)	15 (53.6%)			
		GG	3 (10.7%)	4 (14.3%)	A vs. G	0.108	1.600 (0.886-2.891)
		A	16 (57.1%)	10 (35.7%)			
		G	12 (42.9%)	18 (64.3%)			
6	rs6897932/T	TT	3 (10.7%)	1 (3.5%)	(TT+TC) vs. CC	1.000	1.000 (0.521-1.918)
		TC	8 (28.6%)	10 (35.8%)			
		CC	17 (60.7%)	17 (60.7%)	T vs. C	0.778	0.900 (0.433-1.872)
		T	9 (32.1%)	10 (35.7%)			
		C	19 (67.9%)	18 (64.3%)			

* $P \leq 0.05$ was considered statistically significant. MS: Multiple sclerosis; N/A: Not applicable.

In the intronic region of exon 2, for rs11567705, 7.1% of MS patients and none of the controls were homozygous for G, 32.1% of MS patients and 39.2% of controls were heterozygous and 60.8% of both MS patients and controls were homozygous for C allele. However, 10.7% of both MS patients and controls carried the G mutant allele while 89.3% of both groups carried the wild type allele C. For rs969128, none of MS patients and controls were homozygous for G, 25% of MS patients and 21.4% of controls were heterozygous and 75% of MS patients and 78.6% of controls were homozygous for A allele. All participants carried the wild type allele A. In rs1494555 of exon 4, 46.4% of MS patients and 32.1% of controls were homozygous for A, 42.9% of MS patients and 53.6% of controls were heterozygous and 10.7% of MS patients and 14.3% of controls were homozygous for the G allele. However, 57.1% of MS patients and 35.7% of controls carried the mutant allele A while 42.9% of MS patients and 64.3% of controls carried the wild type allele G showing by this no significant difference between the 2 groups ($P=0.108$).

In rs6897932 of exon 6, 10.7% of MS patients and 3.5% of controls were homozygous for T, 28.6% of MS patients and 35.8% of controls were heterozygous and 60.7% of both MS patients and controls were homozygous for C allele. However, 32.1% of MS patients and 35.7% of controls carried the mutant allele T while 67.9% of MS patients and 64.3% of controls carried the wild type allele C.

No significant difference in terms of allelic and genotypic frequencies between controls and patients was observed for all studied SNPs.

Allelic and genotypic frequency of TP53, HLA-DRB1*1501, and SynIII

TP53

Figure 1 shows a representative agarose gel electrophoresis of TP53 amplified PCR products along with their BstU1 digest from patients and controls. The size of the expected amplified products obtained from exon 4 with C>G substitution and the normal C allele after digestion with BstU1 are 310 bp and 592 bp respectively in homozygous participants. Products from heterozygous participants yielded two fragments of 310 and 592 bp.

The genotypic frequencies for patients and controls were as follow: 37.1% of MS patients and 50% of controls were homozygous for G, 51.8% of MS patients and 42.9% of controls were heterozygous and 11.1% of MS patients and 7.1% of controls were homozygous for C allele (Table 4). No significant difference was obtained in terms of genotypic frequencies.

HLA-DRB1*1501

For rs3135388, 7% of MS patients and 17% of controls were homozygous for A, 43% of MS patients and 29% of controls were heterozygous and 50% of MS patients and 54% of controls were homozygous for G allele. However, 7.1% of MS patients and 17% of controls carried the risk allele A while 92.9% of MS patients and 83% of controls carried the wild type allele G (Table 4).

SynIII

For rs133945, 21.4% of both MS patients and controls were homozygous for A, 57.2% of MS patients and 67.6% of controls were heterozygous and 21.4% of MS patients and 11% of controls were homozygous for G allele. However, 53.6% of MS patients and 43% of controls carried the risk allele A while 46.4% of MS patients and 57% of controls carried the wild type allele G (Table 4).

No significant difference was shown in terms of genotypic and allelic frequencies for HLA-DRB1*1501 rs3135388 and SynIII rs133945.

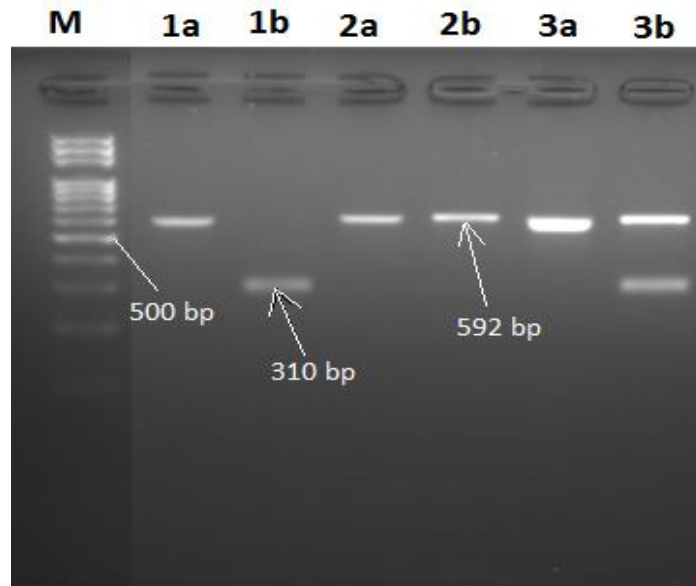


Figure 1. Agarose gel electrophoresis of TP53 PCR products with and without BstU1 digestion (M: 100 bp DNA marker; Letter a represents undigested PCR product. Letter b represents BstU1 digested PCR product. Number 1 shows results from a participant with GG genotype; number 2 shows results from a participant with CC genotype and number 3 shows results from a participant with GC genotype).

Table 4. Allele and genotype frequencies of HLA-DRB1*1501, SynIII and TP53 in MS patients versus controls.

Gene	SNP/ Risk allele	Genotypes and alleles	MS patients	Controls	Risk vs normal genotype or allele	P-value	Odds ratio (95% CI)
HLA-DRB1*1501	rs3135388/A	AA	2 (7%)	5 (17%)	(AA+AG) vs. GG	0.789	1.077 (0.625-1.855)
		AG	12 (43%)	8 (29%)			
		GG	14 (50%)	15 (54%)			
		A	2 (7.1%)	5 (17%)	A vs. G	0.422	0.400 (0.085-1.892)
G	26 (92.9%)	23 (83%)					
SYNIII	rs133945/A	AA	6 (21.4%)	6 (21.4%)	(AA+AG) vs. GG	0.469	0.880 (0.698-1.110)
		AG	16 (57.2%)	19 (67.6%)			
		GG	6 (21.4%)	3 (11%)			
		A	15 (53.6%)	12 (43%)	A vs. G	0.422	1.250 (0.722-2.165)
G	13 (46.4%)	16 (57%)					
TP53	rs1042522/G	GG	10 (37.1%)	14 (50%)	(GG+GC) vs. CC	1.000	0.962 (0.816-1.133)
		GC	14 (51.8%)	12 (42.9%)			
		CC	3 (11.1%)	2 (7.1%)			

* $P \leq 0.05$ was considered statistically significant. MS: Multiple sclerosis; HLA-DRB1*1501: Human Leukocyte Antigen-DRB1*1501; SynIII: Synapsin III; Tp53: Tumor protein p53.

Determination of IL7-R α haplotypes and association testing with MS

Haplotyping of SNPs rs1494555 and rs6897932 of IL7-R α for both MS and control participants generated 4 haplotypes that were used to assess their co-inheritance in multiple sclerosis. Their association with the HLA-DRB1*1501 allele was also analyzed. Similarly, P-values did not reach significant association. However, an increase in the AT haplotype and a decrease in the GC haplotype in MS patients was observed and this was independent of HLA DRB1*1501 as shown in Table 5.

Table 5. Comparison of IL7-R α haplotype (rs1494555 and rs6897932) frequencies in MS patients and control participants, and in HLA-DRB1*1501-positive and -negative ones.

rs1494555		rs6897932		All MS patients (n=28)	All controls (n=28)	P-value
A	T			8 (28.6%)	4 (14.3%)	0.248
A	C			8 (28.6%)	6 (21.4%)	0.593
G	T			3 (10.7%)	5 (17.9%)	0.480
G	C			9 (32.1%)	13 (46.4%)	0.394
				MS patients with HLA-DRB1*1501	Controls with HLA-DRB1*1501	P-value
A	T			0 (0%)	0 (0%)	N/A
A	C			0 (0%)	1 (3.5%)	N/A
G	T			0 (0%)	1 (3.5%)	N/A
G	C			2 (7.1%)	1 (3.5%)	0.564
				MS patients without HLA-DRB1*1501	Controls without HLA-DRB1*1501	P-value
A	T			8 (28.6%)	4 (14.3%)	0.248
A	C			8 (28.6%)	5 (17.9%)	0.405
G	T			3 (10.7%)	4 (14.3%)	0.705
G	C			7 (25%)	12 (43%)	0.251

P \leq 0.05 was considered statistically significant. MS: Multiple sclerosis; HLA-DRB1*1501: Human Leucocyte Antigen-DRB1*1501; N/A: Not applicable.

Association of gender and smoking with MS susceptibility

Statistical analyses were performed to investigate the association between gender and smoking with MS susceptibility. No significant association was found between gender or smoking and MS susceptibility as shown in Table 6.

Table 6. Association of gender and smoking with MS susceptibility.

Gender				Smoking			
MS patients		Controls		MS patients		Controls	
M	F	M	F	Smoker	Non-smoker	Smoker	Non-smoker
13	15	9	19	19	9	13	15
(46.4%)	(53.6%)	(32.1%)	(67.9%)	(67.9%)	(32.1%)	(46.4%)	(53.6%)
P-value 0.788				P-value 0.105			
Odd's ratio (95% CI)				Odd's ratio (95% CI)			
1.156 (0.403- 3.317)				2.436 (0.822-7.220)			

* P \leq 0.05 was considered statistically significant. MS: Multiple sclerosis; M: Male; F: Female.

DISCUSSION

MS is a neurodegenerative inflammatory disease. Although its etiology is not well understood, it has a major heritable component and various genes are thought to be directly correlated with the disease susceptibility.

This preliminary study was conducted on Lebanese MS patients from Bekaa province in Lebanon aiming at an initial investigation of the association between IL7-R α , TP53, HLA-DRB1*1501 and SynIII SNPs with MS.

The heterogeneous clinical profile and the demographic characteristics of the participants were somehow similar to the Kashmiri and Indian participants recruited in Bahoor and Bhatia studies (BHATIA *et al.*, 2015; ZAHOR *et al.*, 2017) where the age of the majority of the MS participants ranged between 31 and 40 years. The female to male ratio was 1.15:1 revealing that the females had 1.15 times higher risk for MS than males. This ratio was 3.1:1 and 1.5:1 in Kashmiri and Indian populations, respectively. Optic nerve involvement is also comparable to previous reports where the optic-spinal form of MS is considered to be more frequent in Asian populations (KIRA, 2003; LI *et al.*, 2014).

Several studies have shown a positive linkage between IL7-R α SNPs and MS (EBERS *et al.*, 1996; OTURAI *et al.*, 1999; MAJDINASAB *et al.*, 2014; SIMSEK *et al.*, 2019). IL7-R α exerts a significant impact on the immune system through development, proliferation and homeostasis of B and T cells as it was proven that it plays a crucial role in the differentiation of autoimmune lymphocytes Th1 allowing the proliferation of pathogenic Th17 cells in experimental autoimmune encephalomyelitis (EAE), in several severe combined immunodeficiency and MS patients (POSER *et al.*, 1983; BEBO *et al.*, 2000; O'DOHERTY *et al.*, 2008; RAJI *et al.*, 2012; KREFT *et al.*, 2012). In this study, seven IL7-R α SNPs were investigated as a susceptible candidates for MS among Lebanese patients. However, no association was found between all IL7-R α exon 2 SNPs studied and susceptibility to MS among the Lebanese participants as seen in the Iranian and Belfast populations (O'DOHERTY *et al.*, 2008; RAJI *et al.*, 2012). Furthermore, no allelic variations or heterozygosity in rs35967524 and rs11567704 of IL7-R α exon 2 were found between controls and Lebanese MS patients of Bekaa showing that they are not implicated in MS susceptibility. In contrast, rs11567705 showed similar allelic and genotypic distribution in comparison with the Asian, European and especially African-American populations according to an Iranian publication (RAJI *et al.*, 2012). As for IL7-R α exon 6 rs6897932 which plays a significant role in determining whether IL7-R α stays as a transmembrane protein or changes into a soluble cytoplasmic form, several studies showed its direct correlation with MS (POSER *et al.*, 1983; ALCINA *et al.*, 2008). It is considered as one of the main representative candidates for MS susceptibility after HLA class II genes (KREFT *et al.*, 2012). However, our study shows no association between this SNP and susceptibility to MS among patients groups. Our results are in the same line with a study performed in the western Balkan population where SNP rs6897932 was not identified as a genetic risk factor for MS susceptibility (STANKOVIC *et al.*, 2010). These differences may originate from geographical location, ethnicity variation and interplay between population genetic background and environmental factors.

As for Human Leukocyte Antigen, HLA-DRB1*1501, is without any doubt, one of the best markers underlying the MS disease (ZHANG *et al.*, 2011). Other HLA alleles that are veritable risk markers of MS include HLA-DRB1*0405 (YOSHIMURA *et al.*, 2012), *15 (KAIMEN-MACIEL *et al.*, 2009; YAMOUT *et al.*, 2016), *17 (WU *et al.*, 2010), and *03 (ZHANG *et al.*, 2011). Similarly, HLA-DRB1*1001,*01,*10,*11,*14,*07 was also shown to play a protective role against MS (KANKONKAR *et al.*, 2003; DEAN *et al.*, 2008; ZHANG *et al.*, 2011). Unlike the majority of previous studies that confirmed a high correlation between HLA-DRB1*1501 and MS (DE BAKKER *et al.*, 2006; SCHMIDT *et al.*, 2007; ALCINA *et al.*, 2012;

AL JUMAH *et al.*, 2018), our preliminary study did not reproduce the contribution of this allele to the disease in the Lebanese MS patients living in the Bekaa region. Similar findings were obtained in the Kuwaiti MS cohort (AL-TEMAIMI *et al.*, 2015). Most studies were performed in European populations and rarely in Arab World populations. The risk allele frequency was very low in both controls and MS patients. Due to the fact that DRB1 alleles present various structural properties for antigen presentation based on the differences in their amino acid sequence, the association between MS and HLA genes is a crucial key used to support the evidence that MS pathogenesis is the consequence of autoimmunity, most probably against myelin antigens as in the case of DRB1*1501 (SMITH *et al.*, 1998).

Concerning SynIII, known class of neuro-specific phosphoproteins regulating neurotransmitter release and playing an important role in neuroplasticity and in synapses' formation (HILFIKER *et al.*, 1999), our results showed no association between rs133945 and susceptibility to MS in our patients group similar to the German patients (AKKAD *et al.*, 2006). However, an association has been established in Italian and Basque MS patients (LIGUORI *et al.*, 2004; OTAEGUI *et al.*, 2009).

Similarly, no association was found between Tp53 SNP and MS in our study. Mutation in Tp53 is widely known to have a high implication in cancer due to its oncogenic effect (OZAKI and NAKAGAWARA, 2011). They are also implicated in MS since it is a polygenic disease. Knowing that MS is characterized by oligodendrocytes death, Tp53 was included in our study to determine if a genetic variant of this gene affects the apoptotic rate of oligodendrocytes, thus the neuronal damage. The mutant amino acid at codon 72 has been known to be more efficient in apoptosis since it reduces the induction of growth arrest (PIM and BANKS, 2004). In our study, most controls and MS patients presented the mutant amino acid Arginine. It has been reported that P53-enhanced apoptosis is driven by the pro-inflammatory cytokines TNF- α and IL-1 β that induce excitotoxic neural damage (ROSSI *et al.*, 2014). Moreover, P53 activation by INF- γ and TNF- α , which are overexpressed in oligodendrocytes, induces an injury and subsequent death of oligodendrocytes; an ultimate consequence of multiple sclerosis (WOSIK *et al.*, 2003).

Although P-values did not reach significance, haplotype analysis of two alleles in IL7-R α (rs1494555 and rs6897932) has demonstrated that (AT) and (GC) haplotypes were more frequent in MS patients and controls respectively. This means that a synergistic effect of alleles may underlie the disease occurrence.

Although smoking is a universal environmental factor associated with MS susceptibility (HANDEL *et al.*, 2011), our results showed no significant correlation between smoking and the disease implying that smoking may not be directly implicated in MS. A possible explanation for this is that smoking might exert differential risk across ethnic populations, or a combination of various mechanisms might be behind the disease occurrence.

CONCLUSION

Our preliminary study showed no association between SNPs of HLA-DRB1*1501, IL7-R α , SynIII and TP53 and MS occurrence in the Lebanese living in the Bekaa region of Lebanon. A larger number of participants are needed to confirm this conclusion.

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