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Original Article

Investigation of IL-21 gene polymorphisms (rs2221903, rs2055979) in cases with multiple sclerosis of Azerbaijan, Northwest Iran

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Abstract: Background: Multiple Sclerosis (MS) is a chronic inflammatory demyelinating disease of the Central Nervous System that is immunologically mediated in genetically susceptible individuals. IL-21, a cytokine produced by TCD4$^+$ cells, particularly by Th-17 cells, is believed to play an important role in the MS pathogenesis. Objective: This study was performed to investigate the impact of genetic polymorphisms in IL-21 gene on MS susceptibility and clinical profiles. Methods: Seventy Iranian patients with clinically definite relapsing-remitting MS and 110 age, sex and ethic matched controls were genotyped for IL-21 gene polymorphisms using PCR-RFLP method. Results: Our results showed that the IL-21 rs2221903 SNP is not polymorphic in our population. Also, the allelic and genotypic frequencies of the IL-21 rs2055979 did not differ significantly between the MS patients and controls (P = 0.413 and P = 0.565 respectively, and OR = 1.122, 95% CI = 0.79-1.87 for T allele). However, our results showed that IL-21 rs2055979 (G/T) T allele positive (TT+GT) MS patients had lower (PI ≤ 1.5) disease progression compared to rs2055979 T allele negative (GG) patients (P = 0.009). Conclusion: Our results showed that no outstanding association exists between IL-21 alleles and susceptibility to MS. However, our clinical analysis showed significant association of IL-21 gene polymorphism with the progression of multiple sclerosis disease. Our results indicate that the G allele promotes, or the T allele protects against disease progression. To clarify the role of IL-21 rs2055979 in MS pathogenesis, further comprehensive studies with larger sample sizes among different ethnicity populations are recommended.

Keywords: Multiple sclerosis, interleukin-21, polymorphism, progression index

Introduction

Multiple sclerosis (MS) is the most common neuro-inflammatory demyelinating disease of the central nervous system (CNS) that can cause severe physical disability and nervous system defects. MS is characterized by demyelination of nervous cells as well as different degrees of axonal damages that leads to neural clinical manifestation including visual and sensory disorders, weakness, spasticity, acute and chronic pains, tiredness, depression, and organ paralysis [1-4]. To date, the precise etiology of MS is unknown. However, it has been shown that MS develops in genetically susceptible individuals following exposure to unidentified environmental triggers [5]. Following activation of auto-reactive T cells through some infectious agents that are molecular mimicking from proteins of the myelin sheath, they pass through the blood brain barrier (BBB) and enter the CNS. These cells demyelinate the neurons through the neuro-inflammatory responses which finally lead to the destruction of myelin and axons of nervous cells [6-9]. Along with TH1 cells, IL-17-producing CD4+ T cells (TH-17) play important roles in the pathogenesis of experimental autoimmune encephalomyelitis (EAE), an animal model of MS which are also found at high frequency in active MS lesions [10, 11]. IL-21, IL-6 and IL-22 are the other products of TH17 cells which also may involve in the dis-

ease process [12-14]. IL-6 is implicated in EAE as the Deletion of IL-6 gene or blockade of its receptor by monoclonal antibody leads to the prevention of EAE [15, 16]. IL-22 knock-out mice develop severe EAE indicating that this cytokine is not among the proinflammatory factors mediating the tissue damage seen in EAE [12].

IL-21 is a pro-inflammatory cytokine produced by activated CD4+ T cells and NK cells. It is expressed at high levels by T follicular helper cells and T helper 17 cells [17]. IL-21 is a potent immunomodulatory cytokine with pleiotropic effects on both innate and adaptive immune responses. This cytokine enhances proliferation of lymphoid cells, increases cytotoxicity of CD8+ T cells and natural killer cells, and differentiation of B cells into plasma cells [18]. IL-21 is found to synergize with TGF-β to induce RORγt and IL-17 in naive T cells [19] and thereby augments the differentiation of Th17 cell, which also secrete IL-21, indicating that IL-21 auto-regulates its own production [17]. In synergy with IL-15 or IL-18, IL-21 also enhances the functional maturation of human and murine NK cells [20]. IL-21R, consisting of a unique IL-21R-α chain and the common γ-chain, is widely expressed on T cells, B cells, NK cells, and dendritic cells in all lymphoid tissues as well as some other cells, such as fibroblasts and epithelial cells [21]. Involvement of IL-21 in EAE is controversial; Studies in IL-21 and/or IL-21 receptor (IL-21R) knock-out mice have yielded apparently conflicting evidence as to the role of IL-21 in EAE. Nurieva and colleagues observed an amelioration of the disease when EAE was induced in IL-21-deficient mice, compared to EAE induced in wild-type mice. They also analyzed the CD4+ cells infiltration to the central nervous system and found that these cells express IFNγ, but not IL-17, while CD4+ cells from wild-type highly express IL-17, suggesting that the lack of IL-21 impaired Th17 differentiation *in vivo* and protected against EAE [14]. However, in other studies in IL-21-/- and/or IL-21R-/- mice, IL-21 was not required for disease promotion and may even have partially protected against EAE [10]. IL-21 gene along with IL-2 gene covers a region of approximately 200 kb that maps in the 4q27 locus. Previously, Genetic association of the IL2/IL21 locus with different autoimmune diseases (AIDs) has been detected. This association was found in Graves' disease, celiac diseases, rheumatoid arthritis, inflammatory bowel diseases, giant cell arthritis and psoriasis [18, 22-25]. Moreover, it was reported that IL21 gene polymorphisms are associated with systemic lupus erythematosus (SLE) [26] On the other hand, in recent genetic study it was also reported that the polymorphisms in IL-21R gene are associated with EAE and MS [27], suggesting that IL-21 is a susceptibility locus for immune disorders. Based on these data we aimed to investigate the possible relevance of IL21 gene polymorphisms to the disease susceptibility and immunopathogenesis of MS.

In this study, we determined the genotype of two IL-21 SNPs (rs2221903 A/G and rs2055979 G/T) located within the second intron of IL-21 in Iranian patients with MS and healthy control group. We analyzed the plausible association of any genotype or allele with susceptibility to MS and also analyzed whether these SNPs have an impact on clinical profiles of disease.

Patients and methods

Patients and control subjects

Seventy (28 male and 42 female) Iranian patients aged between 14-51 years with clinically definite RRMS were enrolled in this study. Patients were diagnosed by experienced neurologists at Neurosciences Research Center of Tabriz University of Medical Sciences according to the revised 2010 McDonald criteria [28]. All the patients were evaluated for their current and past medical histories such as neurology exams and paraclinical data (laboratory and imaging). Only patients with relapsing-remitting multiple sclerosis of duration ≥ 2 years and who had no exacerbations in the previous 3 months were included in the study. Their disability status was quantified by Kurtzke's Expanded Disability Status Scale (EDSS) [29]. The progression index was defined as the ratio of the EDSS score and disease duration [30]. We collected EDSS scores at blood sampling. Also, we determined other clinical variables such as age, sex, onset age of the disease and disease duration. Mean age of patients and controls were 33.23 and 30.77, respectively.

Healthy volunteers consisted of 110 subjects from the same region of Iran without any history of autoimmune, asthma, allergy and chronic

Table 1. The primer sequences used to amplify flanking regions of two IL-21 SNPs

Reference SNP ID	PCR primers
rs2221903 A/G	Forward primer: 5-GGTACCTGGACACTGACGCCCA-3
	Reverse primer: 5-AAGGCAGTTTAGTGGCGACAGCC-3
rs2055979 G/T	Forward primer: 5-GCTCTGAACCCAAACACTCTC-3
	Reverse primer: 5-ACAGCCAGGAAACTCTGGAA-3

Table 2. Demographic and clinical characteristics of the Iranian patients with relapsing-remitting multiple sclerosis at study entry

Characteristic	MS Patients
Gender (M/F), n/n	42/28
Age (years), mean ± SD	34.2±8.3
Age at disease onset (years), mean ± SD	28.3±7.7
Disease duration (years), mean ± SD	5.8±4.6
EDSS score, mean ± SD	3.29±1.6
Progression index, mean ± SD	0.94±0.82

EDSS, Kurtzke's Expanded Disability Status Scale; Progression index = EDSS score/disease duration in years.

infectious diseases and matched for ethnicity and gender that were recruited from the Tabriz Blood Transfusion Center. Informed consent was obtained from all the participants or their legal guardians. A questionnaire was completed for each person and a blood sample was taken from each individual for DNA extraction. This study was approved by The Ethic Committee of Tabriz University of Medical Sciences.

Genotyping of IL-21 SNPs

The whole peripheral blood sample was collected in tubes containing Ethylene Diamine Tetraacetic Acid (EDTA) anticoagulant. Genomic DNA extracting was carried out by standard salting-out method and stored at -20°C until use. Genotyping of the IL21 gene polymorphisms (rs2221903 (A/G) and rs2055979 (G/T)) was carried out using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Primers used to amplify flanking regions of SNPs are shown in **Table 1**. PCR reactions were run in a final volume of 25 µl and in program consisted of initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation (50 seconds), annealing temperature (62°C 50 seconds) and extension (72°C, 30 seconds). The reaction finished after final extension at 72°C for 5 minutes, and specific bands were electrophoresis on 1% agarose gel and visualized by DNA Safe Stain (CinnaGen, Tehran, Iran). The fragments were digested using MBoII and NLaIII enzymes (New England Biolabs) for IL21 rs2221903 and IL-21 rs2055979 polymorphisms respectively, according to the manufacturer's instructions, and analyzed by separating with 2% agarose gels stained with DNA Safe Stain. The genotypes of each polymorphism were determined according to the digestion patterns. To confirm the results of genotyping by the PCR-RFLP, 15 random samples were selected to perform a direct sequencing analysis.

Statistical analysis

Statistical calculations were carried out using SPSS (version17.0, SPSS Inc, Chicago, USA) and Epi Info 2002 (Centers for Disease Control and Prevention, Atlanta, GA USA) statistical software packages. Genotype and allele frequency of IL-21 (rs2055979 G/T) SNP in patients and normal groups were compared using the chi-square test or Fisher's exact test when proper. Association of continuous data (onset age, duration of disease, EDSS and progression index (PI)) with different genotypes was determined by One Way Anova test. Association of polymorphisms with categorized progression index was determined using non-parametric Kruskal-Wallis test. Hardy-Weinberg's proportions were determined by applying the equation (p2 + 2pq + q2). A *p*-value less than 0.05 were considered significant.

Results

We studied 70 patients with MS aged between 14-51 years (mean 34.23±8.33 years) in this study; 64% of subjects were females and 36% were males. All patients had Expanded Disability Status Scale (EDSS) < 6 and mean of EDSS was 3.29±1.6. Patients suffered from MS about 5.8±4.6 years. Index of Progression (IP) was 0.94±0.82. The clinical characteristics of the patient population are shown in **Table 2**. The control group aged from 17 to 50 years (mean 30.77±8.21) of which 40% were males.

We genotyped two SNPs located within the IL-21 gene in 70 MS patients and 110 matched controls. Amplification of the flanking regions of

Table 3. Genotypes and allele frequencies of IL-21 (rs2055979 G/T) in MS patients and control groups

IL-21 SNP			MS patients; n = 70 (%)	Healthy control; n = 110 (%)	χ^2 *P*-value	*P*-value	OR (95% CI)
rs2055979 G/T	Genotype	G/G	18 (25.7)	31 (28.2)			1.122 (0.79-1.87)
		G/T	27 (38.6)	48 (43.6)			
		T/T	25 (35.7)	31 (28.2)	1.14	0.565	
	Allele	G	63 (45)	110 (50)			
		T	77 (55)	110 (50)	0.67	0.413	

OR: odds ratio, CI: confidence intervals.

Table 4. Distribution of different genotypes (and categorized T allele positive and T allele negative patients) of interleukin 21 rs2055979 G/T according to clinical characteristics of multiple sclerosis patients

Characteristic	Genotype	N[€]	Mean ± SD	*P*-Value	Genotype	N[€]	Mean ± SD	*P*-Value
Age at disease onset	GG	18	29.66±8.35	0.70	GG	18	29.66±8.35	0.41
	GT	27	28.07±8.08		GT+TT	51	27.9±7.54	
	TT	24	27.70±7.05					
Duration of disease	GG	18	6.36±5.34	0.47	GG	18	6.36±5.34	0.587
	GT	27	5.00±3.38		GT+TT	51	5.66±4.37	
	TT	24	6.41±5.24					
EDSS	GG	18	3.88±1.25	0.23	GG	18	3.88±1.25	0.094
	GT	27	3.20±1.80		GT+TT	51	3.13±1.72	
	TT	24	3.06±1.65					
Progression index	GG	18	1.22±0.99	0.17	GG	18	1.22±0.99	0.071
	GT	27	0.90±0.64		GT+TT	51	0.83±0.66	
	TT	24	0.75±0.77					

EDSS, Kurtzke's Expanded Disability Status Scale; Progression index = EDSS score/disease duration in years, *P*-value < 0.05 was regarded as significant. [€]One sample was considered as not define.

rs2055979 and 2221903 SNPs resulted in 213 bp and 236 bp bands, respectively. For rs2055979 SNP, 213, 162/51 and 213/162/51 bp bands were interpreted as GG, TT and GT genotypes respectively. Digestion patterns for rs2221903 SNP were as 236, 149/87 and 236/149/87 bp for AA, GG and AG genotypes, respectively. The genotype distributions of the IL-21 rs2055979 polymorphism among the controls and the cases were in Hardy-Weinberg's equilibrium. The results showed that the IL-21 rs2221903 SNP is not polymorphic in our population. **Table 3** shows the allele and genotype frequencies of the IL21 rs2055979 polymorphism in patients with MS and matched controls. There was no statistically significant difference in the distribution of the alleles and genotypes of IL21 rs2055979 among patients and controls (P = 0.413 and P = 0.565 respectively, and OR = 1.122, 95% CI = 0.79-1.87 for rs2055979 T allele).

Next, we analyzed whether age at disease onset, duration of disease, EDSS and progression index (PI) (reflecting the accumulation of disability over time), are modulated by the rs2055979 polymorphism in the patient group. Also, for more analysis, we investigated different alleles (G/T) impact and gene dosage effect on mentioned clinical profile. In this way, we categorized the genotypes in two groups (T allele Positive (TT and GT) and T allele Negative (GG) patients). As shown in **Table 4** all of the mentioned clinical characteristics were not significantly differed between genotypes. However, we found that there was a marginally significant correlation between the PI values and IL21 rs2055979 T allele positive and negative genotypes (PI: P = 0.07). Thus, for more analysis, we investigated whether the categorized progression index (PI) (PI ≤ 1.5 and PI > 1.5) values have correlation with IL-21 rs2055979 genotypes. Our results showed significant differenc-

Table 5. Impact of different IL-21 rs2055979 genotypes and alleles on progression index (PI) of MS

Genotypes	PI ≤ 1.5 N$^{€}$ = 59 (%)	PI > 1.5 N = 10 (%)	*P*-Value
GG	12 (20.3)	6 (60)	
GT	25 (42.4)	2 (20)	0.032*
TT	22 (37.3)	2 (20)	
T Allele positive (GT+TT)	47 (79.7%)	4 (40.0%)	
T Allele Negative (GG)	12 (20.3%)	6 (60.0%)	0.009*

Progression index = EDSS score/disease duration in years, *P-value < 0.05 was regarded as significant, $^{€}$One patient sample was considered as not defined.

es in genotypes distribution (P = 0.03). In addition, for more clarification we also analyzed if the T allele positive (TT+GT) and T allele negative (GG) genotypes frequency have relation with low (PI ≤ 1.5) and high (PI > 1.5) progression index. We found that the results became more significant; patients with the T allele positive (TT and GT) genotypes had a significantly lower progression index compared with those with the T allele negative (GG) genotype (p = 0.009 **Table 5**).

Discussion

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system destroying myelin, oligodendrocytes and axons [31]. A combination of genetic and environmental factors plays an important role in the induction of the disease [32]. Full genome scans have implicated several chromosomal regions for susceptibility to MS [33]. In a HapMap-CEU population, a large block (480 kb) of linkage disequilibrium in chromosome 4q encompassing: testis nuclear RNA-binding protein (*TENR*) gene; a gene encoding a protein of unknown function (*KIAA1109*); IL2 and IL21 genes showed genetic associations with multiple autoimmune diseases including type 1 diabetes mellitus (T1DM) [25], RA [25], juvenile idiopathic arthritis (JIA) [34], psoriasis and psoriatic arthritis (PA) [35, 36]. It has been also reported that the polymorphisms of the IL2/IL21 gene region are associated with celiac disease and ulcerative colitis [37, 38]. Furthermore, the associations of the IL-21 gene or IL-21R gene polymorphisms with autoimmune diseases have recently been reported. Following the analysis of the SNPs in the IL-21 gene, the significant differences of rs907715 and rs2221903 allele and genotype frequencies were found between SLE and controls [26]. IL-21, which is expressed mainly by the activated CD4+ T cells and natural killer (NK) cells, is a novel member of the type I cytokine superfamily. There are much evidences in agreement with the hypothesis that the genetic control of the TH1/TH17 axis is centered on the cytokines (and their receptors) important in TH17 biology [39, 40]. IL-21 is required for differentiation of naive human CD4+ T cells into TH17 cells which have pivotal role in the pathogenesis of the MS disease [14]. Thus, it is reasonable to speculate that the IL-21 is a candidate gene for autoimmune diseases like MS.

In the present study, we examined IL21 rs2055979 and rs2221903 polymorphisms in MS patients. To the best of our knowledge; this is the first study to determine whether these two SNPs in IL-21 gene are associated with the MS. We found that IL21 rs2221903 is not polymorphic in our population. This is in contrast with other studies in different populations [26, 41]. This controversy may be due to our small sample size, or genetic trait differences. It also may be due to the existence of distinct IL-21 genetic polymorphisms amongst specific populations, ethnicities and geographic regions. Furthermore, MS is a multi-factorial disease; Individual exposures to various environmental factors along with genetic susceptibility may have contributed to these mixed results.

The distribution of genotype and allele frequencies of IL21 rs2055979 SNP was not differed between patients and controls. Some studies have investigated the associations between genetic variation in the IL-21 gene and MS. Fedetz and colleagues have investigated the association of *TENR-IL2-IL21* region with Multiple sclerosis in a Spanish population [42]. They did not find evidence for association with any single nucleotide polymorphisms (SNPs) tested. Linden and colleagues also found no evidence of IL21 gene polymorphisms association with multiple sclerosis in a Swedish population [43]. These findings are consistent with our results showing no significant association of the IL-21 gene with susceptibility to MS.

However, we investigated for the first time, the possible interactions of the IL-21 gene with MS

clinical profiles; we found an association of the IL-21 gene polymorphism with the MS disease progression. Our results showed that IL-21 rs2055979 (G/T) polymorphism have an impact on the progression index in relapsing-remitting MS patients. In this regard, as shown in **Table 4**, we found that there was a marginally significant correlation between the PI values and IL21 rs2055979 T allele -positive and- negative genotypes. In additional analysis, we observed that rs2055979 T allele positive patients had lower disease progression compared to rs2055979 T allele negative patients. This may be indicative of the gene dosage effect. In this regard, it seems that the G allele promotes, or the T allele protects against disease progression. However, we did not find any significant relationship between other clinical parameters (EDSS, duration of disease, age at disease onset) and IL-21 rs2055979 gene polymorphism. It is likely that such associations might exist, but our study was underpowered to detect them. A potential weakness of this exploratory study is its relatively small sample size.

This is the first study in Iran reporting IL-21 polymorphism and MS susceptibility and possible interactions of the IL-21 gene with clinical profiles of MS patients. Our results confirm previous studies reporting no outstanding association between any of the IL-21 alleles and susceptibility to MS. However, our clinical analysis showed significant association of IL-21 gene polymorphism with the progression of multiple sclerosis disease. The confirmation of these findings in replicative studies with large cohorts of MS populations is required. Moreover, the elucidation of functional effects of these associations might improve our understanding of the MS disease. Since a complex cytokine network determines the fate of any immune response including autoimmunity, any conclusion on the cytokine functional role in immunopathogenesis of MS disease necessitates further investigations

Disclosure of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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References

[1] Compston A and Coles A. Multiple sclerosis. Lancet 2008; 372: 1502-1517.
[2] Kornek B and Lassmann H. Neuropathology of multiple sclerosis new concepts. Brain Res Bull 2003; 61: 321-326.
[3] McDonald W and Sears T. The effects of experimental demyelination on conduction in the central nervous system. Brain 1970; 93: 583-598.
[4] Noseworthy JH, Lucchinetti C, Rodriguez M and Weinshenker BG. Multiple sclerosis. N Engl J Med 2000; 343: 938-952.
[5] Barten LJ, Allington DR, Procacci KA and Rivey MP. New approaches in the management of multiple sclerosis. Drug Des Devel Ther 2010; 4: 343.
[6] Frohman EM, Racke MK and Raine CS. Multiple sclerosis--the plaque and its pathogenesis. N Engl J Med 2006; 354: 942-955.
[7] Peterson LK and Fujinami RS. Inflammation, demyelination, neurodegeneration and neuroprotection in the pathogenesis of multiple sclerosis. J Neuroimmunol 2007; 184: 37-44.
[8] Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mork S and Bo L. Axonal transection in the lesions of multiple sclerosis. N Engl J Med 1998; 338: 278-285.
[9] Gharibi T, Ahmadi M, Seyfizadeh N, Jadidi-Niaragh F and Yousefi M. Immunomodulatory Characteristics of Mesenchymal Stem Cells and their role in the Treatment of Multiple Sclerosis. Cell Immunol 2015; 293: 113-21.
[10] Tzartos JS, Craner MJ, Friese MA, Jakobsen KB, Newcombe J, Esiri MM and Fugger L. IL-21 and IL-21 receptor expression in lymphocytes and neurons in multiple sclerosis brain. Am J Pathol 2011; 178: 794-802.
[11] Tzartos JS, Friese MA, Craner MJ, Palace J, Newcombe J, Esiri MM and Fugger L. Interleukin-17 production in central nervous system-infiltrating T cells and glial cells is associated with active disease in multiple sclerosis. Am J Pathol 2008; 172: 146-155.
[12] Kreymborg K, Etzensperger R, Dumoutier L, Haak S, Rebollo A, Buch T, Heppner FL, Renauld JC and Becher B. IL-22 is expressed by Th17 cells in an IL-23-dependent fashion, but not required for the development of autoimmune encephalomyelitis. J Immunol 2007; 179: 8098-8104.
[13] Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, McClanahan T, Kastelein RA and Cua DJ. IL-23 drives a pathogenic T cell population that induces auto-

immune inflammation. J Exp Med 2005; 201: 233-240.
[14] Nurieva R, Yang XO, Martinez G, Zhang Y, Panopoulos AD, Ma L, Schluns K, Tian Q, Watowich SS and Jetten AM. Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. Nature 2007; 448: 480-483.
[15] Eugster HP, Frei K, Kopf M, Lassmann H and Fontana A. IL-6-deficient mice resist myelin oligodendrocyte glycoprotein-induced autoimmune encephalomyelitis. Eur J Immunol 1998; 28: 2178-2187.
[16] Serada S, Fujimoto M, Mihara M, Koike N, Ohsugi Y, Nomura S, Yoshida H, Nishikawa T, Terabe F and Ohkawara T. IL-6 blockade inhibits the induction of myelin antigen-specific Th17 cells and Th1 cells in experimental autoimmune encephalomyelitis. Proc Natl Acad Sci U S A 2008; 105: 9041-9046.
[17] Messaoudi S, Al-Khateeb GM, Dendana M, Sater MS, Jazia KB, Nouira M, Almawi WY and Mahjoub T. Genetic variations in the interleukin-21 gene and the risk of recurrent idiopathic spontaneous miscarriage. Eur Cytokine Netw 2011; 22: 123-126.
[18] Diaz-Gallo LM, Simeon CP, Broen JC, Ortego-Centeno N, Beretta L, Vonk MC, Carreira PE, Vargas S, Román-Ivorra JA and González-Gay MA. Implication of IL-2/IL-21 region in systemic sclerosis genetic susceptibility. Ann Rheum Dis 2013; 72: 1233-1238.
[19] Maddur MS, Miossec P, Kaveri SV and Bayry J. Th17 cells: biology, pathogenesis of autoimmune and inflammatory diseases, and therapeutic strategies. Am J Pathol 2012; 181: 8-18.
[20] Vollmer TL, Liu R, Price M, Rhodes S, La Cava A and Shi FD. Differential effects of IL-21 during initiation and progression of autoimmunity against neuroantigen. J Immunol 2005; 174: 2696-2701.
[21] Jia H, Zhang Z, Gu X, Guo T, Cui B, Ning G and Zhao Y. Association between interleukin 21 and Graves' disease. Genet Mol Res 2011; 10: 3338-3346.
[22] Burton PR, Clayton DG, Cardon LR, Craddock N, Deloukas P, Duncanson A, Kwiatkowski DP, McCarthy MI, Ouwehand WH and Samani NJ. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007; 447: 661-678.
[23] Todd JA, Walker NM, Cooper JD, Smyth DJ, Downes K, Plagnol V, Bailey R, Nejentsev S, Field SF and Payne F. Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. Nat Genet 2007; 39: 857-864.
[24] van Heel DA, Franke L, Hunt KA, Gwilliam R, Zhernakova A, Inouye M, Wapenaar MC, Barnardo MC, Bethel G and Holmes GK. A genome-wide association study for celiac disease identifies risk variants in the region harboring IL2 and IL21. Nat Genet 2007; 39: 827-829.
[25] Zhernakova A, Alizadeh BZ, Bevova M, van Leeuwen MA, Coenen MJ, Franke B, Franke L, Posthumus MD, van Heel DA and van der Steege G. Novel association in chromosome 4q27 region with rheumatoid arthritis and confirmation of type 1 diabetes point to a general risk locus for autoimmune diseases. Am J Hum Genet 2007; 81: 1284-1288.
[26] Sawalha AH, Kaufman KM, Kelly JA, Adler AJ, Aberle T, Kilpatrick J, Wakeland EK, Li QZ, Wandstrat AE and Karp D. Genetic association of interleukin-21 polymorphisms with systemic lupus erythematosus. Ann Rheum Dis 2008; 67: 458-461.
[27] Nohra R, Beyeen A, Guo J, Khademi M, Sundqvist E, Hedreul M, Sellebjerg F, Smestad C, Oturai A and Harbo H. RGMA and IL21R show association with experimental inflammation and multiple sclerosis. Genes Immun 2010; 11: 279-293.
[28] Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, Fujihara K, Havrdova E, Hutchinson M and Kappos L. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol 2011; 69: 292-302.
[29] Kurtzke JF. Rating neurologic impairment in multiple sclerosis an expanded disability status scale (EDSS). Neurology 1983; 33: 1444-1444.
[30] Mostert S and Kesselring J. Effects of a short-term exercise training program on aerobic fitness, fatigue, health perception and activity level of subjects with multiple sclerosis. Mult Scler 2002; 8: 161-168.
[31] Chang A, Tourtellotte WW, Rudick R and Trapp BD. Premyelinating oligodendrocytes in chronic lesions of multiple sclerosis. N Engl J Med 2002; 346: 165-173.
[32] Seyfizadeh N, Kazemi T, Farhoudi M, Aliparasti MR, Sadeghi-Bazargani H, Almasi S and Babaloo Z. Association of IL-13 single nucleotide polymorphisms in Iranian patients to multiple sclerosis. Am J Clin Exp Immunol 2014; 3: 124-129.
[33] Akesson E, Oturai A, Berg J, Fredrikson S, Andersen O, Harbo H, Laaksonen M, Myhr K, Nyland H and Ryder L. A genome-wide screen for linkage in Nordic sib-pairs with multiple sclerosis. Genes Immun 2002; 3: 279-285.
[34] Hinks A, Eyre S, Ke X, Barton A, Martin P, Flynn E, Packham J, Worthington J and Thomson W. Association of the AFF3 gene and IL2/IL21 gene region with juvenile idiopathic arthritis. Genes Immun 2010; 11: 194-198.

[35] Zhang J, Xiao XW, Zhu FY, Muhali SF, Xiao L, Jiang JW, Shi HX, Zhou HL and Zhang AJ. Polymorphisms of interleukin-21 and interleukin-21-receptor genes confer risk for autoimmune thyroid diseases. BMC Endocr Disord 2013; 13: 26.

[36] Liu Y, Helms C, Liao W, Zaba LC, Duan S, Gardner J, Wise C, Miner A, Malloy M and Pullinger CR. A genome-wide association study of psoriasis and psoriatic arthritis identifies new disease loci. PLoS Genet 2008; 4: e1000041.

[37] Adamovic S, Amundsen SS, Lie BA, Gudjonsdottir AH, Ascher H, Ek J, van Heel DA, Nilsson S, Sollid LM and Torinsson Naluai A. Association study of IL2/IL21 and FcgRIIa: significant association with the IL2/IL21 region in Scandinavian coeliac disease families. Genes Immun 2008; 9: 364-367.

[38] Garner CP, Murray JA, Ding YC, Tien Z, van Heel DA and Neuhausen SL. Replication of celiac disease UK genome-wide association study results in a US population. Hum Mol Genet 2009; 18: 4219-4225.

[39] Korn T, Bettelli E, Gao W, Awasthi A, Jäger A, Strom TB, Oukka M and Kuchroo VK. IL-21 initiates an alternative pathway to induce proinflammatory TH17 cells. Nature 2007; 448: 484-487.

[40] Babaloo Z, Aliparasti M, Babaie F, Almasi S, Baradaran B and Farhoodi M. The role of Th17 cells in patients with relapsing-remitting multiple sclerosis: interleukin-17A and interleukin-17F serum levels. Immunol Lett 2015; 164: 76-80.

[41] Li N, Zhu Q, Li Z, Han Q, Chen J, Lv Y, Wang Y, Zeng X, Chen Y and Yang C. IL21 and IL21R polymorphisms and their interactive effects on serum IL-21 and IgE levels in patients with chronic hepatitis B virus infection. Hum Immunol 2013; 74: 567-573.

[42] Fedetz M, Ndagire D, Fernandez O, Leyva L, Guerrero M, Arnal C, Lucas M, Izquierdo G, Delgado C and Alcina A. Multiple sclerosis association study with the TENR-IL2-IL21 region in a Spanish population. Tissue Antigens 2009; 74: 244-247.

[43] Lindén M, Nohra R, Sundqvist E, Khademi M, Hillert J, Alfredsson L, Olsson T and Kockum I. No evidence of IL21 association with multiple sclerosis in a Swedish population. Tissue Antigens 2011; 78: 271-274.