

Lack of Association between Promoter Gene Polymorphism (-318 C/T) and Multiple Sclerosis in Iranian Population

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Abstract

Multiple sclerosis (MS), a chronic inflammatory demyelinating disease of the central nervous system is believed to have a T cell-mediated autoimmune etiology. The cytotoxic T lymphocyte antigen 4 (CTLA-4) gene is a strong candidate for the involvement in autoimmune diseases. To examine the genetic association of the CTLA-4 gene locus with MS, in a case-control design, we analyzed (-318 C/T) single nucleotide polymorphism of the CTLA-4 gene in 135 unrelated Iranian relapsing-remitting MS patients and 135 age, gender and ethnicity-matched healthy subjects using PCR-RFLP method. The frequency of CTLA-4 gene (-318) polymorphism among cases and controls was 52.1% vs. 47.9% for CC, 39.2% vs. 60.8% for TC and 75.0% vs. 25% for TT genotypes respectively. The distribution of CTLA-4 (-318 C/T) genotype and allele frequencies did not significantly differ between MS patients and healthy subjects. In conclusion, there may not be any association between CTLA-4 gene (-318 T/C) polymorphism and MS development.

Keywords: Allelic association; CTLA-4; Multiple sclerosis; RFLP, Single nucleotide polymorphism

Introduction

Multiple sclerosis, the most common neurologic disease affecting young adults, is an inflammatory, presumed autoimmune disorder in which lymphocytes and macrophages infiltrate the central nervous system [1,2]. The susceptibility to MS is influenced by a major

genetic component [3] including HLA and T-cell receptor (TCR) genes [4].

However, as HLA accounts for only a part of the genetic susceptibility, other genes must also be involved. Candidate susceptibility genes may be those encoding proteins regulating the immune response such as cytotoxic T-lymphocyte-associated protein 4

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(CTLA4). The CTLA4 protein is a receptor displayed on activated T cells which receive a negative signal upon binding of CTLA4 to B7 on antigen-presenting cells [5]. The human CTLA-4 gene is known to contain polymorphisms in three regions: a C/T polymorphism in the promoter at position (-318), A/G dimorphism in the exon 1 leader sequence at position 49, and a multiallelic dinucleotide repeat in the 3' untranslated region (UTR) of exon 4. Studies on insulin dependent diabetes mellitus (IDDM), Graves' disease, Hashimoto's thyroiditis and rheumatoid arthritis (RA) suggest a susceptibility locus maps close to the CTLA4 gene or association with CTLA4 gene polymorphisms [6]. Numerous studies were aimed to reveal the allelic association of CTLA-4 with MS development, but the results often appeared to be conflicting [7]. The aim of present study is to investigate whether there is any association between the CTLA-4 (-318 C/T) polymorphism and MS susceptibility in Iranian population.

Materials and Methods

One hundred and thirty five unrelated Iranian Relapsing Remitting MS patients with clinically definite MS according to the Poser criteria [8] were recruited in this study (the mean age was 29.47 years, male/female ratio 1/1.5 and mean EDSS 3.5). One hundred patients were from the Iranian MS society and thirty five from Buali-Sina general hospital, medical university of Qazvin-Iran. The control group consisted of 135 age, gender and ethnicity-matched healthy subjects (the mean age was 45.13 years and male/female ratio 1/1.28). Informed consent was obtained from all the subjects or legal guardians of all patients or volunteers. This study was performed in accordance with the Declaration of Helsinki and subsequent revisions and approved by the ethics committee at Tehran University of Medical Sciences.

Table 1. Association between CTLA-4 (-308 C/T) polymorphism and MS development

	Case n(%)	Control n(%)	Odd ratio	P value
<i>Genotype</i>				
CC	112(52.1)	103(47.9)		
CT	20(39.2)	31(60.8)		0.153
TT	3(75.0)	1(25.0)		
<i>Allele</i>				
C	132(49.6)	134(50.4)	0.730	0.304
T	23(41.8)	32(58.2)		

Whole peripheral blood sample was collected in tubes containing EDTA. Genomic DNA was extracted by standard salting out method [9] and stored at -20°C . Genotypes of the -318 C/T polymorphism were determined by PCR restriction fragment length polymorphisms (PCR-RFLP) using TruII enzyme. Primers and PCR conditions were as previously described [5]. The amplified product were digested overnight and analyzed on 2% agarose gel. The -318 C/T polymorphism was determined by a 568 bp fragment (representing the C allele) or two fragments of 249 and 319 bp (representing the T allele). Genotype and allele frequency of CTLA-4 gene (-318 T/C) polymorphism were compared using Chi-square and Fisher's exact test. The odds ratio was calculated for each allele and genotype. Analyses were performed by SPSS statistical package, version 13.0 for windows. P-value less than 0.05 was regarded as significant.

Results

Statistical analysis of CTLA-4 (-318 C/T) genotype showed no significant deviations from the Hardy-Weinberg equilibrium.

Table 1 Shows essential genotyping data of (-318 C/T) SNP in MS patients and healthy controls.

The frequency of CTLA-4 gene (-318) CC, TC and TT genotypes among all subjects were 79.6%, 18.9% and 1.5%, respectively. No significant differences in the distribution of CTLA-4 (-318 C/T) genotype and allele frequencies between patients with relapsing-remitting MS and normal subjects were found.

Discussion

Relatively recent studies did not find any significant differences in genotypes and allelic distribution of the CTLA-4 gene (-318 C/T) between MS patients and normal controls in UK[10], Netherland [11], Norway [5], Japan [12], Denmark and Shanghai Chinese origin populations [13]. In consistent with the above mentioned studies we also could not find any significant differences in the distribution of CTLA-4 (-318 C/T) genotype and allele frequencies between patients with relapsing-remitting MS and normal subjects. In addition a recent meta-analysis of 3375 MS cases and 2930 healthy controls showed no significant association between CTLA-4 gene (-318 C/T) allelic or genotype distribution and MS susceptibility in European Caucasians and Asian origin subjects [14].

Conversely, significant association between the (-318) CC homozygotes and relapsing-remitting MS has been reported in Northern American population [15].

These conflicting results may be a reflection of genetic heterogeneity and potential differences in linkage disequilibrium among various racial populations. As with other autoimmune disorders, MS is a multifactorial disease, meaning that combinations of genetic and still unknown environmental factors, such as microbial agents, may influence the risk of disease. In addition, it cannot be excluded that the etiologic factors involved in MS may differ among different populations in geographically distinct regions.

We were not able to detect any association between this polymorphism and susceptibility to MS. Although this is in line with some other studies it can be due, at least in part, to the relatively small sample size of our study. Further studies with larger sample size are required to verify this point.

In conclusion, there may not be any association between CTLA-4 gene (-318 T/C) polymorphism and MS development.

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