

## Association of forkhead box J3 (FOXJ3) polymorphisms with rheumatoid arthritis

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**Abstract.** The aim of this study was to investigate the correlation between single nucleotide polymorphisms (SNPs) of the forkhead box J3 (FOXJ3) gene and rheumatoid arthritis (RA). For the association studies, 307 patients with RA and 476 control patients without RA were recruited. Eleven SNPs (rs2282404, rs2455084, rs1393009, rs7539485, rs4660616, rs510157, rs343386, rs12732892, rs343389, rs343376 and rs585320) were genotyped using direct sequencing and the resulting data were analyzed using the SNPStats, Haploview and HelixTree programs. Seven SNPs (rs2455084, rs1393009, rs7539485, rs4660616, rs510157, rs343386 and rs343389) were associated with RA in three alternative models (log-additive, dominant and recessive models;  $P < 0.05$ ). A strong linkage disequilibrium block, including all 11 SNPs, was constructed using the Gabriel method. Two haplotypes, TCCTTGCTTT and TCTTCTGTCAC, were significantly associated with RA ( $P < 0.05$ ). In clinical characteristic analysis, the SNP rs585320 was also associated with the anti-cyclic citrullinated peptide. These results suggest that FOXJ3 may be associated with the development of RA.

### Introduction

Rheumatoid arthritis (RA) is an autoimmune disease that affects ~1% of the adult population (1). RA is considered to be a complex disease and its etiology and pathogenesis remain unclear (2). Genetic and environmental factors, and their interactions, are

involved in the development of RA (3). The strongest genetic associations are with genes located in the human leukocyte antigen (HLA) region, particularly the HLA-DRB1 gene (4,5). Rheumatoid factor (RF) production in RA patients is correlated with smoking. Matthey *et al* (6) demonstrated that patients that were HLA-DRB1\*0401 positive and had previously smoked were likely to be RF positive. Various genes, including the protein tyrosine phosphatase non-receptor 22 (PTPN22) gene, are also known to be associated with RA (7). Karlson *et al* (8) demonstrated that HLA-DRB1 shared epitope (HLA-SE) and PTPN22 were correlated with presence of cyclic citrullinated peptide (CCP) and RF. However, the strength of the association between these genes and RA vary geographically. For example, PTPN22 is associated with RA in populations of European origin, but not in non-European populations (9,10). In contrast, peptidyl arginine deiminase type IV, solute carrier family 22 (organic cation/ergothioneine transporter) member 4 and Fc receptor-like 3 are associated with RA in the Japanese population and in other Asian groups (11-13); however, yields weak or negative results in European populations (14,15). These divergent results suggest the genetic heterogeneity of RA across human populations.

Forkhead proteins are a large family of functionally diverse transcription factors that have been implicated in a variety of cellular processes (16). Forkhead box J3 (FOXJ3) is a member of the FOX gene family, which encodes transcription factors composed of a conserved DNA binding motif termed the forkhead domain (17). Based on interpretations of the mouse and human genome sequences, human FOX genes have ~43 members, which have been classified into 17 subclasses: FOXA to FOXQ (18). Numerous members of this family are crucial in the regulation of immune responses and it has been demonstrated that FOXP3 is important in autoimmune diseases (19). Li *et al* (20) showed that a FOXJ1 polymorphism (rs3192453, 3375G>C) was associated with allergic rhinitis and that another FOXJ1 polymorphism was related to systemic lupus erythematosus and RA in the Korean population (21). However, no genetic study of FOXJ3 has currently been conducted.

In the present study, it was investigated whether FOXJ3 polymorphisms were correlated with susceptibility to RA.

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Table I. Primer sequences for FOXJ3 polymorphisms.

SNP	Primer	Size (bp)
rs2282404	Sense: 5'-CCATAACCTGGGTAGCTACCTTGAG-3' Antisense: 5'-TTCCGAGCGACTTGGGGTCCTTGT-3'	441
rs2455084	Sense: 5'-TGCGATGGCTGCTTCTAAATGTGG-3' Antisense: 5'-AAATGACGTCTGAACCCCTAGCCTT-3'	488
rs1393009	Sense: 5'-GAACCTTGCATGCTTAACTCTGTGG-3' Antisense: 5'-ACAAGCCTGTCCATACAAACCCATC-3'	452
rs7539485	Sense: 5'-CATGATCATAGCTCACTGCAGCCT-3' Antisense: 5'-CATCAGACAAGTCAGTGGGGAAAGG-3'	603
rs4660616	Sense: 5'-CTGGCTGACTCAGTATGAGAAGTGA-3' Antisense: 5'-GGAAGATCTGCCATGTGCCCTAAA-3'	492
rs510157	Sense: 5'-CCTGGCCACAAAGCGAGGTTTAAA-3' Antisense: 5'-TGTAATTCCAGGTGATTTGGGGGGT-3'	557
rs343386	Sense: 5'-GAGCTTCCCTTTGGTCCTTCTTGTG-3' Antisense: 5'-AGACAGCTACATCATTCGGGAGCT-3'	757
rs12732892	Sense: 5'-GATTAGTGTGGCTGTCAGTGTATTG-3' Antisense: 5'-CATACCAAACCTATGGAGTATAGC-3'	512
rs343389	Sense: 5'-CCATTCAGGTAGTCTGTACCTTT-3' Antisense: 5'-GCAGAAACCTTACAGACCAGGAGAG-3'	481
rs343376	Sense: 5'-CCATGTAGATTTGTGAGGTCCTGGG-3' Antisense: 5'-CCTAAACTGTCCCTCAGAGTTAAG-3'	661
rs585320	Sense: 5'-GTGTCAACATGGTTTCCAGCTGTAG-3' Antisense: 5'-CTGCTACGTTTTTCTTTCCCGTGTG-3'	433

FOXJ3, forkhead box J3; SNP, single nucleotide polymorphism; bp, base pair.

## Patients and methods

**Patients.** A case-control study was conducted to investigate the correlation between RA and FOXJ3 polymorphisms. Unrelated patients (n=307) with RA (59 males and 248 females) were recruited from rheumatology centers in the Republic of Korea (Kyung Hee University Hospital, Seoul, Korea). Each patient was diagnosed by a rheumatologist based on the ACR 1987 rheumatoid arthritis diagnostic criteria (22). For the control group, 476 subjects (207 males and 269 females) were recruited from participants undergoing a general health check-up program, following the confirmation of no clinical implication of rheumatic disease or any other disorder. All procedures were conducted according to the Declaration of Helsinki guidelines. Written informed consent was obtained from each subject. This study was approved by the Ethics Review Committee of the Medical Research Institute, School of Medicine, Kyung Hee University (Seoul, Korea).

Clinical data, such as gender, age at disease onset and the presence of bone erosion were obtained by reviewing medical records at the time of enrollment. Laboratory data included erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), anti-cyclic citrullinated peptide (anti-CCP) and RF values.

**SNP selection and genotyping.** All tagging SNPs of FOXJ3 analyzed in this study were selected from our unpublished results of a 100K genome-wide association analysis (Illumina, Inc., San Diego, CA, USA) in RA. From the International HapMap Project data set (<http://www.hapmap.org>), the SNPs with an optimal minor allele frequency ( $\geq 5\%$ ), heterozygosity  $>0.1$  and the best coverage to serve as tag SNPs

were genotyped. A total of 11 SNPs [rs2282404 (5'-near gene), rs2455084 (intron 1), rs1393009 (intron 1), rs7539485 (intron 2), rs4660616 (intron 3), rs510157 (intron 4), rs343386 (intron 4), rs12732892 (intron 4), rs343389 (intron 4), rs343376 (exon 5, Ala161Val) and rs585320 (intron 7)] were selected. The locations of the 11 selected SNPs in the region of FOXJ3 are shown in Fig. 1A. Genomic DNA was extracted using a commercially available Qiagen DNA extraction kit (Qiagen, Tokyo, Japan) from blood samples collected in EDTA. SNP genotyping was performed using direct sequencing with genomic DNA that was amplified using primers for each SNP. The sequences of the primers are shown in Table I. The samples were sequenced using an ABI Prism 377 automatic sequencer (Applied Biosystems, Inc., Foster City, CA, USA). Sequence data were analyzed using the SeqManII software (DNASStar, Inc., Madison, WI, USA).

**Statistical analysis.** Hardy-Weinberg equilibrium (HWE) for 11 SNPs was assessed using SNPStats (<http://bioinfo.iconcologia.net/SNPstats>). A linkage disequilibrium (LD) block of polymorphisms was tested using Haploview version 4.2 (Broad Institute of MIT and Harvard, Cambridge, MA, USA). The haplotypes and their frequencies were calculated by the expectation-maximization algorithm. Multiple logistic regression analyses were conducted to calculate the odds ratios, 95% confidence intervals and corresponding P-values. SNPStats, HapAnalyzer, HelixTree software (Golden Helix, Inc., Bozeman, MT, USA) and SNPAnalyzer (ISTECH, Inc., Goyang, Korea) were used to evaluate genetic data. Logistic regression analysis in all three analysis models (log-additive, dominant and recessive models for rare alleles) was utilized to demonstrate alternative effects of the variants (23).

Table II. General characteristics of study subjects.

Characteristic	Patients with RA (n=307)	Controls (n=476)
Age (years, mean $\pm$ SD)	46.56 $\pm$ 12.91	44.45 $\pm$ 12.70
Gender (male/female)	59/248	207/269
Disease duration (years, mean $\pm$ SD)	4.68 $\pm$ 4.53	
Erythrocyte sedimentation rate (mm/h, mean $\pm$ SD)	41.00 $\pm$ 29.29	
C-reactive protein (mg/dl, mean $\pm$ SD)	2.26 $\pm$ 4.84	
Patients with bone erosion (n)	143	
RF positive (n)	268	
Anti-CCP positive (n)	90	

RA patients (n=130) which were not analyzed for the presence of anti-CCP were excluded. RA, rheumatoid arthritis; n, number of subjects; RF, rheumatoid factor; anti-CCP, anti-cyclic citrullinated peptide.

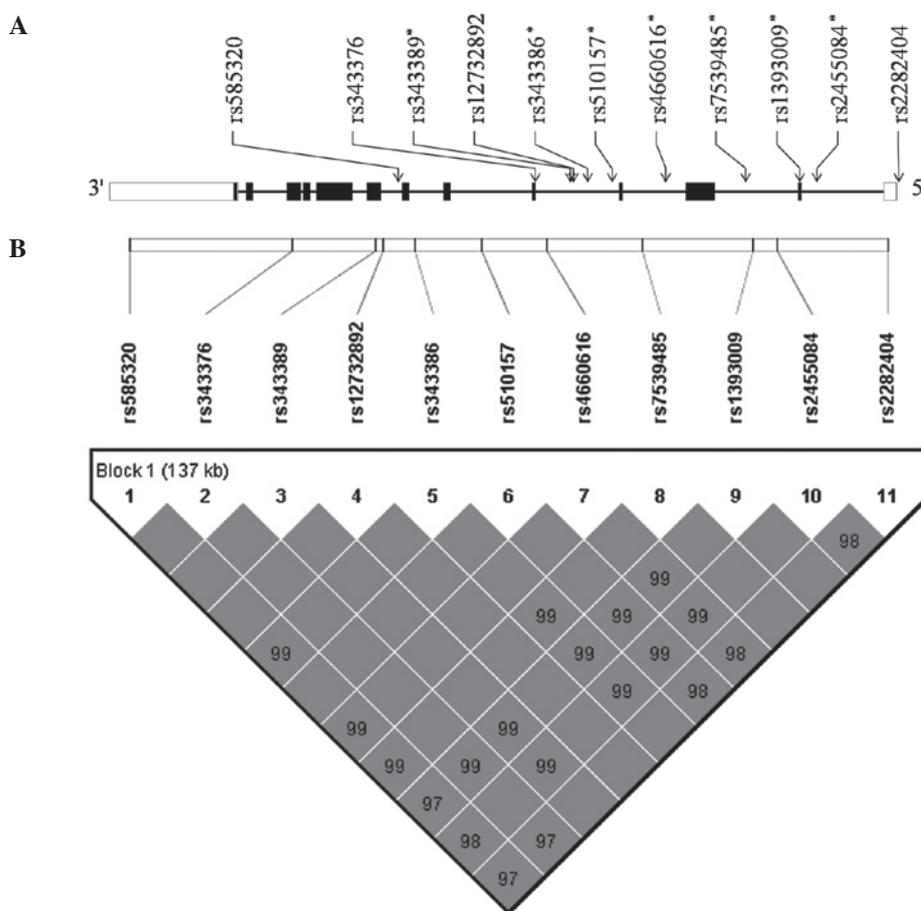


Figure 1. Gene map and LD in FOXJ3. (A) Gene map of SNPs in FOXJ3 on chromosome 1p34.2. Exons are boxed, coding regions are represented by black boxes and untranslated regions by white boxes. \*Indicates significant SNPs. Arrows indicate the location of each SNP. (B) LD coefficient ( $|D'|$ ) and an LD block among FOXJ3 SNPs visualized using Haploview 4.2. LD, linkage disequilibrium; FOXJ3, forkhead box J3; SNPs, single nucleotide polymorphisms.

$P < 0.05$  was considered to indicate a statistically significant difference.

## Results

The clinical characteristics of the 307 RA patients (248 females and 59 males) and 476 control subjects are shown in Table II. The mean age of the RA patients was 45.56 $\pm$ 12.91 years and the mean age of the control subjects was 44.45 $\pm$ 12.70 years.

The mean disease duration was 4.68 $\pm$ 4.53 years. The mean level of ESR in RA patients was 41.00 $\pm$ 29.29 mm/h and CRP was 2.26 $\pm$ 4.84 mg/dl. Among the 307 RA patients, 268 patients were positive for RF (87.3%) and 143 patients exhibited bone erosion (46.6%).

In the control group, the genotype distributions for all SNPs were in HWE (data not shown). The genotype frequencies of the polymorphisms were compared between the RA and the normal control groups using logistic regression models with

Table III. Genotype frequencies of FOXJ3 polymorphisms in patients with rheumatoid arthritis and control subjects.

SNP (Locus)	Genotype	RA	Control	Log-additive		Dominant		Recessive	
		n (%)	n (%)	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
rs2282404 (promoter)	C/C	147 (50.0)	246 (55.7)	0.82 (0.65-1.04)	0.098	0.80 (0.59-1.07)	0.130	0.73 (0.42-1.27)	0.270
	T/C	122 (41.5)	168 (38.0)						
	T/T	25 (8.5)	28 (6.3)						
rs2455084 (intron 1)	T/T	184 (61.3)	252 (53.5)	1.31 (1.04-1.65)	<b>0.020</b>	1.38 (1.03-1.85)	<b>0.032</b>	1.55 (0.90-2.68)	0.110
	A/T	96 (32.0)	172 (36.5)						
	A/A	20 (6.7)	47 (10.0)						
rs1393009 (intron 1)	T/T	183 (62.9)	252 (53.4)	1.37 (1.09-1.73)	<b>0.007</b>	1.48 (1.10-1.99)	<b>0.010</b>	1.58 (0.91-2.76)	0.096
	T/C	89 (30.6)	173 (36.6)						
	C/C	19 (6.5)	47 (10.0)						
rs7539485 (intron 2)	C/C	183 (61.8)	239 (54.6)	1.28 (1.01-1.61)	<b>0.037</b>	1.35 (1.00-1.82)	0.051	1.46 (0.85-2.51)	0.160
	T/C	92 (31.1)	155 (35.4)						
	T/T	21 (7.1)	44 (10.1)						
rs4660616 (intron 3)	T/T	182 (60.1)	251 (53.3)	1.28 (1.02-1.60)	<b>0.033</b>	1.32 (0.98-1.77)	0.063	1.56 (0.92-2.66)	0.095
	T/G	100 (33)	171 (36.3)						
	G/G	21 (6.9)	49 (10.4)						
rs510157 (intron 4)	G/G	184 (62.0)	239 (53.5)	1.33 (1.06-1.68)	<b>0.015</b>	1.42 (1.05-1.91)	<b>0.022</b>	1.55 (0.90-2.68)	0.110
	T/G	93 (31.3)	163 (36.5)						
	T/T	20 (6.7)	45 (10.1)						
rs343386 (intron 4)	T/T	182 (60.3)	249 (53.1)	1.28 (1.02-1.60)	<b>0.032</b>	1.34 (1.00-1.80)	0.050	1.49 (0.87-2.55)	0.140
	T/C	99 (32.8)	173 (36.9)						
	C/C	21 (7.0)	47 (10.0)						
rs12732892 (intron 4)	T/T	217 (72.6)	320 (68.1)	1.17 (0.90-1.52)	0.240	1.24 (0.90-1.71)	0.180	1.08 (0.54-2.18)	0.830
	T/C	69 (23.1)	128 (27.2)						
	C/C	13 (4.3)	22 (4.7)						
rs343389 (intron 4)	C/C	183 (61.0)	252 (53.5)	1.29 (1.02-1.61)	<b>0.029</b>	1.36 (1.01-1.82)	<b>0.040</b>	1.47 (0.86-2.52)	0.150
	T/C	96 (32.0)	172 (36.5)						
	T/T	21 (7.0)	47 (10.0)						
rs343376 (exon 5, Ala161 Val)	C/C	219 (72.5)	321 (68.3)	1.16 (0.90-1.51)	0.250	1.22 (0.89-1.68)	0.210	1.14 (0.57-2.29)	0.700
	T/C	70 (23.2)	126 (26.8)						
	T/T	13 (4.3)	23 (4.9)						
rs585320 (intron 7)	T/T	217 (72.1)	320 (67.7)	1.14 (0.88-1.48)	0.300	1.24 (0.90-1.70)	0.190	0.97 (0.50-1.90)	0.940
	T/C	69 (22.9)	130 (27.5)						
	C/C	15 (5.0)	23 (4.9)						

P-values were obtained from logistic regression analyses with the log-additive, dominant and recessive models controlling age and gender as covariates. Bold numbers indicate significant associations. FOXJ3, forkhead box J3; SNP, single nucleotide polymorphism; RA, rheumatoid arthritis; n, number of subjects; OR, odds ratio; CI, confidence interval.

Table IV: Haplotype association of FOXJ3 in patients with rheumatoid arthritis and control subjects.

Haplotype	Genotype	RA	Control	Log-additive		Dominant		Recessive	
		n (%)	n (%)	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
TCCTTGCTCTC	H/H	77 (25.1)	105 (22.1)	1.00 (0.82-1.22)	0.995	0.86 (0.62-1.18)	0.350	1.18 (0.84-1.66)	0.329
	H/-	140 (45.6)	246 (51.7)						
	-/-	90 (29.3)	125 (26.3)						
TCCTTGCTCTT	H/H	25 (8.1)	28 (5.9)	1.28 (1.02-1.61)	<b>0.035</b>	1.35 (1.01-1.80)	<b>0.043</b>	1.42 (0.81-2.48)	0.221
	H/-	122 (39.7)	165 (34.7)						
	-/-	160 (52.1)	283 (59.5)						
CTTCCTGTCAC	H/H	13 (4.2)	22 (4.6)	0.86 (0.67-1.12)	0.264	0.82 (0.60-1.12)	0.212	0.91 (0.45-1.84)	0.798
	H/-	74 (24.1)	133 (27.9)						
	-/-	220 (71.7)	321 (67.4)						
TCTTCTGTCAC	H/H	3 (1.0)	4 (0.8)	0.66 (0.45-0.96)	<b>0.032</b>	0.60 (0.40-0.91)	<b>0.017</b>	1.16 (0.26-5.24)	0.843
	H/-	34 (11.1)	84 (17.6)						
	-/-	270 (87.9)	388 (81.5)						

P-values were obtained from logistic regression analyses with the log-additive, dominant and recessive models. Bold numbers indicate significant associations. FOXJ3, forkhead box J3; RA, rheumatoid arthritis; n, number of subjects; OR, odds ratio; CI, confidence interval.

Table V. Genotype frequencies of FOXJ3 polymorphisms in rheumatoid arthritis patients with anti-CCP (negative) and anti-CCP (positive).

SNP (Locus)	Genotype	anti-CCP (-)		anti-CCP (+)		Log-additive		Dominant		Recessive	
		n (%)	n (%)	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value		
rs2282404 (promoter)	C/C	36 (43.4)	43 (48.3)	0.89 (0.56-1.40)	0.60	0.83 (0.45-1.52)	0.54	0.94 (0.35-2.50)	0.89		
	T/C	38 (45.8)	37 (41.6)								
	T/T	9 (10.8)	9 (10.1)								
rs2455084 (intron 1)	T/T	45 (52.3)	58 (64.4)	0.64 (0.39-1.05)	0.08	0.62 (0.33-1.14)	0.12	0.44 (0.13-1.53)	0.18		
	A/T	33 (38.4)	28 (31.1)								
	A/A	8 (9.3)	4 (4.4)								
rs1393009 (intron 1)	T/T	44 (55.0)	58 (64.4)	0.69 (0.42-1.14)	0.15	0.68 (0.36-1.26)	0.22	0.48 (0.13-1.70)	0.24		
	T/C	29 (36.2)	28 (31.1)								
	C/C	7 (8.8)	4 (4.4)								
rs7539485 (intron 2)	C/C	44 (54.3)	58 (65.2)	0.66 (0.40-1.09)	0.10	0.65 (0.35-1.21)	0.17	0.42 (0.12-1.46)	0.16		
	T/C	29 (35.8)	27 (30.3)								
	T/T	8 (9.9)	4 (4.5)								
rs4660616 (intron 3)	T/T	44 (51.2)	58 (64.4)	0.63 (0.38-1.03)	0.06	0.59 (0.32-1.09)	0.100	0.44 (0.13-1.52)	0.18		
	T/G	34 (39.5)	28 (31.1)								
	G/G	8 (9.3)	4 (4.4)								
rs510157 (intron 4)	G/G	45 (55.6)	58 (65.2)	0.68 (0.41-1.11)	0.12	0.67 (0.36-1.24)	0.20	0.42 (0.12-1.47)	0.16		
	T/G	28 (34.6)	27 (30.3)								
	T/T	8 (9.9)	4 (4.5)								
rs343386 (intron 4)	T/T	45 (52.3)	58 (64.4)	0.64 (0.39-1.05)	0.08	0.62 (0.33-1.13)	0.12	0.44 (0.13-1.53)	0.18		
	T/C	33 (38.4)	28 (31.1)								
	C/C	8 (9.3)	4 (4.4)								
rs12732892 (intron 4)	T/T	58 (69.0)	66 (73.3)	0.70 (0.40-1.21)	0.20	0.81 (0.42-1.57)	0.53	0.13 (0.02-1.12)	0.03		
	T/C	20 (23.8)	23 (25.6)								
	C/C	6 (7.1)	1 (1.1)								
rs343389 (intron 4)	C/C	45 (54.2)	58 (64.4)	0.67 (0.41-1.09)	0.11	0.66 (0.36-1.22)	0.18	0.43 (0.12-1.48)	0.17		
	T/C	30 (36.1)	28 (31.1)								
	T/T	8 (9.6)	4 (4.4)								
rs343376 (exon 5, Ala161 Val)	C/C	58 (69.0)	66 (74.2)	0.67 (0.38-1.17)	0.16	0.76 (0.39-1.49)	0.43	0.13 (0.02-1.14)	0.03		
	T/C	20 (23.8)	22 (24.7)								
	T/T	6 (7.1)	1 (1.1)								
rs585320 (intron 7)	T/T	58 (67.4)	66 (73.3)	0.65 (0.38-1.12)	0.12	0.75 (0.39-1.44)	0.39	0.12 (0.01-0.99)	<b>0.015</b>		
	T/C	21 (24.4)	23 (25.6)								
	C/C	7 (8.1)	1 (1.1)								

P-values were obtained from logistic regression analyses with the log-additive, dominant and recessive models controlling age and gender as covariates. Bold numbers indicate significant associations. FOXJ3, forkhead box J3; anti-CCP, anti-cyclic citrullinated peptide; SNP, single nucleotide polymorphism; n, number of subjects; OR, odds ratio; CI, confidence interval.

adjustment for age and gender. The genotype distributions of FOXJ3 gene polymorphisms in the RA and control groups are shown in Table III. Of the 11 SNPs, seven (rs2455084, rs1393009, rs7539485, rs4660616, rs510157, rs343386 and rs343389) were significantly associated with the risk of RA. The rare alleles of rs2455084 (rare allele, A), rs1393009 (rare allele, C), rs7539485 (rare allele, T), rs4660616 (rare allele, G), rs510157 (rare allele, T), rs343386 (rare allele, C) and rs343389 (rare allele, T) were associated with a decreased risk of RA. The rest of the SNPs (rs2282404, rs12732892, rs343376 and rs585320) were not associated with RA (Table III).

FOXJ3, located on chromosome 1p34.2, consists of 622 amino acids and has a molecular mass of 68,928 Da. The missense polymorphism rs343376 (Ala162Val) is the only SNP with heterozygosity >0.1 among the coding SNPs located in the FOXJ3 gene region (<http://www.ncbi.nlm.nih.gov/SNP>). CC, CT and TT genotype frequencies are 0.467, 0.417 and 0.117 in European; 0.822, 0.178 and 0.000 in Chinese; 0.750, 0.227 and 0.023 in Japanese and 0.467, 0.433 and 0.100 in Sub-Saharan African populations (<http://www.ncbi.nlm.nih.gov/SNP>).

The CC, CT and TT genotype frequencies in the population of the present study were 0.680, 0.270 and 0.040, which are similar to those in the Japanese population. However, the rs343376 polymorphism was not associated with RA in the study population (Table III). The SNP genotype frequencies obtained from the study population are similar to frequencies observed in the Japanese population, according to the NCBI website.

A strong LD block, including all SNPs, was constructed using the Gabriel method (Fig. 1B). Four haplotypes in the block exhibited frequencies >0.05 and all were used for haplotype association analysis. The frequency of the TCCTTGCTTC haplotype was 0.465, TCCTTGCTTT 0.271, CTCCTGTCAC 0.175 and TCTTCTGTCAC 0.086, respectively. Two haplotypes (TCCTTGCTTT and TCTTCTGTCAC) were significantly associated with RA (Table IV).

To determine whether FOXJ3 SNPs influence the clinical features of RA, the RA group was divided according to clinical characteristics such as ESR ( $\geq 30$  or  $< 30$  mm/h), CRP ( $\geq 0.5$  or

<0.5 mg/dl), bone erosion (positive or not), anti-CCP (positive and negative) and RF (positive or negative). We observed an association between anti-CCP and SNP (rs585320). The SNP rs585320 was significant in the recessive model (OR=0.12, 95% CI=0.01-0.99, P-value=0.015; Table V). However, no association was observed between FOXJ3 polymorphisms and the clinical characteristics (ESR, CRP, bone erosion and RF).

## Discussion

RA is a common autoimmune disease with a complex etiology involving genetic and environmental factors. The association between FOXJ3 polymorphisms and susceptibility to RA was investigated in the present study. The results suggested that certain SNPs in the FOXJ3 gene contribute to RA. The case-control analysis demonstrated that of 11 SNPs analyzed in FOXJ3, seven (rs2455084, rs1393009, rs7539485, rs4660616, rs510157, rs343386 and rs343389) are significantly associated with a susceptibility to RA. Moreover, two haplotypes (TCCTTGCTTT and TCTTCTGTCAC) are significantly associated with RA. To the best of our knowledge, this is the first study to demonstrate that FOXJ3 polymorphisms are associated with RA.

It was also analyzed whether FOXJ3 polymorphisms are related to diagnostic markers reflecting RA disease activity, such as the levels of CRP, ESR, anti-CCP and RF, and the presence of bone erosion. Based on a value for ESR of 30 mm/h, CRP of 0.5 mg/dl, the existence of bone erosion and the presence of anti-CCP (positive or negative) and RF (positive and negative); the RA patient sample was divided into two clinical subgroups and the correlation between clinical data and FOXJ3 polymorphisms was investigated. The results demonstrated that anti-CCP was associated with FOXJ3 SNP.

Forkhead proteins are a large family of functionally diverse transcription factors that have been implicated in a variety of cellular processes. Recently, several studies have demonstrated that this family is involved in the regulation of immune responses. Nik Tavakoli *et al.* (24) reported that FOXP3 is critical in the regulation and development of T<sub>reg</sub> cells. FOXN1 modulates the thymic stroma and regulates thymopoiesis, and FOXOs are also involved in the maintenance of quiescence and tolerance of T and B cells (25). Moreover, it has been suggested that FOXJ1 was associated with autoimmune diseases, such as allergic rhinitis and systemic lupus erythematosus (20,21). However, studies concerning the function of FOXJ3 remain to be conducted. Thus far it has only been suggested that FOXJ3 may be involved in the development of skeletal muscle, and the peripheral and central nervous system (17). Therefore, further studies are required to investigate the function of FOXJ3 (rs585320).

In the present study, seven significant SNPs were located in introns and did not result in an amino acid change. According to the dbSNP database (<http://www.ncbi.nlm.nih.gov/SNP/>) and the HapMap database (<http://www.hapmap.org/>), seven SNPs in exons of the FOXJ3 gene have been identified; however, with the exception of rs343376, these SNPs remain unknown or <0.1 about heterozygosity of them. Since low heterozygosity means little genetic variability, these SNPs were excluded from the present study and only one coding SNP (rs343376)

was analyzed. This SNP (rs343376) was not associated with RA in the study population. It was suggested that significant polymorphisms in introns may be markers rather than direct contributors to genetic functions. The effects of other genetic variations linked to these polymorphisms may exhibit functional significance. However, there remains a possibility that these intronic polymorphisms are involved in its genetic function via the change resulting from alternative splicing (26).

In conclusion, the results of the present study suggest that FOXJ3 polymorphisms are associated with susceptibility to RA in the study population. To the best of our knowledge, this study is the first to analyze the association between FOXJ3 SNPs and RA. Moreover, the polymorphism/association information identified in this study may be useful for further genetic studies of RA.

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