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碩士學位請求論文

Single Nucleotide Polymorphisms of the *Cytotoxic T Lymphocyte Antigen 4* Gene in patients with Behcet's Disease

한국인 베체트병의 *CTLA4* 다형성

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論 文 槪 要

세포독성 T 림프구 항원 4 (Cytotoxic T lymphocyte antigen 4; CTLA4, CD152)는 활성 T 세포에 발현하는 공동자극분자로 항원제시세포(Antigen-presenting Cell; APC)의 B7 분자와 결합하여 T 세포의 분화 및 활성 증가에 영향을 미치는 IL-2의 생성을 감소시켜 T 세포의 활성을 억제한다. 이 연구에서는 *CTLA4* 유전자의 단일염기다형성(SNPs)과 일배체형(haplotype)을 만성염증성 질환인 베체트병의 발병과 임상적인 특징에 연관하여 분석하였다.

베체트병 환자군의 *CTLA4 -1661*G/*G* 유전자형은 건강인보다 통계적으로 유의하게 높은 빈도를 나타냈다 (p=0.019, OR=5.2, 95% CI=1.13-23.86). 특히 안병변 증상을 가진 베체트병 환자와 안병변 증상이 없는 베체트병 환자를 비교하였을 때 *CTLA4 -1722*T/*C* 유전자형은 유의하게 높은 빈도 (p=0.014, OR=1.8, 95% CI=1.13-2.99)를, *CTLA4 -1722*C/*C* 유전자형은 유의하게 낮은 빈도 (p=0.018, OR=0.4, 95% CI=0.20-0.87)를 나타냈다.

베체트병 환자의 프로모터에 위치한 3개 SNPs 일배체형 중 *CTLA4 -1722*T-1661*A-318*T* 일배체형은 건강인에 비하여 유의하게 감소 (p=0.0003, OR=0.1, 95% CI=0.01-0.46)하였고, *CTLA4 -1722*T -1661*G-318*C* 일배체형은 환자에서 유의하게 증가 (p=0.046, OR=2.6, 95% CI=0.99-6.64)했다.

CTLA4 다형성은 베체트병 발병에 연관이 있는 유전자중 하나이며, 특히 *CTLA4*의 프로모터에 위치한 *-1722 T>C* 유전자 다형성은 안병변 증상을 가진 베체트병 환자에서 그 감수성이 높은 것으로 나타났다.

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Introduction

Behcet's Disease (BD) was first described in 1937 by Hulusi Behcet as a trisymptom complex, characterized by recurrent oral and genital ulcers, and uveitis (Behcet *et al.*, 1937). Current studies have shown that BD is a multisystemic inflammatory disorder. With time, this lead to numerous complications such as oral ulcers, skin lesions, genital ulcers, ocular lesions, arthritis, large vessel involvement (LVS), gastrointestinal-involvement (GI) and central nervous system involvement (CNS) (Kaklamani *et al.*, 1998). The prevalence of BD high in Japan, China, Korea, Turkey, Iran, Tunisia, and in the Mediterranean and Middle Eastern countries whereas it is low in Northern Europe and in the United States. The frequency of the disease seems to follow the ancient Silk Route (Saylan *et al.*, 1999). The etiology of BD is still uncertain, but it is generally considered to be a multifactorial disease with important genetic and environmental components that determine susceptibility (Gul, 2001). In genetic factors, a strong association between the HLA class I antigen HLA-B51 and BD has long been known and T cells are considered to be important for controlling the development of HLA-associated diseases that have a chronic inflammatory disease (Ohno *et al.*, 1982). Cytotoxic T lymphocyte antigen 4 (CTLA4, CD152; OMIM 123890), CD28 (OMIM 186760) and their interacting ligands are involved in T cell regulation and are therefore potential candidates conferring susceptibility to chronic inflammatory diseases

(Naluai *et al.*, 2000).

The chromosome region 2q33 has also been implicated in a variety of chronic inflammatory and autoimmune diseases, which contains the three genes *CD28*, *CTLA4* and *Inducible co-stimulator* (*ICOS*; OMIM 604558) (Naluai *et al.*, 2000). *CTLA4* gene is a member of the immunoglobulin superfamily, and is a costimulatory molecule expressed on activated T cells (Brunet *et al.*, 1987). For T cell activation $CD4^+$ T cells recognize antigen-presenting cells by the antigen bound to MHC class II molecules, but antigen recognition alone is not sufficient and costimulation by other receptor-ligand complexes is required (Barreto *et al.*, 2004). CTLA4 is a main costimulatory T cell receptor that binds to B7-1 (CD80; OMIM 112203) and B7-2 (CD86; OMIM 601020) during antigenic stimulation of T cells, and plays a role in down-regulation of T cell activation against another competitive receptor, its homolog CD28 (31% identity), which operates on up-regulation of T cell activation (Harper *et al.*, 1991; Karandikar *et al.*, 1996; Salomon and Bluestone, 2001). Convincing evidence for an inhibitory role of CTLA4 is derived from CTLA4 knockout mice, which mice suffer from fatal T cell hyper-responsiveness that is apparent by 4 weeks after birth (Gough *et al.*, 2005). Unlike CD28, which is expressed on resting T cells, CTLA4 is not detected on the cell surface until 24 hours after activation, peaking at 36 to 48 hours after activation and having approximately 10 to 100 times higher affinity depending on their ligand (Thompson and Allison,

1997; Magistrelli *et al.*, 1999). Thus, the balance between the opposing signals elicited by CD28 and CTLA4 is central to the regulation of T cell responsiveness and homeostasis (Bluestone, 1997) (Figure 1).

CTLA4 have the steady-state mRNA levels of the two known isoform: a full length isoform (flCTLA4) encoded by exon 1 (leader peptide), exon 2 (ligand-binding domain), exon 3 (transmembrane domain) and exon 4 (cytoplasmic tail), and a soluble form (sCTLA4; CTLA4Ig), which lacks exon 3 (Magistrelli *et al.*, 1999). flCTLA4 exists only on the activated T cell surface after antigen presentation as a homodimer, interconnected by a single disulfide bridge (Cys 120) in the extracellular domain (Greenfield *et al.*, 1998). sCTLA4 of the alternative splicing, resulting in the loss of the cystein residue, occurs as for the Ig genes and the recombinant molecule is expressed the nonactivated peripheral blood T cell as a soluble monomeric protein in the serum (Magistrelli *et al.*, 1999) (Figure 2).

Ligation of CTLA4 inhibits production of IL-2 and cell cycle progression, while blockade of CTLA4 increases production of IL-2, IFN-γ, IL-3 and TNF-α in Th1 cell clones, and of IL-3, IL-4, IL-5 and IL-10 in Th2 cell clones (Krummel and Allison, 1996; Oosterwegel *et al.*, 1999; Alegre *et al.*, 1998). Also, it has been shown that signaling via CTLA4 can modulate production of TGF-β and suppress production of TNF-α and IL-1β, yet, CTLA4 knockout T cells can produce TGF-β and IL-10. These findings

support the notion of an inhibitory role for CTLA4 and at the same time indicate that alternative down-regulatory immune pathways may exist (Ligers *et al.*, 2001).

Most of the studies showed an association of *CTLA4* polymorphic alleles with inhibitory function of CTLA4 at the mRNA and protein levels in peripheral blood mononuclear cells, and also with various inflammatory and autoimmune diseases, such as Graves' disease, systemic lupus erythematosus (SLE), multiple sclerosis, type I diabetes (Kouki *et al.*, 2000; Ligers *et al.*, 2001; Heward *et al.*, 1999; Hudson et al., 2002; Bouqbis *et al.*, 2003).

This study was investigated on whether four *CTLA4* SNPs, *-1722 T>C*, *-1661 A>G*, *-318 C>T* in promoter and *+49 G>A* in exon 1, at *CTLA4* gene are associated with BD in Korean.

Materials and Methods

Patients

Two hundred eighty-five patients (136 male and 149 female) from 16 to 66 years of age registered at the Behcet's Disease Specialty Clinic of Severance Hospital at the Yonsei University College of Medicine and Ajou University School of Medicine and 287 healthy controls, were randomly selected included in this case-control study. BD was diagnosed according to the clinical criteria of the International Study Group for Behcet's Disease or the revised criteria of Behcet's Disease (Wechsler *et al.*, 1990). The prevalence of clinical symptoms in patients with BD is presented in Table 1.

Genotyping of the *CTLA4*

Genomic DNA extraction from peripheral blood cells was carried out by using the QIAamp Blood kit (Quiagen, Hilden, Germany). Genotyping for the *CTLA4* variants was determined according to previous studies. Genomic DNA was amplified by GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Foster City, CA, USA) in a total reaction volume of 10 ㎕ containing 15 ng genomic DNA, 10 mM Tris (pH 8.0), 40 mM KCl, 1.5 mM $MgCl^2$, 200 ㎛ dNTP, 5 pmoles of each primers, and 0.38 unit Taq DNA polymerase (Bioneer, Korea). The *CTLA4* promoter and exon 1 polymorphisms were genotyped by polymerase chain reaction-

restriction fragment length polymorphism methods. Promoter-region polymorphisms at positions *CTLA4 -1722 T>C* and *-1661 A>G* were genotyped as follows by using a single primer set (Table 2): forward 5′ CTA AGA GCA TCC GCT TGC ACC T 3′ , reverse 5′ TTG GTG TGA TGC ACA GAA GCC TTT T 3′ and was determined by *Bbv* I and *Mse* I digestion (New England Biolabs, Beverly, MA, USA). Amplification was performed with initial denaturation for 5 min at 94 ℃, followed by thirty cycles for 15 s at 94 ℃, 30 s at 58 ℃, 45 s at 72 ℃, and a final extension for 7 min at 72 ℃ (Hudson *et al.*, 2002). To amplify the region containing the *CTLA4 -318 C>T* polymorphism the following primer pairs were used: forward 5′ AAA TGA ATT GGA CTG GAT GGT 3′ , reverse 5′ TTA CGA GAA AGG AAG CCG TG 3′ and was determined by *Mse* I digestion (New England Biolabs, Beverly, MA, USA). The PCR profile was as follows: initial denaturation at 94 ℃ for 2 min, followed by 40 cycles of 94 ℃ for 30 s, 60 ℃ for 30 s and 72 ℃ for 30 s, with final extension at 72 ℃ for 7 min (Ahmed *et al.*, 2001). The *+49 A>G* polymorphism in exon 1 of *CTLA4* was amplified using a forward primer 5′ AAG GCT CAG CTG AAC CTG GT 3′ , and a reverse primer 5′ CTG CTG AAA CAA ATG AAA CCC 3′ and was determined by *Bst*E Ⅱ digestion (New England Biolabs, Beverly, MA, USA): initial denaturation for 7 min at 95 ℃, followed by 30 cycles for denaturing for 45 s at 95 ℃, annealing for 45 s at 58 ℃, extension for 45 s at 72 ℃, and a final extension for 7 min at 72 ℃ (Marron *et al.*, 1997; Solerio *et al.*,

2005). Products were visualized on a 8% polyacrylamide gel by using ethidium bromide staining and UV illumination.

Statistical analysis

The Hardy-Weinberg equilibrium, Lewontin's D' (|D'|) and the linkage disequilibrium (LD) coefficient r^2 were analyzed using the R program v.2.2.0 (http://cran.r-project.org). The frequencies of genotype, allele, and haplotype of *CTLA4* gene between controls and BD patients were examined by the X^2 or Fisher exact test using SAS version 8.1 (SAS Institute, Cary, NC). By using the PHASE program, v.2.0.1, haplotypes of each individual were inferred (Stephens and Donnelly, 2003). A value of $p < 0.05$ was considered statistically significant. The p-value, where indicated as corrected p (p_c), has been subjected to the Bonferroni correction: the p-value was multiplied by the number of comparisons made.

Results

The frequencies of four *CTLA4* SNPs, *−1722 T>C*, *−1661 A>G*, *−318 C>T* and *+49 G>A*, genotype and allele were analyzed in 285 BD patients and 287 controls among Koreans. The alleles in *CTLA4* gene were in strong LD (Table 3).

The distribution of genotype and alleles frequencies of BD patients and controls at the four SNPs is presented in Table 4. The genotype frequency of the *CTLA4 −1661*G/*G* was significantly higher in BD patients than in control subjects (p=0.019, OR=5.2, 95% CI=1.13−23.86). Furthermore, genotype frequency of *CTLA4 −1722*T/*C* was significantly high (p=0.014, OR=1.8, 95% CI=1.13−2.99), while *CTLA4 −1722*C/*C* was significantly lower (p=0.018, OR=0.4, 95% CI=0.20−0.87) in BD patients with ocular lesions compared to BD patients without this symptom (Table 5).

The haplotype frequency of the *CTLA4 −1722, −1661* and *−318* showed a significant difference between BD patients and controls by permutation test (p=0.01). The frequency of the *−1722*T−1661*A −318*T* haplotype was significantly low in the BD patients as compared to controls (p=0.001, OR=0.1, 95% CI=0.01−0.46), and the *−1722*T−1661*G−318*C* haplotype was significantly high in BD patients (p=0.046, OR=2.6, 95% CI=0.99−6.64) as compared to that in controls (Table 6).

Discussion

This study searching for a possible association among *CTLA4* polymorphisms and BD explore four single nucleotide polymorphisms located at positions −1722, −1661, −318 and +49 of *CTLA4* gene. Although the functional implication of *CTLA4* polymorphisms not well established, the polymorphisms with the promoter and first exon of the *CTLA4* gene have been suggested to be related to the expression levels of CTLA4 molecules (Ligers *et al.*, 2001). Genotype of *CTLA4* polymorphisms is associated with reduced inhibitory function of CTLA4, therefore, the genotype may cause an increase in Th1 cell proliferation and a tendency to the development of inflammatory diseases. Many studies have shown higher levels of Th1 pro−inflammatory cytokines, including TNF−α, IFN−γ, and IL−2, −12 and −18, suggesting a shift towards a Th1 type of immune response that could be responsible for the higher degree of immune activity in BD (Sallakci *et al.*, 2005).

The results of this study show a strong association between the *CTLA4 −1661*G/*G* genotype in promoter region and BD. The functional significance of the *CTLA4* promoter region polymorphisms is not well established. However, this could change the transcript level of the protein, and thus influence the amount of protein produced by altering transcription−factor−binding sites or other regulatory domains. Thus, this could result in the observed interindividual differences in susceptibility to diseases characterized

by aberrations in immune homeostasis (Hudson *et al.*, 2002). This *−1661*G/*G* homozygote could associated with reduced inhibitory function of CTLA4, therefore, this may cause an activation of T cell and increase in Th1 cell proliferation, thus a tendency to the development of chronic inflammatory diseases such as BD.

This results did not find any statistical significant differences between *CTLA4 −1722 T>C*, *−318 C>T* and *+49 A>G* polymorphisms and BD patients. In particular, genotypes of *CTLA4 −1722*T/*C* and *CTLA4 −1722*C/*C* were significant in BD patients with ocular lesions compared with patients without this symptom. −1722T may decrease the transcription level of the gene, and −1722C may be required for the optimal binding of the putative transcription factor (Hudson *et al.*, 2002). This could account for the association of *−1722*T/*C* heterozygotes with susceptibility and *−1722*C/*C* homozygotes with tolerance to the BD. CTLA4 expression is primarily restricted to a subset of T regulatory cells and a possible increase in CTLA4 protein expression by the regulatory T cells in various tissues such as the eye might also explain (Sallakci *et al.*, 2005). Antigen−presenting cells in ocular capture eye−derived antigens and deliver them to the spleen where multicellular clusters of natural killer T cells, marginal zone B cells, and gammadelta T cells create an antigen−presentation environment that leads to alpha/beta T cells such as regulators, suppress induction and expression of Th1 and Th2 immune expression systems (Streilein, 2003). Taken together, it can be speculated that

the *CTLA4 −1722 T>C* in promoter region polymorphism may act to regulate the proliferation of ocular-driving T cells, thus −1722C may suppress Th1 cell function and may cause a relative Th2 cell activation, leading to immune complex-mediated BD with ocular lesions.

An allelic polymorphism in exon 1 of the *CTLA4* gene coding for the peptide leader sequence of CTLA4 was described and has been implicated in several autoimmune disorders (Maurer *et al.*, 2002; Belzen *et al.*, 2004). The *CTLA4 +49 A>G* polymorphism is the most extensively studied, as this is the only polymorphism in the three genes, *CD28*, *CTLA4* and *ICOS*, that alters and amino acid. The *CTLA4 +49***A* allele encodes a threonine at codon 17, whereas the *+49G* allele encodes an alanine (Ueda *et al.*, 2003). This amino acid exchange of the *CTLA4* gene could, therefore, have a negative affect on the down-regulation of T cell function, with subsequent increases in autoantibody formation and the development of disease (Heward *et al.*, 1999). This study did not find any association with BD patients in Korean, however Sallakci and colleagues found that the *CTLA4* **A* allele and **A*/**A* genotype was associated with BD patients in Turkish (Sallakci *et al.*, 2005). Moreover, most of the studies in the literature concerning the *CTLA4 +49 A>G* polymorphism have demonstrated an association with various diseases, such as Graves' disease, inflammatory bowel disease, type 1 diabetes, DR4-positive rheumatoid arthritis and multiple sclerosis (Braun *et al.*, 1998; Machida *et al.*, 2005; Donner *et al.*,

1997; Seidl *et al.*, 1998; Harbo *et al.*, 1999).

In addition, the haplotype analysis performed shows a statistical significant decrease of the *−1722*T−1661*A−318*T* and increase of the *−1722*T−1661*G−318*C* in BD patients compared to controls. The *p*−value of *1722*T−1661*A−318*T* haplotype, where indicated as corrected p_c by using the Bonferroni correction, had significant in BD patients comparisons between the controls (p_c=0.008). These statistical differences reflect a variation of −1661 or −318 polymorphisms in BD patients compared to controls. There are at present suggesting that it has revealed that all of the risk for BD in *CTLA4* can be attributed to a variation of promoter SNPs, *CTLA4 −1661*G* and *CTLA4 −318*T*.

In the table 7, the *CTLA4 −1722*T* allele is present at low frequency in Korean (approximately 60%), whereas higher frequency (93%) in the White population of USA. The allelic frequency found that *−318 C>T* polymorphism was not different in among different ethnic group. *CTLA4 +49*A* allele is lower frequency in Orientals (Korean, Japanese and Chinese; less than 53%, almost 32%) than the Turkish, South Moroccans, African−American and Caucasian populations (Italian, Spanish and White in USA more than 62%). Moreover, *CTLA4 +49*A* allele and **A/*A* genotype were association with BD in Turkish whereas no association in Korean. In this observation, it is difficult to explain but may be based on part in differences in the allelic frequencies among the Korean and other populations.

In conclusion, variations of SNPs in *CTLA4* promoter region were associated with reduced inhibitory function of CTLA4 and may cause an activation of T cell and increase in Th1 and Th2 cells proliferation thus a tendency to the development of BD. It is influence that polymorphisms of *CTLA4* promoter region may contribute to predispose to BD patients and *CTLA4 -1722 T>C* polymorphism may serve as a clinically useful marker Korean BD patients with ocular lesions. Therefore, future expression analyses should consider promoter region polymorphisms in order to detect differences in CTLA4 expression.

Table 1. Demographic characteristics of patients with BD and frequency of clinical symptoms

Clinical features	Total (%)	Male (%)	Female (%)
	285 (100.0)	136 (47.7)	149 (52.3)
Age (years)	3-58	3-58	5-56
Major symptoms			
Oral ulcers	284 (99.6)	135 (99.3)	149 (100.0)
Skin lesions	252 (88.4)	122 (89.7)	130 (87.2)
Genital ulcers	230 (80.7)	101 (74.3)	129 (86.6)
Ocular lesions	179 (62.8)	92 (67.6)	87 (58.4)
Minor symptoms			
Arthritis	122 (42.8)	53 (39.0)	69 (46.3)
Large vessel involvement	41 (14.4)	35 (25.7)	6 (4.0)
Gatrointestinal involvement	17 (6.0)	8 (5.9)	9 (6.0)
Central nervous system involvement	9 (3.2)	2 (1.5)	7 (4.7)

Table 2. Sequences of PCR primer and methods

SNP	Primers (5′ →3′)	Method	Reference
−1722 T>C	F:CTAAGAGCATCCGCTTGCACCT R:TTGGTGTGATGCACAGAAGCCTTTT	*Bbv* I (486bp) T allele: 270bp C allele: 486bp	Hudson *et al.*, 2002
−1661 A>G	F:CTAAGAGCATCCGCTTGCACCT R:TTGGTGTGATGCACAGAAGCCTTTT	*Mse* I (486bp) A allele: 347bp G allele: 486bp	Hudson *et al.*, 2002
−318 C>T	F:AAATGAATTGGACTGGATGGT R:TTACGAGAAAGGAAGCCGTG	*Mse* I (247bp) C allele: 226+21bp T allele: 130+96+21bp	Ahmed *et al.*, 2001
+49 A>G	F:AAGGCTCAGCTGAACCTGGT R:CTGCTGAAACAAATGAAACCC	*Bst*E II (152bp) A allele: 130bp G allele: 152bp	Marron *et al.*, 1997 Solerio *et al.*, 2005

Table 3. Linkage disequilibrium coefficients (| D' | and r^2) between SNPs of *CTLA4* in the Korean population (n=287)

		| D' |			
		−1722 T>C	*−1661 A>G*	*−318 C>T*	*+49 A>G*
r^2	*−1722 T>C*	−	0.94	0.90	0.92
	−1661 A>G	0.090	−	0.88	0.96
	−318 C>T	0.102	0.656	−	0.81
	+49 A>G	0.230	0.348	0.281	−

| D' | value is given above the diagonal; r^2 is given below the diagonal

Table 4. The genotype frequencies of *CTLA4* polymorphisms in BD patients and controls

SNP	BD n=285 (%)	Controls n=287 (%)	*p*	OR (95% CI)
CTLA4 −1722 T>C				
*T/T	97 (34.0)	106 (36.9)		
*T/C	156 (54.8)	136 (47.4)		
*C/C	32 (11.2)	45 (15.7)		
*CTLA4 −1722*T*	0.614	0.606		
CTLA4 −1661 A>G				
*A/A	199 (69.8)	209 (72.8)		
*A/G	76 (26.7)	76 (26.5)		
*G/G	10 (3.5)	2 (0.7)	0.019	5.2 (1.13−23.86)
*CTLA4 −1661*A*	0.832	0.861		
CTLA4 −318 C>T				
*C/C	213 (74.7)	198 (69.0)		
*C/T	69 (24.2)	87 (30.3)		
*T/T	3 (1.1)	2 (0.7)		
*CTLA4 −318*C*	0.868	0.841		
CTLA4 +49 A>G (rs231775)				
*A/A	26 (9.1)	26 (9.0)		
*A/G	115 (40.4)	121 (42.2)		
*G/G	144 (50.5)	140 (48.8)		
*CTLA4 +49*A*	0.293	0.301		

p: BD patiens *vs.* controls

Table 5. The genotype and allele frequencies of *CTLA4* polymorphisms in BD patients with and without various symptoms

SNP	Controls n=287 (%)	BD							
		Genital ulcers		Ocular lesions		Arthritis		LVI	
		with n=230 (%)	without n=55 (%)	with n=179 (%)	without n=106 (%)	with n=122 (%)	without n=163 (%)	with n=41 (%)	without n=244 (%)
CTLA4 −1722 T>C									
*T/T	106 (36.9)	79 (34.3)	18 (32.7)	57 (31.9)	40 (37.7)	43 (35.2)	54 (33.1)	15 (36.6)	82 (33.6)
*T/C	136 (47.4)	125 (54.3)	31 (56.3)	108 (60.3)	48 (45.3)	66 (54.1)	90 (55.2)	20 (48.8)	136 (55.7)
p, OR(95% CI)				0.014, 1.8(1.13−2.99)					
*C/C	45 (15.7)	26 (11.4)	6 (11.0)	14 (7.8)	18 (17.0)	13 (10.7)	19 (11.7)	6 (14.6)	26 (10.7)
p, OR(95% CI)				0.018, 0.4(0.30−0.87)					
*CTLA4 −1722*T*	0.606	0.615	0.609	0.620	0.604	0.623	0.607	0.610	0.615
CTLA4 −1661 A>G									
*A/A	209 (72.8)	161 (70.0)	38 (69.1)	126 (70.4)	73 (68.9)	87 (71.3)	112 (68.7)	26 (63.4)	173 (70.9)
*A/G	76 (26.5)	60 (26.1)	16 (29.1)	46 (25.7)	30 (28.3)	30 (24.6)	46 (28.2)	12 (29.3)	64 (26.2)
*G/G	2 (0.7)	9 (3.9)	1 (1.8)	7 (3.9)	3 (2.8)	5 (4.1)	5 (3.1)	3 (7.3)	7 (2.9)
*CTLA4 −1661*A*	0.861	0.830	0.836	0.832	0.830	0.836	0.828	0.780	0.840
CTLA4 −318 C>T									
*C/C	198 (69.0)	170 (73.9)	43 (78.2)	137 (76.5)	76 (71.7)	93 (76.2)	120 (73.6)	28 (68.3)	185 (75.8)
*C/T	87 (30.3)	57 (24.8)	12 (21.8)	39 (21.8)	30 (28.3)	28 (23.0)	41 (25.2)	12 (29.3)	57 (23.4)
*T/T	2 (0.7)	3 (1.3)	0 (0.0)	3 (1.7)	0 (0.0)	1 (0.8)	2 (1.2)	1 (2.4)	2 (0.8)
*CTLA4 −318*C*	0.841	0.863	0.891	0.874	0.858	0.877	0.862	0.829	0.875
CTLA4 +49 A>G									
*A/A	26 (9.0)	21 (9.1)	5 (9.1)	19 (10.6)	7 (6.6)	14 (11.5)	12 (7.4)	3 (7.4)	23 (9.4)
*A/G	121 (42.2)	95 (41.3)	20 (36.4)	67 (37.4)	48 (45.3)	48 (39.3)	67 (41.1)	19 (46.3)	96 (39.4)
*G/G	140 (48.8)	114 (49.6)	30 (54.5)	93 (52.0)	51 (48.1)	60 (49.2)	84 (51.5)	19 (46.3)	125 (51.2)
*CTLA4 +49*A*	0.301	0.298	0.273	0.293	0.292	0.311	0.279	0.305	0.291

p: BD patients with symptom *vs.* without symptom

Table 6. The haplotype frequencies of *CTLA4* promoter polymorphisms in BD patients and controls

Haplotype	n	*CTLA4*					
		*-1722*T-1661*A-318*C*	*-1722*C-1661*A-318*C*	*-1722*T-1661*G-318*T*	*-1722*T-1661*A-318*T*	-1722*T-1661*G-318*C*	Others
		p, OR (95% CI)	*p*, OR (95% CI)	*p*, OR (95% CI)	*p*, OR (95% CI)	*p*, OR (95% CI)	
Control	574	0.443	0.384	0.125	0.028	0.010	0.012
BD	570	0.455	0.374	0.129	0.002 0.001, 0.1(0.01-0.46)	0.027 0.046, 2.6 (0.99-6.64)	0.013
Oral ulcers	568	0.459	0.369	0.124	0.003 0.001, 0.1(0.03-0.54)	0.029 0.029, 2.7(1.07-7.06)	0.016
Skin lesions	504	0.451	0.372	0.134	0.003 0.002, 0.1(0.03-0.61)	0.027 0.035, 2.7(1.03-7.09)	0.013
Genital ulcers	460	0.454	0.373	0.134	0.003 0.001, 0.1 (0.01-0.58)	0.024 0.091, 2.3(0.85-6.32)	0.012
Ocular lesions	358	0.468	0.364	0.125	0	0.027 0.046, 2.7 (0.98-7.55)	0.016
Arthritis	244	0.468	0.363	0.118	0.005 0.029, 0.1(0.02-1.09)	0.032 0.024, 3.2 (1.10-9.35)	0.014
LVI	82	0.397	0.370	0.155	0.014	0.045 0.001, 4.9(1.34-17.59)	0.019
GI	34	0.507	0.315	0.146	0.001	0.022	0.009
CNS	18	0.389	0.444	0.111	0	0.056	0

p: BD patients *vs.* controls

Table 7. The frequencies of genotype and allele of *CTLA4* in other population

Populations	Genotype frequency n (%)			Allele Frequency	Reference
CTLA4 −1722 T>C	*T/*T	*T/*C	*C/*C	*T	
Korean (n=287)	106 (36.9)	136 (47.4)	45 (15.7)	0.606	This study
〃 (n=200)	66 (33.0)	103 (51.5)	31 (15.5)	0.588	Hudson *et al.*, 2002
African-American (n=72)	48 (66.7)	23 (31.9)	1 (1.4)	0.826	Parks *et al.*, 2004
White in USA (n=202)	175 (86.6)	24 (11.9)	3 (1.5)	0.926	Parks *et al.*, 2004
South Moroccans (n=114)	105 (92.0)	9 (8.0)	0 (0.0)	0.961	Bouqbis *et al.*, 2003
Spanish (n=194)	150 (77.3)	44 (22.7)	0 (0.0)	0.887	Aguilar *et al.*, 2003
CTLA4 −1661 A>G	*A/*A	*A/*G	*G/*G	*A	
Korean (n=287)	209 (72.8)	76 (26.5)	2 (0.7)	0.861	This study
〃 (n=200)	145 (72.5)	50 (25.0)	5 (2.5)	0.850	Hudson *et al.*, 2002
African-American (n=72)	46 (63.9)	25 (34.7)	1 (1.4)	0.813	Parks *et al.*, 2004
White in USA (n=202)	129 (63.9)	68 (33.6)	5 (2.5)	0.807	Parks *et al.*, 2004
South Moroccans (n=114)	76 (67.0)	31 (27.0)	7 (6.0)	0.803	Bouqbis *et al.*, 2003
CTLA4 −318 C>T	*C/*C	*C/*T	*T/*T	*C	
Korean (n=287)	198 (69.0)	87 (30.3)	2 (0.7)	0.841	This study
〃 (n=86)	67 (77.9)	15 (7.4)	4 (4.7)	0.866	Lee *et al.*, 2002
〃 (n=200)	146 (73.0)	49 (24.5)	5 (2.5)	0.853	Hudson *et al.*, 2002
Japanese (n=200)	163 (81.5)	36 (18.0)	1 (0.5)	0.905	Machida *et al.*, 2005
〃 (n=200)	157 (78.5)	43 (21.5)	0 (0.0)	0.893	Ahmed *et al.*, 2001
Chinese (n=204)	105 (51.5)	93 (45.6)	6 (2.9)	0.743	Hou *et al.*, 2005
African-American (n=72)	69 (95.8)	3 (4.2)	0 (0.0)	0.979	Parks *et al.*, 2004
White in USA (n=202)	174 (86.1)	26 (12.9)	2 (1.0)	0.926	Parks *et al.*, 2004
South Moroccans (n=114)	110 (96.0)	4 (4.0)	0 (0.0)	0.982	Bouqbis *et al.*, 2003
Spanish (n=194)	155 (80.0)	39 (20.1)	0 (0.0)	0.899	Aguilar *et al.*, 2003

Populations	Genotype frequency n (%)			Allele frequency	Reference
CTLA4 +49 A>G	**A/*A*	**A/*G*	**G/*G*	**A*	
Korean (n=287)	26 (9.0)	121 (42.2)	140 (48.8)	0.301	This study
〃 (n=86)	8 (9.3)	29 (33.7)	49 (57.0)	0.262	Lee *et al.*, 2002
〃 (n=200)	24 (12.0)	83 (41.5)	93 (46.5)	0.328	Hudson *et al.*, 2002
Japanese (n=200)	33 (16.5)	93 (46.5)	74 (37.0)	0.398	Machida *et al.*, 2005
〃 (n=200)	62 (31.0)	105 (52.5)	33 (16.5)	0.573	Ahmed *et al.*, 2001
Chinese (n=204)	42 (20.6)	113 (55.4)	49 (24.0)	0.483	Hou *et al.*, 2005
〃 (n=203)	18 (9.0)	100 (49.2)	85 (41.8)	0.335	Lee *et al.*, 2003
Turkish (n=99)	43 (43.4)	49 (49.5)	7 (7.1)	0.682	Sallakci *et al.*, 2005
Italian (n=238)	128 (53.8)	91 (38.2)	19 (8.0)	0.729	Solerio *et al.*, 2005
African-American (n=72)	26 (36.1)	38 (52.8)	8 (11.1)	0.625	Parks *et al.*, 2004
White in USA (n=202)	83 (41.1)	85 (42.1)	33 (16.3)	0.621	Parks *et al.*, 2004
South Moroccans (n=114)	59 (52.0)	47 (41.0)	8 (7.0)	0.724	Bouqbis *et al.*, 2003
Spanish (n=194)	110 (56.7)	67 (34.5)	17 (8.8)	0.740	Aguilar *et al.*, 2003

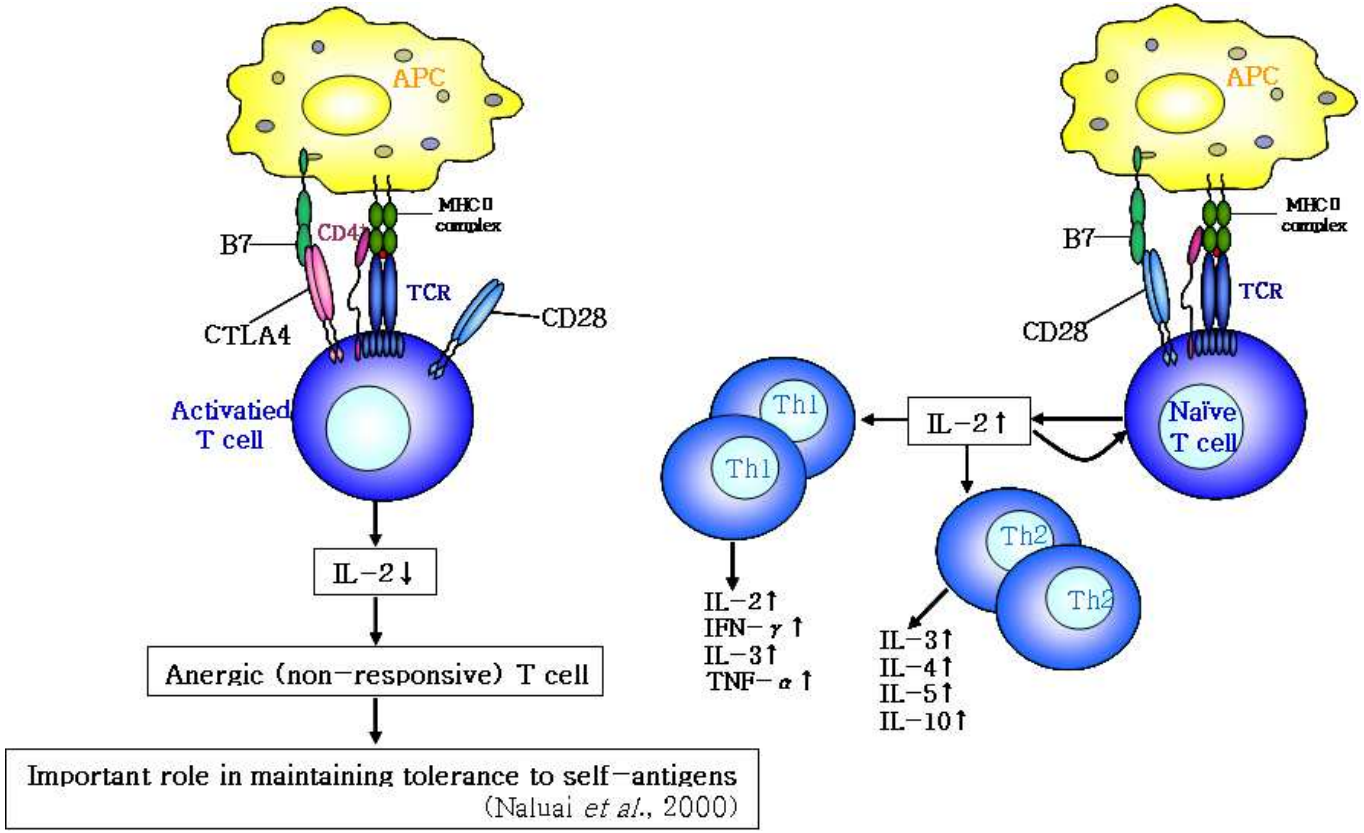

Figure 1. Immunologic role of CTLA4

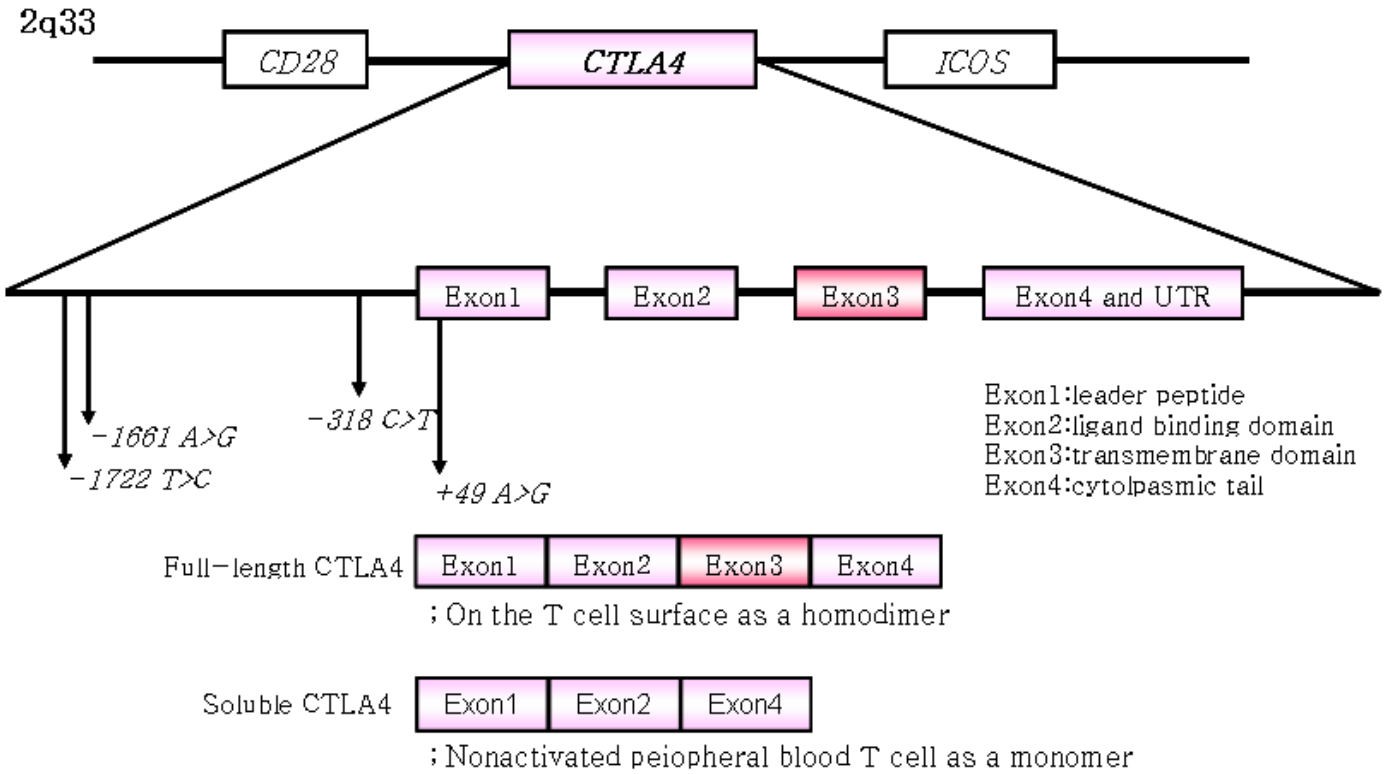

Figure 2. Structure of *CTLA4* gene and location of *CTLA4* polymorphisms

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Abstract

Single Nucleotide Polymorphisms of the Cytotoxic T Lymphocyte Antigen 4 Gene in patients with Behçet's Disease

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The cytotoxic T lymphocyte antigen 4 (CTLA4; CD152) is a costimulatory molecule expressed on activated T cells and plays a key role of inhibitory regulator of the T lymphocyte activation, and may help to limit T cell response under conditions of inflammation. The aim of this study was to investigate the association between the polymorphisms of *CTLA4* gene and BD. *CTLA4* gene promoter region (*-1722 T>C*, *-1661 A>G* and *-318 C>T*) and exon 1 (*+49 G>A*) polymorphisms were determined by PCR-RFLP in 285 BD patients and 287 controls. The frequency of *CTLA4 -1661*G/*G* genotype was significantly higher in BD patients than in controls (p=0.019, OR=5.2, 95% CI=1.13-23.86). Moreover, genotype frequency of *CTLA4 -1722*T/*C* was significantly high (p=0.014, OR=1.8, 95% CI=1.13-2.99), while *CTLA4 -1722*C/*C* was significantly low (p=0.018, OR=0.4, 95% CI=0.20-0.87) in the BD with ocular lesions compared

to the patients without this symptom. The promoter haplotype frequency of *CTLA4 −1722*T−1661*A−318*T* was low (p=0.0003, OR=0.1, 95% CI=0.01−0.46), however *CTLA4 −1722*T−1661*G −318*C* was high (p=0.046, OR=2.6, 95% CI=0.99−6.64) in BD patients as compared to controls. These results indicate that SNPs of promoter region in *CTLA4* gene have a candidate predispose site to BD and suggest that the *CTLA4 −1722 T>C* may contribute to the clinical useful marker of BD with ocular lesions.

감사의 글

논문을 완성하기까지의 지난 시간들은 참으로 많은 것을 배우고 경험할 수 있었던 잊지 못할 순간의 연속이었습니다. 그 순간마다 많은 힘이 되어주신 분께 감사의 마음을 전하고자 합니다.

부족한 저를 꾸준한 관심과 애정으로 지켜봐주시고 세심한 지도와 격려로 이끌어주신 박경숙 교수님께 감사드립니다. 교수님의 제자라는 이름을 잊지 않고 성실하고 최선을 다하는 마음가짐으로 항상 앞을 향하는 과학자가 되도록 노력하겠습니다. 바쁘신 가운데 논문심사를 맡아주시고 아낌없는 조언을 해주신 김호연 교수님과 성주헌 교수님께 감사의 말씀을 드리고 싶습니다. 학부 시절부터 많은 가르침을 주신 배인하 교수님, 오용자 교수님, 김진일 교수님, 강혜순 교수님, 윤진호 교수님, 전용필 교수님께 감사드립니다. 그리고 어린 시절의 저에게 과학 공부의 즐거움을 가르쳐주신 김정혁 은사님께 감사의 마음을 전해드립니다.

말도 많고 탈도 많았던 우리 유전학 연구실의 식구인 정현언니와 미영언니, 맘고생 많았을 동기이자 후배인 진이에게 고마움을 전합니다. 특히 지난 2년 동안 울고 웃고 싸우며(!?), 단 한 순간도 빼놓지 않고 함께 同居同苦 해온 사랑하는 나의 동기 나영이랑 진실이에게 정말 고맙다는 말을 전하고 싶습니다. 그리고 언제, 어떤 황당한 일로 연락을 하더라도 많은 조언을 해준 진선선배, 정아선배, 미정선배와 생물학과 대학원에서 함께 시간을 했던 여러 선배님과 후배들에게도 감사드립니다.

힘들고 지쳤을 때 옆에 있는 것만으로 많은 위로가 되어준 pcaqn의 유진, 희연, 희정, 정태, 성호, 내 자랑스러운 친구 민정, 화영, 영지, 일섭, 유별난 탈선 동기를 언니같이 보살펴주는 대구대 유특 97동기 숙란, 진숙, 유선, 지선, 윤경, 은경 그리고 다은엄마 지선, 친구같이 편한 동생 소운, 세진, 호식, 홍근, 형준, 언제나 어느 곳에서나 큰 힘이 되는 성신여대 생물 00동기 pcr의 지영, 미희, 은정, 민정, 윤정, 그리고 현진, 순임, 수민, 후배 수아에게 고마움을 전하고 앞으로도 서로 의지하며 언제까지나 함께 할 수 있기를 바랍니다. 여유 없는 대학원 생활에 심적 여유를 불어 넣어 준 청년성서모임 창세기 361차 조명준로마노 신부님과 이하늘나리유스티나 자매님, 사랑합니다. Make A Wish 어린왕자팀의 김영민팀장님, 윤우영총무님, 은정, 유진, 유정씨에게도 감사의 마음 전하고 싶습니다. 그리고 우리의 wish kids 길모, 주용, 진민. 언제나 건강하고 밝고 이쁘게 자라나렴! ^^

짧은 만남 긴 그리움, 항상 옆에 있어주는 그리운 명칭만 사촌인 내 가족 선아언니, 민아언니, 민영이, 인덕이. 마냥 좋은 미혜이모, 지금까지도 늘 보고 싶은 우리 할머니. 세상에서 제일 사랑하는 내 동생 진주, 그리고 인학이에게 감사의 마음을 전합니다. 늘 부족한 딸이지만 헤아릴 수 없는 큰 사랑과 인내로 언제나 자신감 있는 사람이 될 수 있게 키워주신 우리 마빠, 엄마. 어디 내놓아도 부끄럽지 않도록 항상 노력하는 큰 딸이 되겠습니다. 감사합니다 그리고 사랑합니다. 마지막으로, 항상 돌보아주시고 마련해주시는 나의 하느님, 감사합니다.