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LETTER TO THE EDITOR

Genotypic analysis of Asp299Gly and Thr399Ile polymorphism of Toll-like receptor 4 in systemic autoimmune diseases of Korean population

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Dear Editor,

Systemic autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus are mainly characterized by chronic inflammation amplified by large quantities of proinflammatory cytokines released from immune cells including macrophages and dendritic cells. Although the pathogenesis of autoimmune diseases are largely unknown, adaptive immune responses in various systemic autoimmune diseases can be driven by innate immune system as exemplified in pathogenic autoantibody production triggered by infectious agents in the pathogenesis of rheumatoid arthritis and systemic lupus erythematosus [1, 2]. Toll-like receptors (TLRs) are phylogenetically conserved receptors expressed on various cells, mainly on macrophages and dendritic cells that recognize pathogen-associated molecular patterns and are key players in cellular innate immunity with an emerging role as a bridge between innate and adaptive immune responses [3]. TLRs, especially TLR4, are predominantly activated by bacterial lipopolysaccharides [4] and by endogenous ligands such as heat shock proteins [5], fragments of hyaluronic acid [6] and fibronectin [7], which are released by damaged cells. Activation of TLRs finally leads to activation of nuclear transcription factors such as NF-κB via signal transduction involving various intracellular signaling systems and to expression of various inflammatory cytokines. There are two variants of *TLR4* gene, Asp299Gly and Thr399Ile; both are present in a putative coreceptor-binding region of TLR-4 and in linkage disequilibrium [8]. The polymorphisms at these two *TLR4* variants appeared to be correlated with the susceptibility to gram-negative septic shock [9], the development of atherosclerosis [10] and ischemic stroke [11], and with the host susceptibility to autoimmune process in human [12, 13]. Therefore, aberrant TLR functions, especially TLR4 involved in host defense against Gram-negative bacteria, may alter the response of this receptor to stimulation with LPS and probably endogenous ligands and eventually may induce the loss of self-tolerance and triggering of autoimmune diseases. The expression and function of TLR4 in systemic autoimmune diseases have been studied by different groups, but the frequencies of variant alleles and the clinical significance were different depending on diagnosis or on race [9–14]. The frequencies of *TLR4* gene polymorphism in these two alleles among Korean population, is not known. Therefore, we report the frequencies of *TLR4* Asp299Gly and Thr399Ile polymorphism in

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systemic autoimmune diseases among Korean population and the possible contribution to susceptibility and clinical outcome of there diseases.

Ninety-five patients diagnosed as having systemic autoimmune diseases according to the Criteria of the 1997 American College of Rheumatology at Rheumatology clinic of Ewha Womans University, Mokdong Hospital, Seoul, Korea, were subjected to *TLR4* genotyping. The patient group was diagnosed with diseases including 44 RA, 31 SLE, 5 Bechet disease and 15 unclassified autoimmune diseases as a diagnosis. Male to female ratio was 17:78 and mean age was 48 (range 17–79 year old). The clinical and laboratory findings were obtained from medical records. A group of controls comprised of 80 healthy female individuals with mean age of 36 (range 27–58 year old), was included. The ethnic background of all patients and controls was Korean and informed consent was obtained from all participants. PCR–RFLP analysis was performed with sets of specific primers, modified to create specific restriction enzyme recognition sites to differentiate wild and mutant alleles. Three different conditions using three sets of primer pairs and three kinds of restriction enzymes were applied for confirmation of the results from each conditions for Asp299Gly variant and a pair of primer and *Hinf*I restriction enzyme were used for investigation of Thr399Ile variant (Table 1) [15, 16].

None of the individuals in 95 patients and 80 control groups had Asp299Gly and Thr399Ile polymorphisms. Of note, the absence of Asp299Gly polymorphism was double checked with PCR–RFLP which was designed to create the restriction enzyme recognition sites for mutant allele alone and for both wild and mutant allele to exclude technical insufficiency of the other condition. Because the Asp299 Gly polymorphism is in linkage disequilibrium with the Thr399Ile, absence of Thr399Ile polymorphism could be explained. This result is comparable with no detectable Asp299Gly and Thr399Ile mutant alleles among Japanese [14, 15], and with no detectable Asp299Gly mutant allele among Taiwanese [11].

In conclusion, when considering the genetic similarity among East Asian population, the Asp299Gly and Thr399Ile polymorphisms of *TLR4* gene might be very rare not only among Japanese and Chinese but also among Koreans, which is in contrary with Caucasians. Though there are preferable results about the influence of *TLR4* gene polymorphisms on disease susceptibility and outcome of systemic autoimmune diseases such as RA and SLE in the study on Caucasians, TLR4 polymorphisms may not be implicated as a significant genetic factor in the pathogenesis of systemic autoimmune diseases among Korean population. Ethnic variation in allele frequencies of *TLR4* gene might explain the differences of disease susceptibility to infections, septic shock and certain conditions manifested by inflammatory cytokines among different races.

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Appendix

Table 1.

Table 1 Sequences of primers, restriction enzymes and length of the resulting restriction fragments for the analysis of *TLR4* gene polymorphisms

Polymorphism	Primers	Restriction enzyme	Restriction fragments		References
Asp299Gly	First condition				[15]
	F 5′-AGCATACTTAGACTACTA**C**CTCCATG-3′	*Nco*I	Wild	188 bp	
	R 5′-GAGAGATTTGAGTTTCAATGTGGG-3′		Mutant	168 bp	
	Second condition				[16]
	*F*1 5′-TTAGAAATGAAGGAAACTTGGAAAAG-3′	*Bsa*BI	Wild	112 bp	
	*R*1 5′-TTTGTCAAACAATTAAATAAGT**GA**T**T**AATA-3′		Mutant	139 bp	
	Third condition				[16]
	*F*2 5′-AGCATACTTAGACTAC**C**ACCTCGATG-3′	*Bst*XI	Wild	112 bp	
	*R*2 5′-GTTGCCATCCGAAATTATAAGAAAAG-3′		Mutant	108 bp	
Thr399Ile	*F* 5′-GGTTGCTGTTCTCAAAGTGATTTTGGGA**G**AA-3′	*Hinf*I	Wild	124 bp	[15]
	R 5′-GGAAATCCAGATGTTCTAGTTGTTCTAAGCC-3′		Mutant	98 bp	

Bold characters indicate mismatched nucleotides

F, forward primer; *R*, reverse primer

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