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Polymorphisms of Natural Killer Cell Receptor *NKG2-A, -C* and *CD94* genes in Behcet's Disease

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論 文 槪 要

자연살해세포 (Natural Killer cells; NK cells)는 세포막의 다양한 수용체로부터 외부의 신호를 받아 표적 세포 주변으로 싸이토카인을 불러 모으거나 표적 세포를 직접 자연살해, 또는 그 기능을 억제하여 염증반응을 조절하는 내재 면역의 중요 구성 세포로, CD94/NKG2-A 수용체는 억제신호를, CD94/NKG2-C 수용체는 활성신호를 자연살해세포 내로 전달한다. 베체트병은 전신 복합적 만성 염증성 질환으로, 자연살해세포의 활성도가 건강인에서보다 베체트병 환자군에서 저하되었으며 비정상 자연살해세포 수용체가 베체트병 환자군에서 발현되었다. 이 논문에서는 자연살해세포의 세포독성 기능을 억제하는 CD94/NKG2-A 수용체 유전자와 활성 *CD94/NKG2-C* 수용체 유전자의 단일염기다형성을 베체트병 환자군과 연관하여 분석하였다.

*NKG2-A c.-4258C>G, c.284_67-62del, c.338-90A>G, c.1077C>T, NKG2-C c.305C>T, CD94 c.-134A>T*의 여섯 부위의 단일염기다형성을 실험하였다. NKG2-A, -C와 각각 헤테로다이머를 이루는 CD94 유전자의 5'UTR에 있는 *c.-134*A* allele은 베체트병 환자군에서 건강인보다 통계적으로 유의하게 높은 빈도를 나타냈다 (P = 0.021, OR = 1.3, 95% CI = 1.04 - 1.58). *NKG2-A*의 프로모터부위에 있으며 자연살해세포의 세포독성을 높인다고 알려진 *c.-4258*G* allele (P = 0.015, OR = 1.3, 95% CI = 1.05 - 1.60)과, *NKG2-A*의 인트론 4에 있는 *c.338-90*A* allele (P <.0001, OR = 1.9, 95% CI = 1.47 - 2.33)은 베체트병 환자군에서 건강인보다 통계적으로 유의하게 높은 빈도를 나타냈다. 특이하게도 *NKG2-C c.305*T* allele은 안병변을 가지거나 (P <.0001, OR = 2.1, 95% CI = 1.61 - 2.75) 류마티즘 증상을 가진 (P = 0.003, OR = 1.8, 95% CI = 1.22 - 2.68) 베체트병 환자군에서만 유의하게 높은 빈도를 나타냈다.

*NKG2-A*와 *NKG2-C*, *CD94*의 SNP간의 linkage는 보이지 않았으며, risk SNP간의 combined test에서도 특별한 addictive effects는 보이지 않았다.

결과적으로, NKG2-A와 NKG2-C 수용체와 각각 헤테로다이머를 이루는 CD94 유전자의 *c.-134A>T* 단일염기다형성과 억제 수용체로 작용하는 NKG2-A 유전자의 *c.-4258C>G*와 *c.338-90A>G* 단일염기다형성은 베체트병 발병과 연관이 있으며, 활성수용체 NKG2-C 유전자의 *c.305C>T* 단일염기다형성은 안병변과 류마티즘 증상을 가진 베체트병 환자군에서 그 감수성이 높은 것으로 나타났다.

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Introduction

Natural killer (NK) cells are lymphocytes that play an important role in the regulation of innate immune response through participating in inflammatory response inducing inflammatory cytokines (IFN-ɣ and TNF-α) and chemokines and directly attack target cells that lack self-MHC class I molecules such as tumorigenic and virus-infected cells (Lieto et al., 2006, Borrego et al., 2005, Biron et al., 1999, David et al., 2001). The role of NK cells is regulated by the balance of signals of receptors, killer cell immunoglobulin-like receptor (KIR) family and C-type lectin-like NK cell receptors CD94/NKG2 heterodimers in human. CD94/NKG2 consist of one CD94 molecule and one NKG2 family member which contain a C-type lectin domain. These receptors capable of recognizing target cells transmit inhibitory and activating signals into NK cells (Gunturi et al., 2004). The CD94/NKG2-A (or its isoform NKG2-B) heterodimer has immunoreceptor tyrosine-based inhibitory motifs essential for the recruitment of phosphatase (SHP-1, SHP-2, and SHIP) in their cytoplasm and transmits inhibitory signal. The CD94/NKG2-C (or -E, -H (isoform of NKG2-E)) heterodimer serve as activating receptor and pair with the DAP12 adapter protein for stable expression on the cell surface and for signaling (Lanier et al., 1998). But, NKG2-D is unique in that it does no dimerize with CD94; instead, it associates with the adaptor molecules DAP10 and DAP12 for its activating function (Gilfillan et al., 2002). The CD94 does not have a proper cytoplasmic domain, but is required for the transport

and membrane expression of NKG2 protein (O'Callaghan., 2000). The CD94/NKG2 heterodimeric receptors recognize the nonclassical MHC class Ⅰ (class Ⅰb) molecule, HLA-E, presenting MHC class Ⅰ (class Ⅰa)-derived leader peptides. The HLA-E/leader peptide complex is sufficient either to inhibit NK cells expressing inhibitory CD94/NKG2-A receptors or to enhance NK cells expressing activatory CD94/NKG2-C receptors. The peptides bound to HLA-E can differentially affect recognition by the inhibitory and activating receptors. HLA-E/HLA-G peptide complex triggered cytotoxicity and HLA-E/HLA-C peptide complex bound to inhibitory receptor. The CD94/NKG2-A inhibitory receptor appears to bind ligand with a higher affinity than the CD94/NKG2-C activating receptor (Llano et al., 1998, Vales-Gomez et al., 1999).

Behcet's disease (BD; MIM 109650) is a chronic inflammatory disorder characterized by recurrent oral and genital ulcerations, uveitis, skin lesions, ocular symptoms, arthritis, large vessel involvements (LVI), central nervous systems (CNS) and gastrointestinal lesions (GI) (Marshall., 2004). There is a tendency for a higher incidence of BD among the Asian and Eurasian populations along the Silk Route stretching to the countries of the Mediterranean region (Saylan et al., 1999). Although the etiology remains unclear, the combinations of multiple genetic and environmental factors have been implicated in its pathogenesis (Zierhut et al., 2003).

In several studies on BD, natural killer cells have been considered to regulate BD and suggested that immunological abnormalities and variants of inflammatory molecules may be involved in the etiology of

BD. Kaneko et al. (1985) observed that the number of NK cells was markedly increased in peripheral blood of patients with active stage of BD but supporting the results of Onder et al. (1994) and Hamzaoui et al. (1988), the cytotoxic activity of NK cells was lower among BD patients than that of healthy controls. Takeno et al. (2004) reported the expression of abnormal and skewing inhibitory/activating NK cell receptors in patients with BD. These results may be relevant to the presence of abnormal forms and/or isoforms of NK cell receptors.

The gene encoding the CD94 (MIM; 602894, KLRD1) and NKG2-A (MIM; 161555, KLRC1), NKG2-C (MIM; 602891, KLRC2) molecules are located on chromosome 12p12.3-13.2 called the natural killer complex (NKC) (Plougastel et al., 1996) that shown to be the strong susceptibility region for BD (Karasneh et al., 2005). This region contains 19 genes encoding C-type lectins, including the other members of NKG2 family. The *NKG2-A* gene has seven exons including non-coding initial exon (Plougastel et al., 1996) that, by alternative splicing of the pre-mRNA, encode both NKG2-A and NKG2-B transcripts. The *NKG2-C* has six coding exons and the *CD94* gene has six coding exons (Lieto et al., 2003).

This study is to identify the polymorphisms of the *NKG2-A*, *NKG2-C* and *CD94* gene, and to test their association with the susceptibility to Behcet's Disease in a Korean population. The previous reports (Hikami et al., 2003, Hayashi et al., 2006) and National Center for Biothchnology Information (NCBI) and International HapMap Project (http://www.hapmap.org/) database were used to identify SNPs. Among the allele frequency over 5% in database, Six SNPs *NKG2-A*

c.-4258C>G (rs1983526) in promoter region, *c.284-67_62del* in intron 3, *c.338-90A>G* (rs2734440) in intron 4, *c.1077C>T* and *NKG2-C c.305C>T* (Ser102Phe) in exon 3 and *CD94 c.-134A>T* in 5'UTR, were investigated (locations displayed in Figure 1). The *c.-4258C>G* in promoter region of *NKG2-A* was reported that it is closely associated with the natural cytotoxic activity but the functional links and importance of the other polymorphisms are still uncertain (David et al., 2001, Hayashi et al., 2006).

Materials and Methods

Subjects

A total of 345 patients with BD registered at the Behcet's Disease Specialty Clinic of Severance Hospital at the Yonsei and The Ajou University School of Medicine, KOREA, and 368 healthy controls were included in this case-control study. The prevalence of clinical features in patients with BD is presented in the Table 1.

Genotyping

Genomic DNA was extracted from peripheral blood using a QIAamp Blood kit (Quiagen, Hilden, Germany). Genotyping for NKG2-A, -C and CD94 variants was determinded according to the reference (http://www.dmd.nl/mutnomen.html/). Genomic DNA was amplified by GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, U.S.A.) in 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 μM dNTP, 5 pmoles of each primers and 0.38 unit Taq DNA polymerase (Bioneer, Korea). The genotyping of *NKG2-A c.-4258C>G, c. 338-90A>G* and *c.1077C>T,* digested with the *Cac8 I , SnaB I and Hpy188Ⅲ* restriction enzyme, respectively, and *NKG2-C c.305C>T* (Ser102Phe), digested with the *MboⅡ* restriction enzyme, were done using PCR-RFLP method. The digested PCR products were electrophoresed on a 8% or 5% polyacrylamide gel and were stained with ethidium bromide to visualize the DNA fragments. The *NKG2-A c.284-62_67del* PCR

products was electrophoresed on 10% polyacrylamide gel. The *CD94 c.-134A>T* was genotyped by PCR-SSP method. In Table 2, the primer pairs and experimental methods of *NKG2* and *CD94* variants are listed.

Statistical analysis

The differences of allele frequencies and genotype distribution between BD patients and controls were examined by the χ^2 test using SAS v.9.1 (SAS Institute, Cary, NC). The *P*-values <0.05, and odds ratios (OR) with 95% CI were regarded as statistically significant. For multiple comparison analysis, the *P*-value, where indicated as corrected *P* (P_c), has been subjected to Bonferroni correction: the *P*-values was multiplied by the number of comparisons made (Bonferroni corrected P_c <0.008 as significant level). The Hardy-Weinberg equilibrium and linkage disequilibrium (LD) were analyzed using the R program v.2.2.0 (http://cran.r-projects.org/) in healthy controls. LD; the two most frequently used are |D'| and r^2. The stringent threshold were |D'| > 0.8 or r^2 > 0.5 (Carlson et al., 2004). The PHASE program v.2.0.1 was used to infer haplotypes.

Results

A total of 345 BD patients and 368 healthy controls in Korean were genotyped for 6 SNPs in the *NKG2-A, -C* and *CD94* gene. The genotypic distributions of *NKG2-A, -C* and *CD94* gene fulfilled Hardy-Weinberg Equilibrium in the healthy control group. The *NKG2-A c.-4258 *G* allele frequencies (P = 0.015, OR = 1.3, 95% CI = 1.05 - 1.60) was significantly higher in BD patients than in healthy controls. The *NKG2-A c.338-90*A/*A* genotype (P <.0001, OR = 2.2, 95% CI = 1.63 - 2.96, Bonferroni corrected P_c = 0.0006, Power = 96%) and *A allele frequencies (P <.0001, OR = 1.9, 95% CI = 1.47 - 2.33) were significantly higher in BD patients than in healthy controls. The *CD94 c.-134*A>*A* genotype (P = 0.041, OR = 1.4, 95% CI = 1.01 - 1.98) and **A* allele frequencies (P = 0.021, OR = 1.3, 95% CI = 1.04 - 1.58) were significantly higher in BD patients than in healthy controls. However, no significant differences were detected in the genotype and allele frequencies of the *NKG2-A c.284-67_62del, c.1077C>T* between the BD patients and the healthy controls. The *NKG2-C c.305C>T* was not significant between the BD patients and the healthy controls (Table 3).

In view of clinical features (Table 4-1, -2), the *NKG2-A c.-4258*G* allele was observed significantly increased in BD patients with clinical features of major symptoms (oral ulcers: P = 0.018, OR = 1.3; genital ulcers: P = 0.027, OR = 1.3; ocular lesions: P = 0.025, OR = 1.3) excepting skin lesions (P = 0.054, OR = 1.2). The *NKG2-A c.338-90*A*

allele was observed significantly increased in BD patients with clinical features of major symptoms (oral ulcers: P <.0001, OR = 1.8; skin lesions: P <.0001, OR = 1.8; genital ulcers: P <.0001, OR = 1.9; ocular lesions: P <.0001, OR = 2.3) and arthritis (P <.0001, OR = 2.1). The *CD94 c.-134*A* allele was observed significantly increased in three of major symptoms (oral ulcers: P = 0.014, OR = 0.3; skin lesions: P = 0.030, OR = 1.3; genital ulcers: P = 0.010, OR = 1.3, respectively) except ocular lesions and in one of minor symptoms GI (P = 0.012, OR = 2.7) in BD patients than in healthy controls. Interestingly, the *NKG2-C c.305*T* allele was observed significantly increased in BD patients with ocular lesions (P <.0001, OR = 2.1) and arthritis (P = 0.003, OR = 1.8) than in healthy controls.

The haplotype frequencies in *NKG2-A* containing *c.388-90*A* allele were increased in BD patients than in healthy controls except *c.-4258*G-c.284-67_62*del-c.338-90*A-c.1077*C/T* (P = 0.010, OR = 0.5, 95% CI = 0.34 - 0.87). The haplotype frequencies containing *c.388-90*G* allele were decreased in BD patients than in healthy controls except *c.-4258*G-c.284-67_62*del-c.338-90*G-c.1077*C/T* (although, there were no significance) (Table 5).

The linkage disequilibrium was not found among SNPs of *NKG2-A*, *-C* and *CD94* (Table 6). The individuals carrying risk alleles or genotypes of *NKG2-A c.-4258*C/*C* and **C/*G, c.338-90*A/*A* and *CD94 c.-134*A/*A* were significantly higher in BD patients than in healthy controls (P = 0.005, OR = 2.8, 95% CI = 1.32 - 5.91) (Figure 4). But the individuals carrying risk alleles or genotypes of *NKG2-C c.305C>T* and *CD94 c.-134A>T* were not significant by higher in patients with

BD compared with healthy controls (Figure 3).

Discussion

The results of this study indicate that the inhibitory receptor gene *NKG2-A c.-4258C>G* in promoter region and c.*338-90A>G* in intron 4 polymorphisms are associated with BD. *CD94 c.-134A>T* in 5'UTR polymorphism is weakly associated with BD. But the other polymorphisms of *NKG2-A c.284-67_62del* in intron 3, *c.1077C>T* in 3'UTR and *NKG2-C c.305* in exon 3 were not associated with BD. But, interestingly, *NKG2-C c.305***T* allele was associated with BD with clinical features of ocular lesions (P <.0001, OR = 2.1, 95% CI = 1.61 - 2.75) and of arthritis (P = 0.003, OR = 1.8, 95% CI = 1.22 - 2.68).

It has been suggested that NK cells play a role in susceptibility to BD. No concrete evidence shows whether NK cells are directly involved in induction or regulation of BD, but NK cells play a role in induction and/or regulation of various types of immune responses, including several autoimmune diseases, through cytotoxicity and cytokine production (Zierhut et al., 2003, Carnaud et al., 1999). Actually, the cytotoxic activity of NK cells in the clinically active stage of BD was lower than that of healthy controls and patients in the inactive stage (Onder et al., 1994, Hamzaoui et al., 1988) and the expression of abnormal NK cell receptors has been reported among patients with BD (Takeno et al., 2004, Lanier, 2001).

The *NKG2-A, -C* and *CD94* gene polymorphisms in RA and SLE reported in Japanese, statistically significant difference between patients and healthy controls was not detected in any of the

polymorphisms (Hikami et al., 2003).

In the Table 7, there are the frequencies of genotype of *NKG2-A*, *-C* and *CD94* gene SNPs in other ethnic groups. For the most part of the genotype and major allele frequencies of SNPs were not different in Asian ethnic groups.

CD94 heterodimerized with NKG2-A, -C recognize HLA-E as a ligand. The individuals carrying HLA-E *01032 risk allele and *CD94 c.-134*A/*A* genotype were higher in BD than healthy controls. But, there were no addictive effects (data not shown).

Although both of inhibitory CD94/NKG2-A and activating CD94/NKG2-C receptors are highly homologous in their extracellular domains and recognize HLA-E as a ligand, the inhibitory receptor CD94/NKG2-A has a higher affinity for its ligand HLA-E than the activating receptor CD94/NKG2-C (Vales-Gomez et al., 1999). The NKG2-B is isoform of the *NKG2-A* gene missing the 54 base pair of the exon 5 by differential splicing (Ploufastel., 1996). The inhibitory receptor gene *NKG2-A c.338-90A>G* polymorphism located in intron 4, this region may be important to alternative splicing. Although the functions of *NKG2-A c.338-90*A* allele is unclear, this polymorphism located in intron 4 may influence alternative splicing of *NKG2-A* receptor gene resulting in an unexpected or abnormal CD94/NKG2-A receptor allotypes. So, the inhibitory receptor CD94/NKG2-A may have a lower affinity for its ligand HLA-E than the activating receptor CD94/NKG2-C, the CD94/NKG2-C receptor is to have hyper-cytotioxicity of NK cells, relatively. The *NKG2-A c.-4258C>G* polymorphism located in the promoter region of *NKG2-A* gene and

*c.-4258*C* allele associated with low cytotoxic activity and *c.-4258*G* allele associated with high cytotoxic activity of NK cells (Hayashi et al., 2006). In this study, BD patients has higher frequency of *c.-4258*G* allele than healthy control (0.607 vs. 0.543, P = 0.015, OR = 1.3, 95% CI = 1.05 - 1.60), this result suggests that higher *c.-4258*G* allele in BD patients enhances cytotoxicity of NK cells and/or induces inflammatory cytokines in BD patients.

In conclusion, although the functional implication of *NKG2-A*, *-C* and *CD94* variants is not well established, in several past studies on BD, NK cells play a role in induction and/or regulation of various types of immune responses through cytotoxicity and cytokine production. Although the functions of *NKG2-A c.338-90*A* allele is unclear, this variant located in intron 4 and may influence alternative splicing of *NKG2-A* receptor gene resulting in an unexpected or abnormal CD94/NKG2-A receptor allotypes. The activating receptor CD94/NKG2-C may have hyper-cytotoxicity of NK cells by a higher affinity for its ligand HLA-E than the inhibitory receptor CD94/NKG2-A, relatively. And *c.-4258*G* risk allele in BD patients may increase cytotoxicity of NK cells resulting up-regulation of inflammation of BD patients.

Table 1. Clinical characteristics of patients with Behcet's Disease

Clinical features	n = 345 (Female / Male)	%
Female/Male	345 (181/ 164)	
Age range (years)	16 ~ 66	
Major symptoms		
Oral ulcers	341 (180/ 161)	98.8
Skin lesions	296 (155/ 141)	85.8
Genital ulcers	282 (159/ 123)	81.7
Ocular lesions	200 (95/ 105)	58.0
Minor symptoms		
Arthritis	142 (75/ 67)	41.2
Large vessel involvement	44 (7/ 37)	12.8
Central nervous system symptoms	11 (8/ 3)	3.2
Gastrointestinal lesions	16 (9/ 7)	4.6

Table 2. Method for genotyping for SNPs of *NKG2-A, -C* and *CD94*

SNPs	Primer sequence	PCR cycle		Method	Reference
NKG2-A					
c.-4258C>G (rs1983526) Promoter	S: GGCCCTCTGAGGCACTAAATAG A: CAGAGTGGGATCTTTGGTTCATGAT	95℃ 10min 95℃ 15sec 60℃ 60sec 72℃ 30sec 72℃ 7min	40 cycle	PCR-RFLP	Hayashi et al., 2006
c.284-67_62del Intron3	S: GAGATGGTGAAATTTGGTTCT A: TTTGTACAGCCTAAGATCAAG	96℃ 10min 96℃ 30sec 52℃ 30sec 72℃ 30sec 72℃ 10min	35 cycle	PCR	Hikami et al., 2003
c.338-90A>G (rs2734440) Intron4	S: AGCCCATGAAGATGTATAGAT A: ATATTATCGACCGAAAGAAGC	96℃ 10min 96℃ 30sec 46℃ 30sec 72℃ 30sec 72℃ 10min	35 cycle	PCR-RFLP	Hikami et al., 2003
c.1077C>T 3'UTR	PCR 1 S: AGCATAAGCTTTAGAGGTAAAGCG PCR 1 A: CACAGTACATTGAAGGAAACACT PCR 2 S: TAGAATAGTGGTTGCCAATGTCTG PCR 2 A: GATGTTTAGTATATTCGCAGAGTTA	96℃ 10min 96℃ 30sec 60℃(PCR1) 58℃(PCR2) 30sec 72℃ 60sec 72℃ 7min	35cycle	PCR-SSLP-RFLP	Hikami et al., 2003

Table 2. (Continued)

SNPs	Primer sequence	PCR cycle		Method	Reference
NKG2-C					
c.305C>T	S: GAAACTAAACTCGTTATGGTTCATC	95℃ 10min	50 cycle	PCR-RFLP	Hikami et
(Ser102Phe)	A: TTTTCTGCAAAAATGCCACT	95℃ 5sec			al., 2003
Exon3		54℃ 10sec			
		72℃ 15sec			
		72℃ 7min			
CD94					
c.-134A>T	S (-134A):	94℃ 5min	40 cycle	PCR-SSP	Hikami et
5'UTR	ATCATTTAAATACACAATTTTTCATTCTCTA	94℃ 30sec			al., 2003
	S (-134T):	55℃ 60sec			
	ATCATTTAAATACACAATTTTTCATTCTCTT	72℃ 30sec			
	A : CCAAAATCAGCCAATCCAAG	72℃ 7min			

PCR: polymerase chain reaction, RFLP: restriction fragment length polymorphism, SSLP: simple sequence length polymorphism, SSP: sequence-specific primer

Table 3. Genotype and allele frequencies of *NKG2-A,-C* and *CD94* gene

SNPs	BD n=345 (%)	Control n=368 (%)	*P*	OR (95% CI)	P_c
NKG2A					
c.-4258C>G (Promoter)					
**G/*G*	121 (35.1)	105 (28.5)			
**G/*C*	177 (51.3)	190 (51.6)			
**C/*C*	47 (13.6)	73 (19.9)	0.027	0.6 (0.43 - 0.95)	
**G* allele	0.607	0.543	0.015	1.3 (1.05 - 1.60)	
c.284-67_62del (Intron3)					
**Ins/*Ins*	255 (73.9)	258 (70.1)			
**Ins/*del*	78 (22.6)	90 (24.5)			
**del/*del*	12 (3.5)	20 (5.4)			
**Ins* allele	0.852	0.823			
c.338-90A>G (Intron4)					
**A/*A*	200 (58.0)	142 (38.6)	<.0001	2.2 (1.63 - 2.96)	0.0006
**A/*G*	121 (35.1)	176 (47.8)			
**G/*G*	24 (6.9)	50 (13.6)	0.004	0.5 (0.29 - 0.79)	
**A* allele	0.755	0.625	<.0001	1.9 (1.47 - 2.33)	
c.1077C>T (3'UTR)					
**C/*C*	132 (38.2)	141 (38.3)			
**C/*T*	148 (43.0)	166 (45.1)			
**T/*T*	65 (18.8)	61 (16.6)			
**C* allele	0.597	0.609			
NKG2C					
c.305C>T(Ser102Phe) (Exon3)					
**C/*C*	208 (60.3)	225 (61.1)			
**C/*T*	121 (35.1)	128 (34.8)			
**T/*T*	16 (4.6)	15 (4.1)			
**C* allele	0.778	0.785			
CD94					
c.-134A>T (5'UTR)					
**A/*A*	103 (29.9)	85 (23.1)	0.041	1.4(1.01 - 1.98)	
**A/*T*	198 (57.4)	216 (58.7)			
**T/*T*	44 (12.7)	67 (18.2)	0.045	0.7(0.43 - 0.99)	
**A* allele	0.586	0.524	0.021	1.3(1.04 - 1.58)	

P: BD patients vs. controls

Table 4-1. The allele frequencies of *NKG2-A* polymorphisms for clinical features of BD patients

	n	*c.-4258*G*	*P*	OR (95% CI)	*c.284*Ins*	*P*	OR (95% CI)	*c.338-90*A*	*P*	OR (95% CI)	*c.1077*C*	*P*	OR (95% CI)
Control	368	0.543			0.823			0.625			0.609		
BD	345	0.607	0.015	1.3(1.05-1.60)	0.852			0.755	<.0001	1.9(1.47-2.33)	0.597		
Oral ulcers	341	0.606	0.018	1.3(1.04-0.59)	0.853			0.754	<.0001	1.8(1.46-2.31)	0.600		
Skin lesions	296	0.596	0.054	1.2(0.99-1.54)	0.855			0.753	<.0001	1.8(1.44-2.33)	0.603		
Genital ulcers	282	0.605	0.027	1.3(1.03-1.60)	0.855			0.755	<.0001	1.9(1.45-2.36)	0.596		
Ocular lesions	200	0.612	0.025	1.3(1.04-1.70)	0.845			0.793	<.0001	2.3(1.73-3.04)	0.537		
Arthritis	142	0.595			0.852			0.775	<.0001	2.1(1.50-2.83)	0.563		
LVI	44	0.636			0.820			0.693			0.602		
CNS	11	0.545			0.773			0.818			0.636		
GI	16	0.531			0.844			0.688			0.500		

LVI: Large vessel involvement, CNS: Central nermous system symptoms, GI: Gastrointestinal lesions

P: BD patients vs. controls

Table 4-2. The allele frequencies of *NKG2-C* and *CD94* polymorphisms for clinical features of BD patients

	n	*NKG2-C c.305**C*	*P*	OR (95% CI)	*CD94 c.-134**A*	*P*	OR (95% CI)
Control	368	0.215			0.524		
BD	345	0.222			0.586	0.021	1.3 (1.04-1.58)
Oral ulcers	341	0.221			0.589	0.014	1.3 (1.06-1.61)
Skin lesions	296	0.225			0.584	0.030	1.3 (1.02-1.58)
Genital ulcers	282	0.218			0.596	0.010	1.3 (1.07-1.67)
Ocular lesions	200	0.363	<.0001	2.1 (1.61-2.75)	0.578		
Arthritis	142	0.330	0.003	1.8 (1.22-2.68)	0.563		
LVI	44	0.227			0.614		
CNS	11	0.182			0.500		
GI	16	0.312			0.750	0.012	2.7 (1.21-6.13)

LVI: Large vessel involvement, CNS: Central nermous system symptoms, GI: Gastrointestinal lesions

P: BD patients vs. controls

Table 5. Haplotype frequencies of *NKG2-A* in BD patients and in controls

Haplotype *c.-4258*C>*G-c.284-67_62*del-c.338-90*A>*G-c.1077*C>*T*	BD	Control	*P*	OR (95% CI)
G-*Ins/*del-*A-*C/*T	**0.559**	**0.491**	**0.009**	**1.3 (1.07 - 1.63)**
**G-*Ins-*A-*C/*T*	0.518	0.419	0.0001	1.5 (1.21 - 1.85)
**G-*del-*A-*C/*T*	0.041	0.072	0.010	0.5 (0.34 - 0.87)
C-*Ins/*del-*A-*C/*T	**0.195**	**0.134**	**0.002**	**1.6 (1.18 - 2.08)**
**C-*Ins-*A-*C/*T*	0.166	0.115	0.005	1.5 (1.13 - 2.07)
**C-*del-*A-*C/*T*	0.029	0.019		
C-*Ins/*del-*G-*C/*T	**0.199**	**0.323**	**<.0001**	**0.5 (0.41 - 0.66)**
**C-*Ins-*G-*C/*T*	0.137	0.243	<.0001	0.5 (0.37 - 0.65)
**C-*del-*G-*C/*T*	0.062	0.080		
G-*Ins/*del-*G-*C/*T	**0.047**	**0.052**		
**G-*Ins-*G-*C/*T*	0.031	0.047		
**G-*del-*G-*C/*T*	0.016	0.005		

P: BD patients vs. controls

Table 6. Linkage disequilibrium coefficients (| D' | and r^2) between SNPs of *NKG2-A, -C* and *CD94* in the Korean population
(n = 368)

			| D' |				
			CD94	*NKG2-A*				*NKG2-C*
			c.-134A>T	*c.-4258C>G*	*c.284-67_62del*	*c.338-90A>G*	*c.1077C>T*	*c.305C>T*
	CD94	*c.-134A>T*	-	0.074	0.025	0.074	0.039	0.145
r^2	*NKG2-A*	*c.-4258C>G*	0.005	-	0.140	0.768	0.570	0.599
		c.284-67_62del	0.001	0.005	-	0.091	0.012	0.026
		c.338-90A>G	0.004	0.421	0.003	-	0.434	0.635
		c.1077C>T	0.001	0.248	0.001	0.175	-	0.281
	NKG2-C	*c.305C>T*	0.005	0.117	0.001	0.184	0.034	-

Table 7. The frequencies of genotype of *NKG2-A, -C* and *CD94* gene in other ethnic groups

SNPs	[1)]Korean	[2)]Japanese	[3)]Chinese	[3)]European	[3)]African
NKG2-A					
c.-4258C>G (Promoter)	(n=368)	[3)] (n=44)	(n=45)	(n=56)	(n=60)
*G/*G	105 (28.5)	21 (47.7)	15 (33.3)	9 (16.1)	2 (3.3)
*G/*C	190 (51.6)	15 (34.1)	20 (44.4)	25 (44.6)	21 (35.0)
*C/*C	73 (19.9)	8 (18.2)	10 (22.2)	22 (39.3)	37 (61.7)
c.284-67_62del (Intron3)		(n=215)			
*Ins/*Ins	258 (70.1)	178 (82.8)	-	-	-
*Ins/*del	90 (24.5)	36 (16.7)	-	-	-
*del/*del	20 (5.4)	1 (0.5)	-	-	-
c.338-90A>G (Intron4)			(n=23)	(n=58)	(n=59)
*A/*A	142 (38.6)	113 (52.6)	11 (47.8)	25 (43.1)	4 (6.8)
*A/*G	176 (47.8)	88 (40.9)	10 (43.5)	24 (41.4)	25 (42.4)
*G/*G	50 (13.6)	14 (6.5)	2 (8.7)	9 (15.5)	30 (50.8)
c.1077C>T (3'UTR)					
*C/*C	141 (38.3)	98 (45.6)	-	-	-
*C/*T	166 (45.1)	86 (40.0)	-	-	-
*T/*T	61 (16.6)	31 (14.4)	-	-	-
NKG2-C					
c.305C>T (Exon3)		(n=201)			
*C/*C	225 (61.1)	135 (67.2)	-	-	-
*C/*T	128 (34.8)	38 (18.9)	-	-	-
*T/*T	15 (4.1)	28 (13.9)	-	-	-
CD94					
c.-134A>T (5'UTR)		(n=210)			
*A/*A	85 (23.1)	80 (38.1)	-	-	-
*A/*T	216 (58.7)	98 (46.7)	-	-	-
*T/*T	67 (18.2)	32 (15.2)	-	-	-

[1)] In this study, [2)] Hikami et al, 2003., [3)] http://www.hapmap.org/

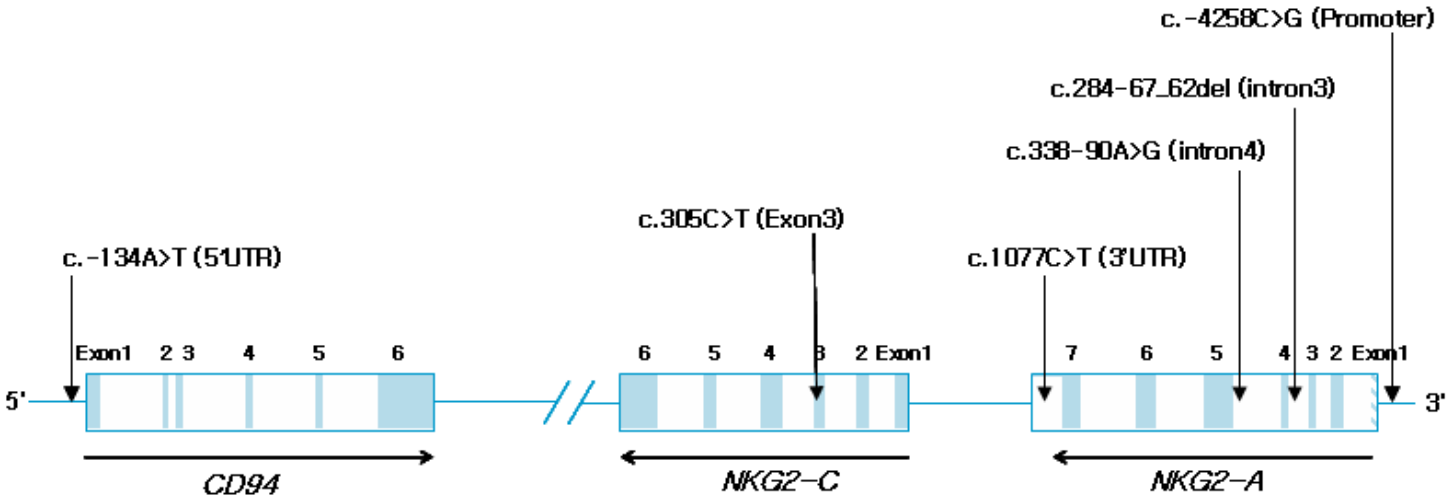

Figure 1. Gene structure of *NKG2-A, -C* and *CD94* and location of SNPs (12p12.3-13.2)

NKG2-A has a non-coding initial exon represented by the diagonal lines. This figure is not drawn to scale.

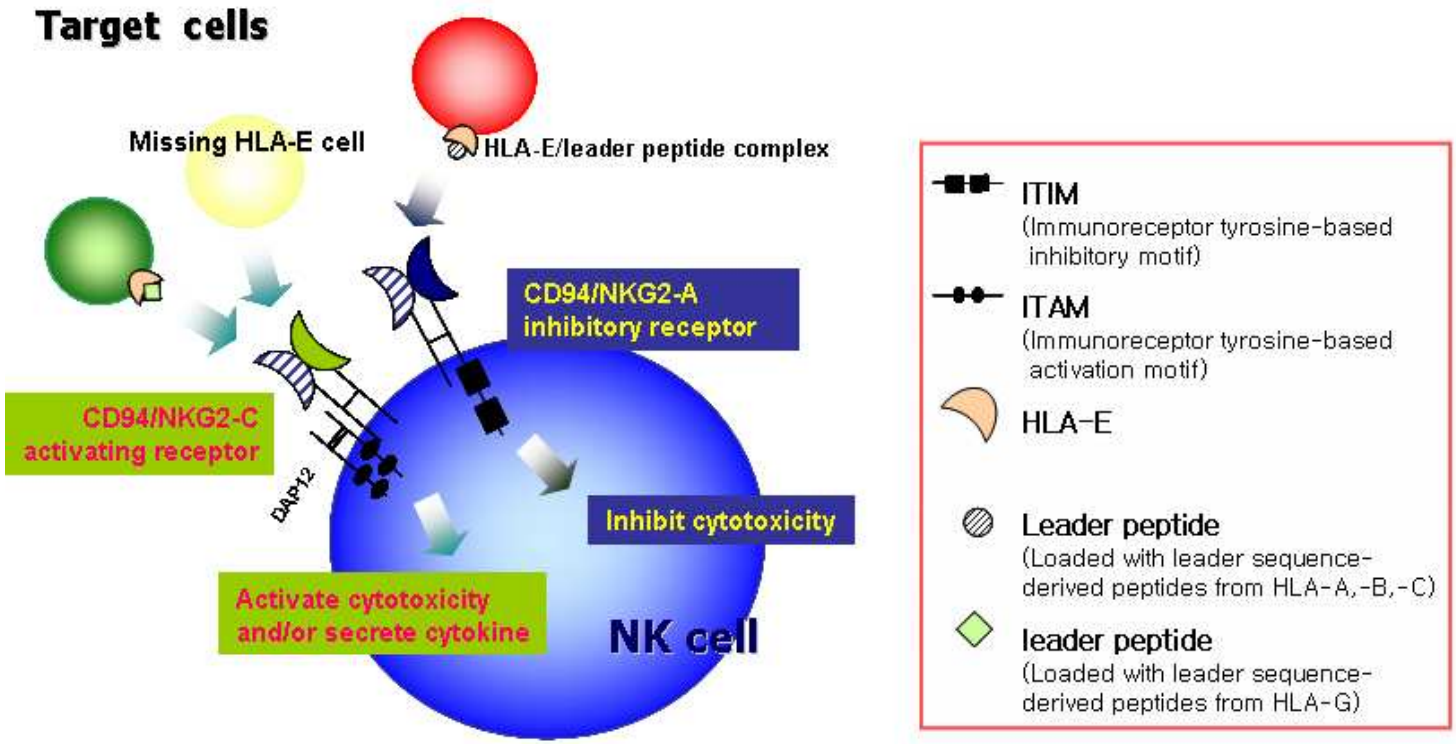

Figure 2. The function of NK cells receptor CD94/NKG2-A and CD94/NKG2-C

BD n=345(%)	*NKG2-C c.305*T-*	*NKG2-C c.305*T+*
*CD94 c.-134 *T-*	51(14.8)	52(15.1)
*CD94 c.-134 *T+*	157(45.5)	85(24.6)

Control n=368(%)	*NKG2-C c.305*T-*	*NKG2-C c.305*T+*
*CD94 c.-134 *T-*	46(12.5)	39(10.6)
*CD94 c.-134 *T+*	179(48.6)	104(28.3)

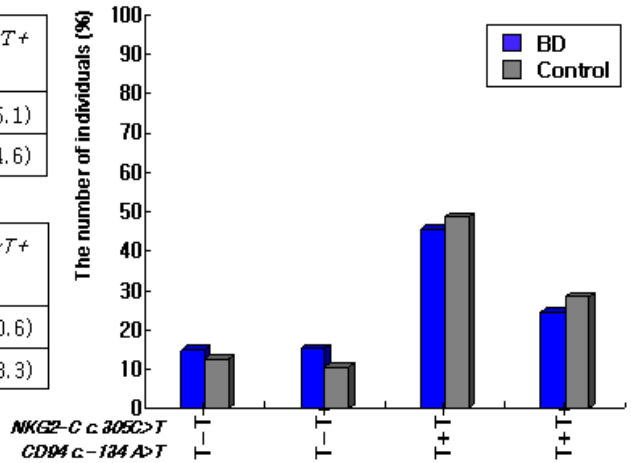

Figure 3. *CD94 c.-134A>T* vs. *NKG2-C c.305C>T* Combined Genotype

The significant differences were not observed in comparison with BD patients and controls.

- *NKG2-C c.305*T-* : *c.305*C/*C*
- *NKG2-C c.305*T+* : *c.305*C/*T*, *c.305*T/*T*
- *CD94 c.-134 *T-* : *c.-134*A/*A*
- *CD94 c.-134 *T+* : *c.-134*A/*T*, *c.-134*T/*T*

BD n=345(%)	*NKG2A*			
	*c.-4258 *C+, c.-338_90 *G-*	*c.-4258 *C+, c.-338_90 *G+*	*c.-4258 *C-, c.-338_90 *G-*	*c.-4258 *C-, c.-338_90 *G+*
*CD94 c.-134 *T-*	[1]25(7.3)	47(13.6)	28(8.1)	3(0.9)
*CD94 c.-134 *T+*	[2]69(20.0)	[3] 83(24.0)	78(22.6)	12(3.5)

Control n=368(%)	*NKG2A*			
	*c.-4258 *C+, c.-338_90 *G-*	*c.-4258 *C+, c.-338_90 *G+*	*c.-4258 *C-, c.-338_90 *G-*	*c.-4258 *C-, c.-338_90 *G+*
*CD94 c.-134 *T-*	[1]10(2.7)	48(13.1)	24(6.5)	3(0.8)
*CD94 c.-134 *T+*	[2]43(11.7)	[3] 162(44.0)	65(17.7)	13(3.5)

P : BD patients vs. controls
[1] *P* = 0.005, OR = 2.8(95% CI = 1.32 - 5.91)
[2] *P* = 0.002, OR = 1.9(95% CI = 1.25 - 2.86)
[3] *P* < .0001, OR = 0.4(95% CI = 1.29 - 0.56)

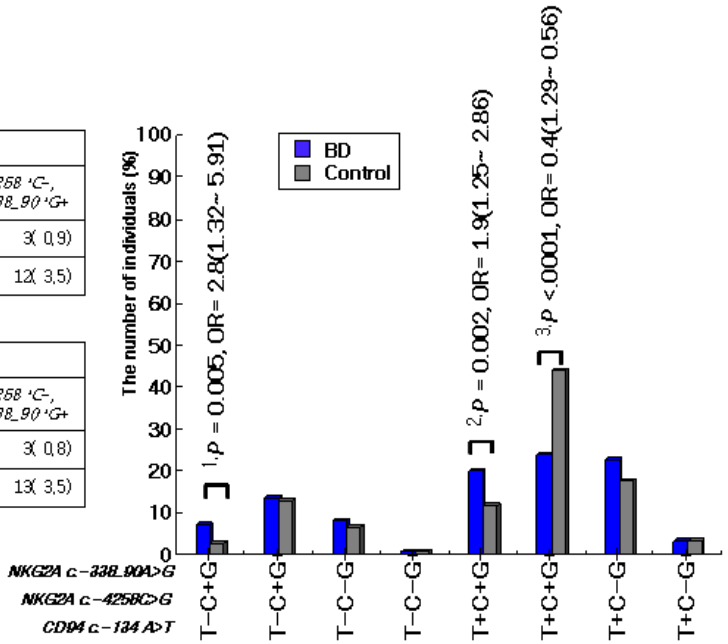

Figure 4. *NKG2A c.-4258C>G* and *c.-338_90A>G* vs. *CD94 c.-134A>T* Combined Genotype

- *NKG2A c.-4258*C+* : *c.-4258*C/*C, c.-4258*C/*G,*
- *NKG2A c.-4258*C-* : *c.-4258*G/*G*
- *NKG2A c.-338_90*G-* : *c.-338_90*A/*A*
- *NKG2A c.-338_90*G+* : *c.-338_90*A/*G, c.-338_90*G/*G*
- *CD94 c.-134*T-* : *c.-134*A/*A*
- *CD94 c.-134*T+* : *c.-134*A/*T, c.-134*T/*T*

Web Resources

Accession numbers and URLs for data presented herein are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/

Online Mendelian Inheritance in Man (OMIM),

http://www.ncbi.nlm.nih.gov/Omim/

Entrez Gene, http://www.ncbi.nlm.nih.gov/entrez/

Human Gene Nomenclature Database,

http://www.gene.ucl.ac.uk/nomenclature/

International HapMap Project, http://www.hapmap.org/

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Abstract

Polymorphisms of Natural Killer Cell Receptor *NKG2-A, -C* and *CD94* genes in Behcet's Disease

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Natural Killer (NK) cells regulate inflammatory response inducing inflammatory cytokines around the target cells and/or directly killing the target cells by the balance of signals of receptors, inhibitory CD94/NKG2-A and activating CD94/NKG2-C receptors. The expression of abnormal NK cell receptors and the decreased cytotoxic activity of peripheral blood NK cells among patients with Behcet's Disease (BD). BD is an immune-mediated disease with a multisystemic inflammatory disorder. This study focused on the association between *NKG2-A*, *NKG2-C* and *CD94* polymorphisms and BD risk. The six SNPs, *NKG2-A c.-4258C>G, c.284-67_62del, c.338-90A>G*, *c.1077C>T* and *NKG2-C c.305C>T* (Ser102Phe) and *CD94 c.-134A>T*, were genotyped in 345 BD patients and 368 healthy controls. The allele frequencies of *NKG2-A c.-4258***G* (P = 0.015 OR = 1.3), *c.338-90***A* (P <.0001, OR = 1.9) and *CD94 c.-134***A* (P = 0.021, OR = 1.3) were associated with the increased risk of BD and that of *NKG2-A c.284-67_62del,*

*c.1077*C* and *NKG2-C c.305*C* were not associated with BD. Interestingly, in BD patients with ocular lesions and arthritis, *NKG2-C c.305*T* allele were significantly higher than healthy controls (P <.0001, OR= 2.1; P = 0.003, OR = 1.8, respectively). Although it is not yet clear whether these SNPs actually have an effect on BD mostly, these results suggest that *NKG2-A c.-4258C>G, c.338-90A>G* and *CD94 c.-134A>T* SNPs are associated with BD.

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