Neurology Asia 2013; 18(1) : 83 – 86

The influence of -330 IL-2 gene polymorphism on relapsing remitting and secondary progressive multiple sclerosis in Iranian patients

[1,2]Aida Sayad, [2]Abdolamir Allameh, [3]Arezou Sayad, [3]Mehrdad Noruzinia, [4]Ali Sarzaeem

[1]Department of Biology, Payame Noor University; [2]Department of Biochemistry and [3]Department of Medical Genetics,, Faculty of Medical Science, Tarbiat Modares University, Tehran; [4]Department of Venomous Animals and Antivenom Production, Razi Vaccine and Serum Research Institute, Karaj, Iran

Abstract

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system. Interleukin-2 (IL-2) is identified as the crucial and main immunoregulatory cytokines. Previously, we showed significant association between -330 T/T IL-2 genotype and relapsing remitting MS among Iranian population. In this study we investigated 100 relapsing remitting, 30 secondary progressive MS and 125 healthy controls to compare the relapsing remitting and secondary progressive course MS in association to -330 IL-2 polymorphism. Our results showed that the -330 T/T IL-2 genotype was significantly more frequent in relapsing remitting and secondary progressive MS than controls. The significant increased frequency of -330 T/T IL-2 genotype in secondary progressive than relapsing remitting MS, imply -330 T/T IL-2 genotype can cause higher susceptibility to secondary progressive MS than relapsing remitting.

INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS). It has unknown etiology, but a T cell mediated inflammatory process and an abnormal immune responses in the CNS have been suggested. MS is the second common reason of disability after trauma in the young and middle-aged persons.[1-12] A number of experiments indicated single nucleotide polymorphisms of genes which are involved in immune system are associated to the MS. Interleukin-2 (IL-2) is identified as the crucial and main immunoregulatory cytokines.IL-2 acts as pro-inflammatory and anti-inflammatory factor which elevates proliferation of T cell.[13] Increased expression of IL-2 gene among MS patients was reported.[14,15] IL-2 gene is located on 4q26. Kikuchi *et al*. showed no significant association between -330 IL-2 polymorphisms and MS.[16-19]

For the first time, among Iranian population, Amir Zargar *et al*.[20] showed that -330 G/G IL-2 genotype was more frequent in MS group than controls, but Shokrgozar *et al*.[21] reported no significant association and finally, Shahbazi *et al*. [17]detected susceptibility association between -330 T/T and G/T IL-2 genotypes and MS. Matesanz *et al*.[18] reported -330 T/T IL-2 genotype was not associated with relapsing remitting (RR) course of disease but had susceptibility association to secondary progressive (SP) MS. Therefore, in this study we investigated the comparison of RR and SP MS in association to -330 IL-2 polymorphism.

METHODS

One hundred unrelated RR course MS and thirty unrelated SP course MS were selected. MS subjects were diagnosed by neurologist according to the McDonald criteria[22], and chosen from Medical Genetics Department of Sarem Women Hospital. One hundred and twenty five healthy controls were included and matched to patient group. MS patients and controls had neither personal nor family history of autoimmune disorders. All individuals gave informed and written consent form.

Genotyping and data collection

Total DNA was extracted from EDTA-blood leukocyte by using salting out method and was used for PCR.[23] Promoter of IL-2 gene at -330 position was genotype by using RLFP-PCR according to Matesanz *et al*.[18]

Address correspondence to: Abdolamir Alameh, Department of Biochemistry, Faculty of Medical Sciences, Tarbiat Modares University, P.O. Box 14115-331, Tehran, Iran. Tel.: +98 21 88013030; Fax: +98 21 88006544, E-mail: allameha@modares.ac.ir

Table 1: The frequencies of -330 IL-2 genotypes in patients with relapsing remitting multiple sclerosis and controls

	RR	control	P	OR(95%CI)
Genotype	**N=100(%)**	**N=125(%)**		
G/G	20 (20)	47 (37.6)	0.004	0.415 (0.226-0.763)
G/T	38 (38)	43 (34.4)	0.576	1.169 (0.676-1.468)
T/T	42 (42)	35 (28)	0.028	1.186 (1.067-3.25)

RR: relapsing remitting multiple sclerosis; N: number of individuals.

Statistical methods

The data were evaluated using chi square (χ^2) test and with Fisher's exact test when the criteria of (χ^2) were not fulfilled. The p values of less than 0.05 were accepted significant. Odds ratio (OR) and 95% confidence interval (CI) were determined. Analysis was performed with the SPSS version 18 for windows statistical package.

RESULTS

We compared and analyzed the frequency of -330 position of IL-2 gene promoter among 100 RR, 30 SP course MS and 125 healthy controls to determine which course of MS was influenced by-330 IL-2 polymorphism.

The -330 T/T IL-2 genotype were significantly more frequent in RR MS compared to controls (p: 0.02; OR: 1.186) (Table 1). The -330 T/T IL-2 genotype was significantly more frequent in SP course MS than controls (p:<0.0001; OR: 4.442) (Table 2). Also, the -330 G/G IL-2 genotype was significantly more frequent in control individuals than both RR and SP course MS (Table 1 and 2). However, comparisons between SP and RR course MS identified that -330 T/T IL-2 genotype was significantly more frequent in SP than RR course MS (p: 0.04; OR: 0.419) (Table 3).

DISCUSSION

MS is a neuro-inflammatory and autoimmune disease. The genes which are involved in immune system, may effect on susceptibility to MS. IL-2 is an important cytokine gene that effect on susceptibility to MS. Previously, we identified that the -330 T/T IL-2 genotype was significantly associated with susceptibility to RR MS (not published). In the present study, for the first time, we investigated the frequency of -330 IL-2 gene polymorphism in SP MS compared to RR disease in an Iranian population. Our results showed that the -330 T/T IL-2 genotype was significantly more frequent in SP and RR MS than controls. Furthermore, -330 T/T IL-2 genotype was significantly more frequent in SP than RR disease. This polymorphism may have influence on the course of disease.

There are three studies on the effect of -330 IL-2 gene polymorphism on Iranian MS patients that were reported three different results. Amirzargar *et al*.[20] found that G/G genotype was associated with susceptibility to MS among Iranian population. In contrary, Shokrgozar *et al*.[21] showed no significant association, but Shahbazi *et al*.[17] reported that T/T and G/T genotypes were significantly associated to MS in Iranian population. One study in Japanese population demonstrated no significant association between -330 IL-2 polymorphism and MS disease.[16] A study among Spanish population identified that G/T and T/T genotypes were significantly associated with susceptibility to SP course MS

Table 2: The frequencies of -330 IL-2 genotypes in patients with secondary progressive course multiple sclerosis and controls

	SP	control	P	OR(95%CI)
Genotype	**N=30(%)**	**N=125(%)**		
G/G	3 (10)	47 (37.6)	0.004	0.184 (0.053-0.641)
G/T	8 (26.7)	43 (34.4)	0.418	0.693 (0.285-1.688)
T/T	19 (63.3)	35 (28)	<0.0001	4.442 (1.919-10.278)

SP: secondary progressive multiple sclerosis; N: number of individuals.

Table 3: The comparison of -330 IL-2 genotypes frequencies between patients with relapsing remitting and secondary progressive course multiple sclerosis

	RR	SP	P	OR(95%CI)
Genotype	**N=100(%)**	**N=30(%)**		
G/G	20 (20)	3 (10)	0.208	2.250 (0.620-8.170)
G/T	38 (38)	8 (26.7)	0.255	1.685 (0.682-4.164)
T/T	42 (42)	19 (63.3)	0.04	0.419 (0.181-0.973)

RR: relapsing remitting multiple sclerosis; SP: secondary progressive multiple sclerosis; N: number of individuals.

(not to RR course MS).[18] But we found that T/T genotype was associated with susceptibility to RR and SP course disease. Hence, ethnicity may have an effect on the susceptibility association of this polymorphism to MS disease. Moreover, The significant increased frequency of -330 T/T IL-2 genotype in SP than RR course MS implies that -330 T/T IL-2 genotype may be associated with higher susceptibility to SP course MS than RR.

In conclusion, we demonstrated that the-330 T/T IL-2 genotype was associated with susceptibility to SP and RR course MS. Also the -330 T/T IL-2 genotype was significantly more frequent in SP than RR course disease. These implies that -330 IL-2 gene polymorphism may influence on the course of disease. To verify these subjects further studies with large sample size are recommended.

ACKNOWLEDGEMENT

Medical Genetics Department of Sarem Women Hospital supported this research work.

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