박 경 숙 교수지도

석사학위청구논문

Genetic association study between the T cell immunoglobulin mucin *(TIM)-3* gene and rheumatoid arthritis

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성신여자대학교 교육대학원

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이 논문을 석사학위논문으로 제출함

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박 재 희

인 준 서

박재희의 석사학위 논문으로 인준함.

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성신여자대학교 교육대학원

논 문 개 요

T cell immunoglobulin mucin (TIM) gene family는 5q33.2에 위치하며 *TIM-1, TIM-3, TIM-4*로 구성되어 있고, 세포표면당단백질, signal peptide, Ig domain, mucin domain, transmembrane region, phosphorylation site를 가진 intercellular tail을 부호화하고 있다. *TIM-3* gene *(HAVCR2,* MIM:606652*)* 은 Th1 세포에서 발현되며, Th1 면역반응과 대식세포 반응에 필수적인 역할을 한다. IL-12에 의해 유도된 Th1 세포는 주로 interferon-ɣ와 tumour-necrosis factor-α를 분비하며, 이러한 cytokine에 의해 염증반응과 특정 체액성 반응을 증진시켜 류마티스관절염와 같은 자가면역질환을 활성화시킨다. 이 논문에서는 류마티스관절염 환자 206명과 건강인 215명으로 *TIM-3*유전자의 6 곳의 단일염기다형성 *rs11742259* (intron), *rs10515746* (*-574G>T*; 5′near gene), *rs35960726* (*Ile97Met*; exon2), *rs1036199* (*Leu140Arg*; exon3), *rs4704846* (3′UTR), *rs11134551* (intron)을 분석하였다. *TIM3 c.-574*G/*G (rs10515746), 97*Ile/*Ile (rs35960726), rs11742259 *C/*C* (intron)의 동종접합자의 빈도는 류마티스관절염 환자군에서 건강인보다 통계적으로 유의하게 감소하였다 (*p*= 0.009, OR= 0.3, 95% CI= 0.12 - 0.78; *p*= 0.0001, OR= 0.2, 95% CI= 0.05 - 0.45; *p*= 0.001, OR= 0.4, 95% CI= 0.27 - 0.74). 그 외 다른 단일염기다형성은 환자군과 건강인에서 그 빈도의 차이가 없었다. *rs11742259 *C/*C, c.-574*G/*G (rs10515746) 97*Ile/*Ile (rs35960726)* 를 가진 류마티스 환자군은

건강인보다 유의하게 감소하였다 (61.8% vs. 85.6%; p<.0001, OR = 0.3, 95% CI = 0.17 – 0.44). *rs11742259 *C/*T, *T/*T, TIM3c.-574*G/*G, TIM3 97*Ile/*Met, 97*Met/*Met* 를 가진 환자군은 건강인보다 통계적으로 유의하게 높게 나타났다 (3.4% vs. 0.5%; p= 0.028, OR = 7.5, 95% CI = 0.91 – 61.42). *TIM-3* (*rs10515746, rs35960726, rs11742259)* SNP와 류마티스 환자에서의 임상적 특징 (rheumatoid factor, antinuclear antibody and erosion level) 과의 통계적 유의한 결과를 찾을 수 없었다. 일배체분석에서 *TIM-3*의 *rs11742259C-rs10515746G-rs35960726T-rs1036199T-rs4704846A-rs11134551A* 를 단일염기다형성 분석과 비교하였을 때, 환자군에서 건강인 보다 유의하게 감소하였다(p= 0.0001, OR = 0.5, 95% CI = 0.39 – 0.74).

결론적으로, *rs10515746 (-574G>T), rs35960726 (Ile97Met), rs11742259* (intron)는 류마티스관절염 환자군에서 낮은 감수성을 보이며, 환자군에서의 임상적 특징과의 연관성은 찾을 수 없었다. *TIM-3* 와 류마티스관절염과의 유전적 관련성은 질병의 발생과 중증도 및 치료에 대한 반응에 영향을 미치는 유전인자를 밝혀내는 기존의 연구 자료에 중요한 기초가 될 수 있을 것으로 기대된다.

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Introduction

Rheumatoid arthritis (RA, MIM: 180300) is a chronic inflammatory and destructive joint disease that affects 0.5–1% of the population in the industrialized world and commonly leads to significant disability and a consequent reduction in quality of life. It is two to three times more frequent in women than in men and can start at any age, with a peak incidence between the fourth and sixth decade of life. In addition to the joint swelling and pain caused by the inflammatory process, the ultimate hallmark of RA is joint destruction. The most commonly involved joints are those of the hands, feet and knees, and distal interphalangeal joints are usually spared. RA is a disease in which the immune and inflammatory systems are intimately linked to the destruction of cartilage and bone. The discordance in developing rheumatoid disease between identical twins clearly suggests that nongenetic factors are also important, and infectious agents are the most plausible explanation. It has long been speculated that RA could be triggered by infectious agents, but proof of this is still lacking (Marc et al. 1996; Smolen et al. 2003).

The family of T-cell immunoglobulin domain and mucin domain (TIM) proteins is expressed on T cells. The *TIM* gene family consists genes of three members (*TIMs 1, 3* and *4*) on the chromosome 5q33.2 without any other intervening genes. This gene family in human encodes cell surface glycoprotein with common

structural motifs, including a signal peptide, Ig domain, mucin domain, transmembrane region, and intracellular tail with phosphorylation sites. *TIM-1 (HAVCR)* is preferentially expressed on Th2 cells, whereas *TIM-4* is expressed on antigen-presenting cells and represents the only natural ligand reported for *TIM-1* (Meyer et al. 2005). *TIM-3* (*HAVCR2,* MIM:606652) is expressed at highest level in Th1 cells and has essential role in Th1 responses by macrophage activation (Monney et al. 2002). In addition to TIM-3 expression in T cells, TIM-3 mRNA is present in CD11b and other non-T cells, although the functional relevance of this is not known (Gielen et al. 2005; Khademi et al. 2004; Sui et al. 2006). A subset of CD4 Th-17, characterized by the production of IL-17 express TIM-3, but at lower levels than Th1 cells (Langrish et al. 2005; Chen et al. 2006), raising the possibility that *TIM-3* might have a role in regulation of this subset as well. Indeed, high levels of IL-17 have been found in the joints of patients with rheumatoid arthritis, which suggests the involvement of Th-17 cells in the development of various human autoimmune diseases as well (Kolls et al. 2004). Anderson et al. (2007) suggested that human dendritic cells (DCs) expressed high levels of TIM-3, although it was also detected at low levels in human monocytes. The TIM-3 is highly expressed by cells of the innate immune system in humans, and its expression on antigen-presenting cells promotes the secretion of proinflammatory cytokines from monocytes and DCs (Anderson et al. 2007).

Th1 cells mainly secrete interferon-ɣ (IFN-ɣ) and tumour-necrosis factor-α (TNF-α) and are important for the activation of macrophages and for the clearance of intracellular pathogens; Th2 cells produce IL-4, IL-5, IL-13 and IL-10. Th1 cells exert pro-inflammatory activities and promote cellular immune responses, whereas Th2 cells have anti-inflammatory potential and promote humoral responses, including immunoglobulin (Ig) E production. The pathological consequences of an inappropriate Th1 response include delayed type hypersensitivity responses and the induction of organ-specific autoimmune disease. Many other cytokines and chemokines are involved in RA pathogenesis, including IL-15 and IL-18 and angiogenic factors. These soluble molecules lead to the activation of transcription factors and the subsequent induction of genes whose products mediate inflammation and tissue degradation. Among these products are various cytokines, chemokines and tissue-degrading enzymes, such as the matrix metalloproteinases, but also cell-surface molecules that enhance cell activation and cell-cell interactions, such as co-stimulatory and adhesion molecules (Marc et al. 1996). The latter comprise selectins, integrins and their counterparts, which mediate cell rolling, adhesion and migration into inflammatory sites via endothelial cells and other important intercellular interactions, and so are pivotal in the generation of the inflammatory response (Smolen et al. 2003).

TIM-3-TIM-3 ligand (TIM-3L) interactions play an important role in tolerance (Zhu et al. 2005). Galectin-9, a member of the galectin family, as a ligand for TIM-3. Sabatos et al (2003) suggested that TIM-3-deficient mice exhibit increased T-cell proliferation and production of IL-2 after administration of high-dose aqueous antigen relative to controls, its cannot be tolerized. Galectin-9 triggering of TIM-3 on Th1 cells induces calcium flux, cell aggregation and cell death *in vitro.* In contrast to TIM-3 Ig fusion protein treatment, *in vivo* administration of galectin-9 causes selective loss of IFN-ɣ-producing T cells and ameliorates autoimmune encephalomyelitis (Zhu et al. 2005). These data collectively support a model whereby the TIM-3-galectin-9 pathway serves to dampen Th1 immunity by eliminating effector Th1 cells. The IFN-ɣ upregulates galectin-9, which in turn eliminates TIM-3 Th1 cells, thereby controlling inflammation and autoimmunity (Krakowski et al. 1996). Thus, inability to upregulate galectin-9 (because of low or absent levels of IFN-ɣ) will result in failure to delete Th1 cells. Similarly, cells that express lower levels of TIM-3 will escape deletion in tissue sites, despite significant production of IFN-ɣ and upregulation of galectin-9 (Anderson et al. 2006).

TIM-3 is primarily expressed on DCs in the naïve state and that TIM-3 synergizes with Toll-like receptors to promote TNF-α secretion. Once Th1 response are generated, TIM-3 is expressed on terminally differentiated Th1 cells, which will outnumber DCs and

induce the upregulation of galactin-9 via their production of IFN-ɣ (Asakura et al. 2002; Imaizumi et al. 2002). Finally, galectin-9 triggers TIM-3 Th1 cells to terminate Th1 immunity. TIM-3, by virtue of differential expression on cells of the innate and adaptive immune systems, can both promote inflammation and terminate Th1 immunity. Thus, Anderson et al. (2007) suggested that *TIM*-3 may represent a valid therapeutic target and organ-specific human inflammatory disease such as RA.

RA has a polygenic basis, although the genes involved have not yet been defined. There is an evidence of triggering microbial infection such an Epstein-Barr virus in RA. The products of foreign agent(s) could activate the innate immune system by binding to Toll-like receptors or CD14, and subsequently triggering a T cell response (Smolen et al. 2003). The T cells infiltrating the synovial membrane are primarily CD4 memory cells, which produce IL-2 and IFN-ɣ to a similar extent as antigen-triggered T cells and so clearly have a Th1 bias. This polarity of the T cell response in RA is further supported by a vast preponderance of Th1 cell clones derived from RA patients and by the presence of a milieu favouring the generation of myeloid DCs that preferentially activate Th1 cells. These T cells, by cell-cell contact and activation by different cytokines, such as IFN-ɣ, TNF-α and IL-17, activate monocytes, macrophages and synovial fibroblasts. These latter cells then overproduce pro-inflammatory cytokines (TNF-α, IL-1 and IL-6)

which seem to constitute the pivotal event leading to chronic inflammation. Overall, the T cells, B cells, monocyte/macrophages, mast cells, dendritic cell (DCs), fibroblasts and cytokines are found in highly increased numbers in the synovial membrane in RA (Smolen et al. 2003; Weyand et al. 2003; Figure 1).

In particular, there is a strong association between RA and several types of autoantibodies; rheumatoid factor (RF), which is directed against the Fc fragment of IgG. Aside from RF, responses to other autoantigens occur very commonly, both at the B and T cell level (Steiner et al. 2001). Whether such autoantigens initiate the T cell activation cascade and the consequent inflammatory changes, or step in at a later point in time to bolster and/or perpetuate the process, is unknown (Fritsch et al. 2002; Schellekens et al. 1998).

The genetic association with RA is found in particular alleles of *HLA-DRB1* and remains as the best characterized, and the association of the 1858C/T polymorphism of the *PTPN22* gene has been reported in Causuan RA patients (Newton et al. 2004; Begovich et al. 2004). However, it has been estimated that only 30% of the genetic contribution to RA can be attributed to *HLA* genes, and it is suggested that other non-HLA genes may play a relevant role in RA susceptibility (Orozco et al. 2006). Several other SNPs in the candidate genes, such as *CTLA4, HAVCR1* and *SUMO4* suggesting the association with RA. Recently, it has been reported that the

TIM-3 polymorphism also is significantly associated with susceptibility to RA (Chae et al. 2004b).

In the light of these evidence, *TIM-3* is a transmembrane protein expressed on differentiated Th1 cells, which may play a role in Th1-mediated diseases RA. This study was performed to investigate the polymorphism of *TIM-3* in RA patients and their association with the presence of RF, antinuclear antibody (ANA) and bony erosion.

Materials and Methods

Subjects

A total of 206 RA patients and 215 non-RA healthy controls were recruited from Seoul National University Hospital in Korea. All individuals were Koreans. Patients with RA, defined by the American College of Rheumatology 1987 revised criteria for RA and disease duration for >2 years, gave informed consent prior to their participation in this study, which was approved by the Seoul National University Hospital. RF levels and presence of ANA in RA patients were determined in a routine laboratory at the Seoul National University Hospital. The prevalence of clinical features in patients with RA is presented in the Table 1. The ratio of the female:male was the 8.4:1.

Genotyping

Genomic DNA was extracted from peripheral blood using a QIAamp Blood kit (Quiagen, Hilden, Germany). Six single nucleotide polymorphisms (SNPs) for *TIM-3* genotyping were chosen for genotyping based on the NCBI SNP database (1 in promoter, 2 in exon, 2 in intron, 1 in 3′UTR; Figure 2). The TaqMan-Allelic Discrimination method using the Applied Biosystems 7300 Real Time PCR System (Applied Biosystems) was used for the detection of 6

SNPs. Context sequences were as follows for the *rs11742259* (intron), *rs10515746* (*-574G>T*), *rs35960726* (*Ile97Met*), *rs1036199* (*Leu140Arg*), *rs4704846* (3′UTR), *rs11134551* (intron) SNPs: *rs11742259*- TTGACTACCTCTCACAGCATGACTA[C/T]GGCGTGTTC TTATATTGATTTTGT, *rs10515746*- CCTTATCCTCACATTTACAGAC CAT[A/C]GCAACTCCCAGCATAAGCCTCACTC, *rs35960726*- CACT GTCTGCTAGAGTCACATTCTC[C/T]ATGGTCAGGGACACATCTCCTT TGC, *rs1036199*- AAAGGCTGCAGTGAAGTCTCTCTGC[A/C]GAGTCG GTGCAGGGGTGACCTTGGC, *rs4704846*- GAATCTTCAAGATCAAGG TAGACCT[A/G]GTCCAGTCATCTCTTAATCTTTTAA, *rs11134551*- TCTTTGTAAAAAGAGTCACACCTTA[A/G]GCAAAAGAAATTGGAATG TTAATTC. After PCR was completed, results were analyzed using the Allelic Discrimination software.

Statistical analysis

The differences of allele frequencies and genotype distribution between RA patients and controls were examined by the χ^2 test using SAS v.9.1 (SAS Institute, Cary, NC). Odds ratios (OR) with 95% CI were obtained. *p*-values <0.05 were regarded as statistically significant. The Hardy-Weinberg equilibrium and linkage disequilibrium (LD) were analyzed using the R program v.2.6.2 (http://www.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

Association of genotype and allele frequencies between RA patients and healthy controls at the SNPs of *TIM-3* gene (6 SNPs; 1 in promoter, 2 in exon, 2 in intron, 1 in 3′UTR) was analyzed (Table 2). The homozygous *TIM-3* SNPs of *c.-574*G/*G (rs10515746), 97*Ile/*Ile (rs35960726)* and *rs11742259 *C/*C* (Intron) revealed a reduced risk of RA as compared with controls (p= 0.009, OR = 0.3, 95% CI = 0.12 - 0.78; p= 0.0001, OR = 0.2, 95% CI = 0.05 - 0.45; p= 0.001, OR = 0.4, 95% CI = 0.27 - 0.74, respectively). In contrast, *rs11742259*T/*T* genotype was associated with increased risk (p= 0.020, OR = 2.1, 95% CI = 1.87 - 2.28). There was no significant difference in the genotype and allelic frequencies of the *TIM-3 Leu140Arg, rs4704846* (3′UTR) and *rs11134551* (intron). These results strongly suggest that *rs10515746 (-574G>T), rs35960726 (Ile97Met)* and *rs111742259* (intron) of *TIM-3* polymorphisms might be associated with susceptibility to RA. A significantly reduced risk was observed in RA patients who carried the *rs11742259 *C/*C, rs10515746 *G/*G* and *rs35960726 97*Ile/*Ile* as compared with controls (61.8% vs. 85.6%; $p < 0.0001$, OR = 0.3, 95% CI = 0.17 - 0.44). Conversely, a increased risk of RA patients who carried the *rs11742259*C/*T, *T/*T, rs10515746 *G/*G and rs35960726 97*Ile/*Met, 97*Met/*Met* were found compared with controls (3.4%

vs. 0.5%, *p*= 0.028, OR = 7.5, 95% CI = 0.91 – 61.42; Table 3). Association of *rs11742259, TIM3* c.*-574G>T, 97*Ile/*Met* genotypes with the presence of RF, ANA, and bony erosions was analyzed in RA patients. No significant association between the *rs10515746, rs35960726, rs11742259* genotypes and the presence of RF, ANA, and bony erosions was observed in RA (Table 4). This result suggests that the polymorphisms of the *TIM-3* might not be associated with the RF, ANA, and bony erosions in RA.

Linkage disequilibrium analysis was performed on the basis of the 6 SNPs listed in Figure 3. Although an absolute LD (| D' | = 1) was not observed, the strong LD between SNPs of *TIM-3* were observed. 1, 2 and 3 SNPs are pro-inflammatory activity related SNPs with P< 0.010. When looking at the lower triangle, many of these are closely linked to each other; close linkage disequilibrium with D′ ≥ 0.9 (snp1-snp2, snp1-snp5, snp2-snp4, snp2-snp5, snp2-snp6, snp4-snp5, snp4-snp6, snp5-snp6); 0.9 > D′ ≥ 0.8 (snp1-snp3). Analysis of major haplotype (*rs11742259C-rs10515746G-rs35960726T-rs1036199T-rs4704846A-rs11134551A*) consisting of 6 SNPs on the *TIM-3* gene revealed a reduced susceptibility to RA as compared with controls (*p*= 0.0001, OR = 0.5, 95% CI = 0.39 – 0.74; Table 5). In summary, these results strongly suggest that the polymorphisms of *TIM-3* on Th1 cell might confer decreased susceptibility to RA.

Discussion

This study revealed that *rs10515746* (*-574G>T*) in promoter region, *rs35960726* (*Ile97Met*) in exon 2 and *rs11742259* in intron polymorphisms were associated with RA. Other polymorphisms, *rs1036199 (4259T>G, Leu140Arg)* in exon 3, *rs4704846* in 3′UTR and *rs11134551* in intron, were not associated with RA. Analysis of the haplotype, from the person who carried the major genotypes of 6 SNPs on *TIM-3* gene revealed a reduced susceptibility to RA as compared with controls. The negative immune receptor *TIM-3* on Th1 may be able to influence on inflammatory disease such as RA. Chae et al. (2004b,c) suggested that the *TIM-3* polymorphisms also are significantly associated with susceptibility to RA. The association of the genotype and allele frequencies between RA patients and the healthy controls using samples (295 RA patients and 319 healthy controls) at the *rs10053538* (*−1516G>T*), *rs10515746 (−574G>T*) and *rs1036199* (*4259T>G; Leu140Arg;* exon3) polymorphic sites of *TIM-3* gene. The genotype and allele frequencies of the *−574T>G* (p= 0.001 and 0.001, respectively) as well as the *4259T>G* (p= 0.001 and 0.003, respectively) between RA patients and healthy controls were significantly different. These results strongly suggested that *−574T>G* and *4259T>G* polymorphism of the *TIM-3* might be associated with susceptibility to RA. In this study the *rs10515746*

(*-574G>T*) genotype and allele frequencies was associated in RA. But, the *rs1036199 (4259T>G, Leu140Arg)* in exon 3 were shown to have no significant association with RA. The different result might be caused by difference of genotyping method or patient population.

In the Table 6, there are the frequencies of genotype of *TIM-3* gene SNPs in other ethnic groups. For the most part of the genotype and major allele frequencies of SNPs were not different from of those SNPs in Asian groups, but quite a different from them in African.

RA is a chronic systemic inflammatory disease of undetermined etiology involving primarily the synovial membranes and articular structures of multiple joints. The association of human leukocyte antigen (HLA) with RA has been known and confirmed in numerous population studies. The genome-wide screens have been performed by different groups which suggested that the genes in HLA region (*HLA-DRB1*) provide the largest genetic contribution to RA as well as the non-HLA loci involved in RA susceptibility identified on the several regions including chromosomes 1p and 18q. Recently, the case-control association study by Suzuki et al. (2006). showed that the haplotype of peptidylarginine deiminases (*PADI4*) gene located on chromosome 1p36 is associated with susceptibility to RA (Chen et al. 2008; Nishimoto et al. 2008; Takata et al. 2008). Several other SNPs in the candidate genes, such as *PTPN22, CTLA4, NFKBIL1, STAT4, C5* suggested the associations. Also, *TIM-1* (*HAVCR1*) and *TIM-3*

(*HAVCR2*) polymorphisms showed the strong association with RA susceptibility (Chae et al. 2004b, 2004d, 2005). Chae et al. (2004d, 2005) had suggested that the variations of *TIM-1* might be associated with the susceptibility to RA. Chae et al. (2004d) reported that the exon 4 (*5383_5397del* and *5509_5511delCAA*; p= 0.015, p= 0.0002, respectively) might be associated with the susceptibility to RA. Chae et al. (2005) investigated the association between genotype and allele frequencies of the *TIM-1* gene promoter region, exon 1, and intron 1 regions (containing *−1454G>A, −416G>C, −232A>G* and *−1637A>G*) polymorphic sites in both RA patients and the healthy controls, using polymerase chain reaction-restriction fragment length polymorphism and single-base extension methods. The genotype and allele frequencies of *−232A>G, −416G>C,* and *−1454G>A* polymorphisms were not found to be significantly different among RA patients and the healthy controls. On the other hand, the genotype and allele frequencies of *−1637A>G* in RA patients were significantly different from those in the controls group (p= 0.0004 and p= 0.001, respectively). These results strongly supported that the exon 4 variations and *−1637A>G* (promoter) polymorphism of *TIM-1* are associated with RA susceptibility and might have influence on the development of Th1/Th2 differentiation. And Chae et al. (2004b) showed that the *TIM-3* polymorphisms are also significantly associated with susceptibility to RA. In conclusion, *TIM-3* polymorphisms might have some influence on susceptibility to RA.

The soluble TIM-3 contains the signal peptide, immunoglobulin V and cytoplasmic domains, and lacks the mucin domain and transmembrane region. Sabatos et al.(2003) and Ueda et al.(2003) suggested that most of the immunoregulatory activity of TIM-3 may be mediated through its immunoglobulin domain. The soluble molecules of the receptors are important in susceptibility and resistance to autoimmune disease such as type 1 diabetes (Ueda et al. 2003). Among the 6 SNPs in *TIM-3*, two SNPs (*rs35960726 and rs1036199*) induce change of amino acid. *rs1036199 (Leu140Arg)* SNPs of Th1 cell surface gene *TIM-3* precisely identified was associated with susceptibility to rheumatoid arthritis (Chae et al. 2004b). But, in this study genotype and allele frequencies of *rs1036199 (Leu140Arg)* was not associated with RA. The change of allele *rs35960726 (Ile97Met)* of exon 2, presumed polymorphic site located in mucin domain of the *TIM-3,* seems to be important for function in structural binding manner. These results provide that the *TIM-3* (*rs10515746, rs35960726, rs11742259*) polymorphisms might be related to reduced risk of RA. Although it is not yet known whether these polymorphisms are associated with some functions, it will be important in future studies to determine whether or not this intron, 5′ near gene polymorphism affects the levels of *TIM-3* production.

Table 1. Clinical characteristics of RA patients

	RA n=206 (%)	Controls n=215
Age at entry(yrs)	(Men22/Women184)	(Men14/Women201)
≤40	50	48
41-50	59	55
51-60	49	52
61-70	41	45
71-80	7	15
Mean	49.2	51.9
Anatomic stage		
early	48 (33.3)	
moderate	75 (52.1)	
severe	3 (2.1)	
terminal	18 (12.5)	
Bony erosion		
positive	142 (72.1)	
negative	55 (27.9)	
Rheumatoid factor (RF)		
positive	162 (79.8)	
negative	41 (20.2)	
Antinuclear antibody (ANA)		
positive	121 (60.8)	
negative	78 (39.2)	

Table 2. Genotype and allele frequencies of *TIM-3* gene in RA patients

SNPs	RA n= 207(%)	Controls n= 215 (%)	*p*	OR(95% CI)
TIM-3				
rs11742259 (Intron)				
**C*/*C*	155 (74.9)	187 (87.0)	0.001	0.4 (0.27 - 0.74)
**C*/*T*	47 (22.7)	28 (13.0)		
**T*/*T*	5 (2.4)	0 (0.0)	0.020	2.1 (1.87 - 2.28)
**C* allele	0.862	0.935		
rs10515746(*-574G>T*) 5′near gene				
**G*/*G*	189 (91.3)	209 (97.2)	0.009	0.3 (0.12 - 0.78)
**G*/*T*	18 (8.7)	5 (2.3)		
**T*/*T*	0 (0.0)	1 (0.5)		
**G* allele	0.957	0.984		
rs35960726(*Ile97Met*) exon2				
**T*/*T*	184 (88.9)	211 (98.1)	0.0001	0.2 (0.05 - 0.45)
**T*/*C*	22 (10.6)	3 (1.4)		
**C*/*C*	1 (0.5)	1 (0.5)		
**T* allele	0.942	0.988		
rs1036199 (*4259T>G*) (*Leu140Arg*) exon3				
**T*/*T*	181 (87.4)	196 (91.2)		
**T*/*G*	26 (12.6)	16 (7.4)		
**G*/*G*	0 (0.0)	3 (1.4)		
**T* allele	0.937	0.949		
rs4704846 (3′UTR)				
**A*/*A*	199 (96.1)	209 (97.2)		
**A*/*G*	8 (3.9)	6 (2.8)		
**G*/*G*	0 (0.0)	0 (0.0)		
**A* allele	0.981	0.986		
rs11134551 (Intron)				
**A*/*A*	159 (76.8)	175 (81.4)		
**A*/*G*	46 (22.2)	36 (16.7)		
**G*/*G*	2 (1.0)	4 (1.9)		
**A* allele	0.881	0.898		

p: RA patients vs. controls

Table 3. The combined genotype of three SNPs (*rs10515746, rs35960726* and *rs11742259)* of *TIM-3* in RA patients

Combination of variables	RA patients *n*=207 (%)	Controls *n*=215 (%)	*p*	OR (95% CI)
*rs11742259 *C/*C + rs10515746 *G/*G + rs35960726 *T/*T*	128 (61.8)	184 (85.6)	<.0001	0.3 (0.17 - 0.44)
*rs11742259 *C/*T, *T/*T+ rs10515746 *G/*G + rs35960726 *T/*T*	39 (18.8)	21 (9.8)	0.008	2.1 (1.21 - 3.79)
*rs11742259 *C/*C + rs10515746 *G/*G + rs35960726*C/*T, *C/*C*	15 (7.2)	3 (1.4)	0.003	5.5 (1.57 - 19.36)
*rs11742259 *C/*T, *T/*T+ rs10515746 *G/*G + rs35960726*C/*T, *C/*C*	7 (3.4)	1 (0.5)	0.028	7.5 (0.91 - 61.42)
*rs11742259 *C/*C+ rs10515746*G/*T, *T/*T + rs35960726 *T/*T*	12 (5.8)	0 (0.0)	0.0003	2.1 (1.90 - 2.33)
*rs11742259 *C/*T, *T/*T+ rs10515746*G/*T, *T/*T + rs35960726 *T/*T T*	5 (2.4)	6 (2.8)		
*rs11742259 *C/*C + rs10515746*G/*T, *T/*T + rs35960726*C/*T, *C/*C*	0 (0.0)	0 (0.0)		
*rs11742259 *C/*T, *T/*T + rs10515746*G/*T, *T/*T + rs35960726*C/*T, *C/*C*	1 (0.5)	0 (0.0)		

p: RA patients vs. controls

Table 4. Frequency of *TIM-3 (rs10515746, rs35960726* and *rs11742259)* genotypes in RA patients, stratified by the positivity of RF, ANA and radiographic bony erosion

	RF			ANA			Bony erosion		
	positive	negative	*p*	positive	negative	*p*	positive	negative	*p*
*rs11742259*C/*C*	121 (59.6)	31 (15.3)		91 (45.7)	58 (29.1)		102 (51.8)	47 (23.8)	
*rs11742259*C/*T*	37 (18.2)	9 (4.4)		27 (13.6)	18 (9.0)		36 (18.3)	7 (3.6)	
*rs11742259*T/*T*	4 (2.0)	1 (0.5)	0.992	3 (1.5)	2 (1.0)	0.991	4 (2.0)	1 (0.5)	0.134
*rs10515746*G/*G*	149 (73.4)	36 (17.7)		107 (53.8)	75 (37.7)		129 (65.5)	51 (25.9)	
*rs10515746*G/*T*	13 (6.4)	5 (2.5)		14 (7.0)	3 (1.5)		13 (6.6)	4 (2.0)	
*rs10515746*T/*T*	0 (0.0)	0 (0.0)	0.401	0 (0.0)	0 (0.0)	0.057	0 (0.0)	0 (0.0)	0.673
*rs35960726*T/*T*	146 (71.9)	34 (16.7)		105 (52.8)	71 (35.7)		127 (64.5)	47 (23.8)	
*rs35960726*T/*C*	15 (7.4)	7 (3.4)		15 (7.5)	7 (3.5)		14 (7.1)	8 (4.1)	
*rs35960726*C/*C*	1 (0.5)	0 (0.0)	0.319	1 (0.5)	0 (0.0)	0.537	1 (0.5)	0 (0.0)	0.538

Table 5. Haplotype frequencies of *TIM-3* in RA patients and in controls

Haplotype	Frequency		*p*	OR (95% CI)
	RA	Controls		
rs11742259-rs10515746-rs35960726-rs1036199-rs4704846-rs11134551				
C G T T A A	0.711	0.820	0.0001	0.5 (0.39 - 0.74)
C G T T A G	0.062	0.068	0.011	0.5 (0.28 - 0.85)
C G T G A A	0.010	0.033	0.021	0.3 (0.09 - 0.88)
C G T G A G	0.003	0.001		
C G C T A A	0.020	0.007		
C G C T A G	0.006	0.003		
C G C G A A	0.009	0.0	0.042	2.0 (1.91 - 2.19)
C G C G A G	0.014	0.0	0.013	2.0 (1.91 - 2.19)
C T T T A A	0.014	0.0	0.023	2.0 (1.91 - 2.19)
C T T T A G	0.004	0.0	0.014	4.0 (3.50 - 4.66)
C T T G A A	0.007	0.002		
C T T G G A	0.005	0.0002	0.014	4.0 (3.50 - 4.66)
T G T T A A	0.084	0.019	<.0001	4.8 (2.22 - 10.55)
T G T T A G	0.029	0.030		
T G T G A A	0.0002	0.0006		
T G T G A G	0.0	0.00009		
T G C T A A	0.009	0.002		
T G C T A G	0.0001	0.0		
T G C G A A	0.00002	0.0		
T G C G A G	0.00007	0.0		
T T T G G A	0.012	0.014		

p: RA patients vs. controls

Table 6. The frequencies of genotype of *TIM-3* gene in other ethnic groups

SNPs	[1)]Korean	[2)]Korean	African	[4)]Asian	[4)] European
Tim-3					
rs11742259 (Intron)			[3)] (n=86)	(n=90)	(n=120)
*C/C	187 (87.0)	-	33 (38.4)	70 (77.8)	80 (66.7)
*C/T	28 (13.0)	-	42 (48.8)	20 (22.2)	32 (26.7)
*T/T	0 (0.0)	-	11 (12.8)	0 (0.0)	8 (6.7)
rs10515746(*-574G>T*) 5′near gene	(n=215)	(n=319)	[4)] (n=10)	(n=12)	(n=20)
*G/G	209 (97.2)	319 (100)	10 (100)	12 (100)	16 (80.0)
*G/T	5 (2.3)	0 (0.0)	0 (0.0)	0 (0.0)	4 (20.0)
*T/T	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
rs35960726(*Ile97Met*) exon2			[4)] (n=72)		
*T/T	211 (98.1)	-	70 (97.2)	-	-
*T/C	3 (1.4)	-	2(2.8)	-	-
*C/C	1 (0.5)	-	0 (0.0)	-	-
rs1036199(*4259T>G*) (*Leu140Arg*) exon3		(n=319)	[4)] (n=46)	(n=48)	(n=46)
*T/T	196 (91.2)	256 (80.3)	28 (60.9)	33 (69.6)	32 (69.6)
*T/G	16 (7.4)	63 (19.7)	18 (39.1)	13 (26.1)	12 (26.1)
*G/G	3 (1.4)	0 (0.0)	0 (0.0)	2 (4.3)	2 (4.3)
rs4704846 (3′UTR)				(n=88)	(n=114)
*A/A	209 (97.2)	-	-	86 (97.7)	80 (70.2)
*A/G	6 (2.8)	-	-	2 (2.3)	26 (22.8)
*G/G	0 (0.0)	-	-	0 (0.0)	8 (7.0)
rs11134551 (Intron)			[3)] (n=87)	(n=90)	(n=120)
*A/A	175 (81.4)	-	46 (52.9)	66 (73.3)	100 (83.3)
*A/G	36 (16.7)	-	39 (44.8)	20 (22.2)	20 (16.7)
*G/G	4 (1.9)	-	2 (2.3)	14 (4.4)	0 (0.0)

[1)] In this study, [2)] Chae et al, 2004b, [3)] Gao et al., 2005, [4)]http://www.hapmap.org

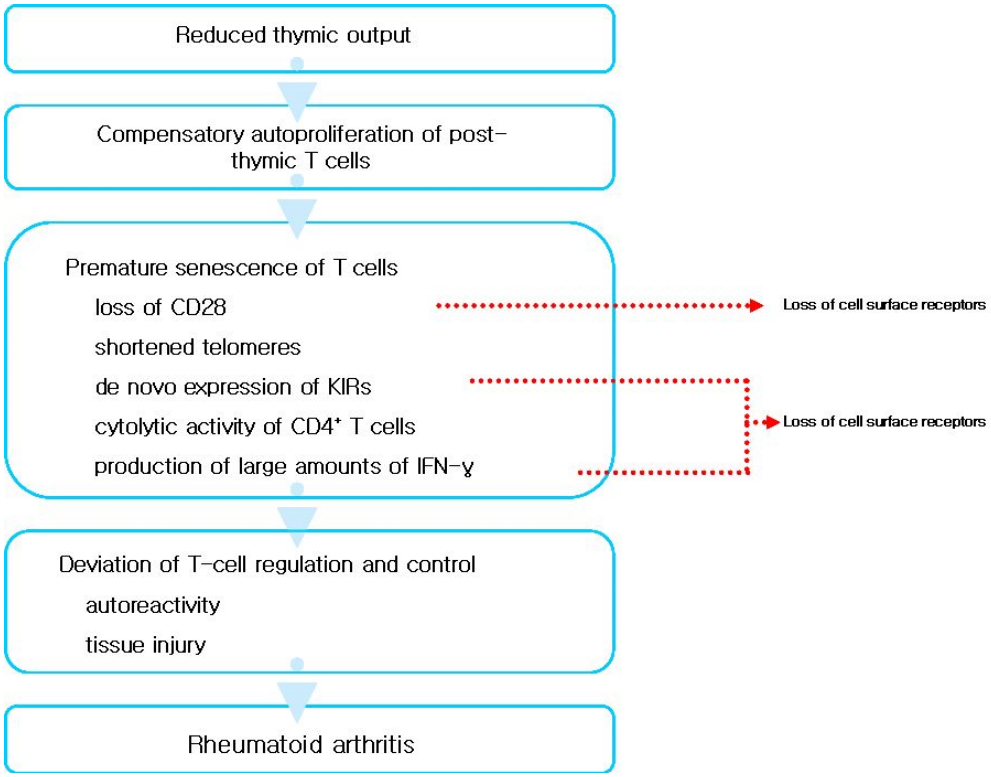

Figure 1. Premature immunosenescence and RA- a disease model

(after Weyand et al., 2003)

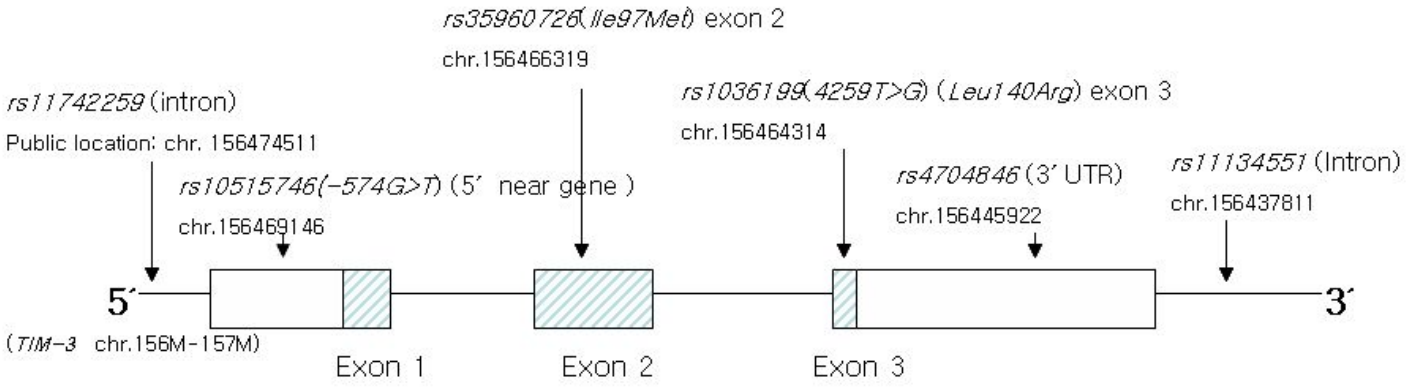

Figure 2. SNPs position in the *TIM-3* (*HAVCR2,* MIM:606652) on 5q33.2

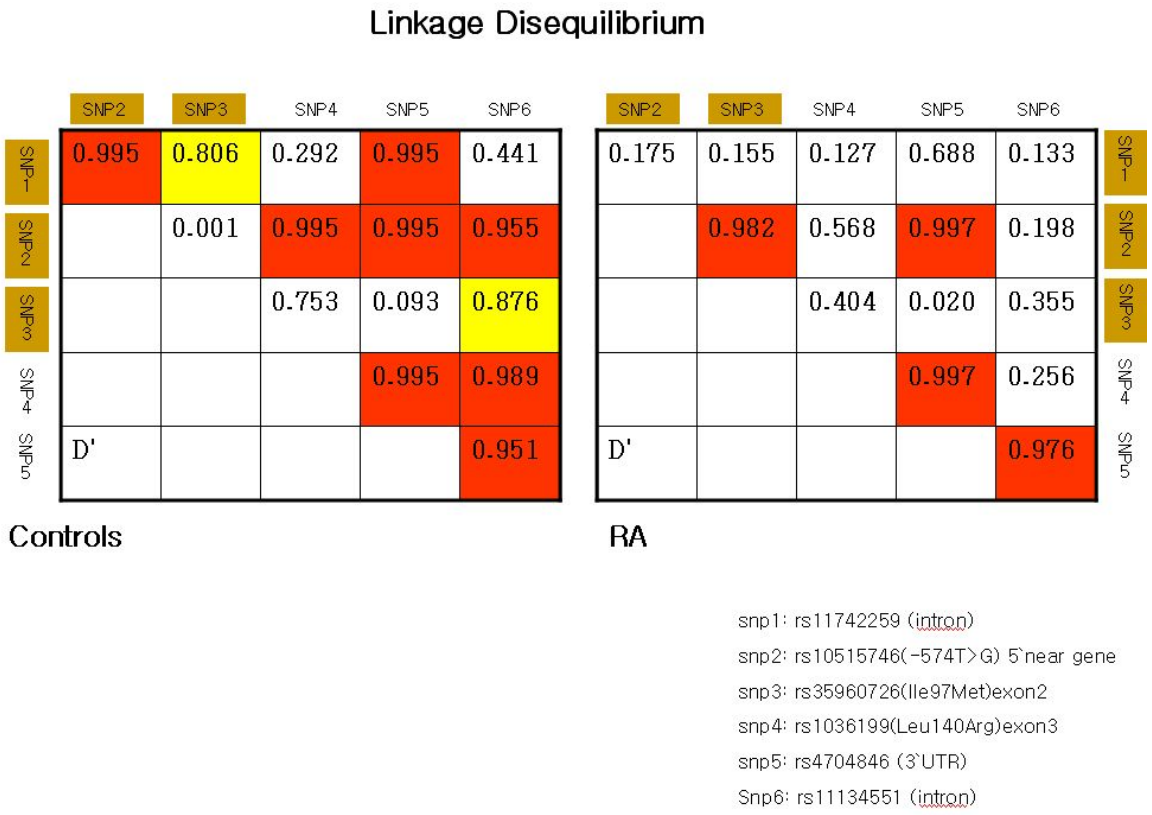

Figure 3. Linkage disequilibrium coefficient | D' | between SNPs of *TIM-3* in the Korean population

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/

http://www3.appliedbiosystems.com/

http://www.r-project.org/

http://bibs.snu.ac.kr/R/

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Abstract

Genetic association study between the T cell immunoglobulin mucin *(TIM)-3* gene and rheumatoid arthritis

Jae Hee Park
Department of Education
Graduate School of Education
Sungshin Women's University

The family of T-cell immunoglobulin domain and mucin domain (TIM) proteins is expressed on T cell. TIM-3 protein, a member of TIM family, is specifically expressed on Th1 cells might have an important role in the induction of autoimmune disease by regulating macrophage activation and interacts with TIM-3 ligand to regulate Th1 responses negatively. The objective of this study was to investigate genetic association of the sequence variants for *TIM-3 (HAVCR2,* MIM:606652*)* gene with RA in Korean population. A total of 206 RA patients and 215 non-RA healthy controls were studied. The TaqMan-allelic discrimination method using the Applied Biosystems 7300 Real Time PCR System (Applied Biosystems, Foster City, Calif) was used for detection of 6 SNPs (1 in promoter site, 2

in exon, 2 in intron, 1 in 3′UTR). PCR results were analyzed using the Allelic Discrimination software. The major *TIM3 c.-574*G/*G,* 97*Ile/*Ile,* and *rs11742259*C/*C* genotypes in RA were significantly associated compared with controls (p= 0.009, OR = 0.3, 95% CI = 0.12 - 0.78; p= 0.0001, OR = 0.2, 95% CI = 0.05 - 0.45; p= 0.001, OR = 0.4, 95% CI = 0.27 - 0.74, respectively). No significance was observed between the *TIM-3* genotype and the presence of RF, ANA, or bony erosions in RA. Analysis of major haplotype (*rs11742259C-rs10515746G-rs35960726T-rs1036199T-rs4704846A-rs11134551A*) consisting of 6 SNPs on the *TIM-3* gene revealed a reduced susceptibility to RA as compared with controls (p= 0.0001, OR = 0.5, 95% CI = 0.39 - 0.74).

In conclusion, the polymorphisms, *rs10515746 (-574G>T)*, *rs35960726* (*Ile97Met*) and *rs111742259* (intron) of *TIM-3* were associated with RA, but were not associated with RF, ANA, and bony erosion status in RA patients.