Cyclooxygenase-2 Polymorphisms and Risk of Rheumatoid Arthritis in Koreans

HYE-RYEON YUN, SOO-OK LEE, EUN JU CHOI, HYOUNG DOO SHIN, JAE-BUM JUN, and SANG-CHEOL BAE

ABSTRACT. ***Objective.*** To determine the association of single-nucleotide polymorphisms (SNP) in the cyclooxygenase-2 (*COX-2*) gene with the risk and radiologic severity of rheumatoid arthritis (RA) in Koreans.
Methods. Sequencing of the *COX-2* gene using a DNA analyzer revealed genetic variants in 24 Korean DNA samples. A total of 1201 Korean patients with RA and 973 controls were genotyped using the TaqMan method. HLA-DRB1 was genotyped by polymerase chain reaction and sequence-specific oligonucleotide probe hybridization techniques. Logistic regression models were used to calculate odds ratios (OR) and 95% confidence intervals (95% CI) and the corresponding probability values for each SNP site and haplotype.
Results. Direct sequencing identified 23 SNP of *COX-2* gene, from which 2 common SNP (*–1329A→G* and *6365T→C*) were selected based on the linkage disequilibrium status among SNP and minor allele frequencies. The *–899G→C* SNP was also studied because it is reportedly associated with the risk of RA. The *–1329A→G* SNP was not significantly associated with the risk of RA. However, the risk of RA was significantly lower in the presence of the C allele for *6365T→C* (OR 0.50, 95% CI 0.29–0.85, in a recessive model, and OR 0.80, 95% CI 0.67–0.97, in a codominant model). The C allele for *–899G→C* was also associated with a significantly lower risk of RA (OR 0.67, 95% CI 0.48–0.95, in a codominant model). The radiologic severity of RA was not associated with *COX-2* polymorphisms.
Conclusion. Our study revealed a possible protective influence of the *C* allele for *6365T→C* and for *–899G→C* in RA. (First Release Mar 15 2008; J Rheumatol 2008;35:763–9)

Key Indexing Terms:
CYCLOOXYGENASE-2 RHEUMATOID ARTHRITIS
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Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by chronic inflammation and destruction of the synovial joints, hyperplasia, and overgrowth of synoviocytes. RA affects roughly 0.8% of the population worldwide[1], and is 3 times more common and has an earlier onset in women, frequently beginning in the childbearing years[2]. Its etiology and pathogenesis are not completely understood. Epidemiologic genetic data suggest that the heritability of RA is about 60%[3]. The relative risk for siblings of patients with RA ranges from 2 to 17[4], which suggests that both genetic and environmental factors contribute to RA susceptibility. The most thoroughly examined genes associated with RA are the HLA class II genes, particularly shared-epitope (SE) alleles. The HLA-DRB1 SE consistently shows the strongest association with the risk of RA[5,6]. However, HLA has been estimated to account for only one-third of the genetic component in RA[7], indicating that genes outside the HLA region also contribute to the disease.

Cyclooxygenase (COX) is the key enzyme in the conversion of arachidonic acid to prostaglandin (PG) H_2, the precursor of a diverse family of bioactive lipid mediators including prostaglandins, thromboxane, and prostacyclin. It exists in 2 isoforms, COX-1 and COX-2: the former is constitutively expressed in most tissues and acts as a housekeeping enzyme regulating vascular homeostasis, protecting gastric mucosa, and maintaining renal integrity[8,9]; whereas the latter is less widely distributed but inducible in response to growth factors, cytokines, and other proinflammatory molecules[10–12].

The expression of COX-2 (but not COX-1) was found to be higher in synovial tissue from patients with RA than in osteoarthritis or normal synovial samples[13–15]. Many studies

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have indicated that COX-2 is actively involved in the inflammatory process and the resulting pain[16]. There is also evidence supporting a role of COX-2 in joint destruction in RA. Prostaglandins are known to be potent agonists that can stimulate bone absorption by activated osteoclasts[17], and a study has demonstrated that prostaglandin E_2 is produced by rheumatoid synovia and can stimulate bone resorption[18]. Several reports also indicate that COX-2 inhibition by nonsteroidal antiinflammatory drugs (including selective COX-2 inhibitors) reduces joint inflammation and pain in patients with RA[19,20].

In a study involving 258 Korean patients with RA, Lee, *et al*[21] showed that the *rs20417 (–765G→C)* of the *COX-2* gene was associated with a lower risk of RA in subjects without the SE. We reinvestigated whether *rs20417 (–899G→C)* is associated with risk of RA using a large population-based case–control design. The difference of nomenclature for *rs20417* was due to the different start site (+1), since *–765G→C* was calculated from transcription start site, whereas *–899G→C* was calculated from translational start site. Thus *COX-2 –899G→C* in our study is identical with *–765G→C*.

In addition, we scrutinized sequence variations in the *COX-2* gene in a Korean population (n = 24), and then also examined whether common single-nucleotide polymorphisms (SNP) of the *COX-2* gene are associated with the risk and radiologic severity of RA in a Korean population.

MATERIALS AND METHODS

Study participants. A total of 1201 Korean patients with RA and 973 controls were recruited from the Hospital for Rheumatic Diseases, Hanyang University, Seoul. The study was approved by the Institutional Review Board of Hanyang University Medical Center, and all subjects provided written informed consent.

The patients were aged 52.5 ± 12.3 years, and were 40.9 ± 12.5 years old at the onset of RA, and the controls were aged 37.3 ± 12.6 years. All patients satisfied the American College of Rheumatology (ACR) 1987 revised criteria for a diagnosis of RA[22]. Genomic DNA was extracted from blood leukocytes from all subjects using a standard protocol. Clinical data including sex, current age, age at disease onset, age at the time of diagnosis, time from disease onset to initiation of therapy, and disease duration were obtained from medical records and from interviews performed at the time of enrollment.

Clinical variables. Functional class was determined according to the ACR criteria for the classification of global functional status in RA[23]. Subjects completed the Korean-language version of the Health Assessment Questionnaire[24]. The staging system proposed by Steinbrocker, *et al* was used to assess the radiographic severity of RA[25]. Each subject was initially classified as having disease at stage I, II, III, or IV, and all subjects were then dichotomized into 2 groups, stage I and stages II to IV.

Sequencing analysis of COX-2 gene. Sequencing of the exons of the *COX-2* gene and their boundaries using a DNA analyzer (ABI Prism 3700; Applied Biosystems, Foster City, CA, USA), including the promoter region, revealed genetic variants in 24 Korean DNA samples. The 15 primer sets used for the amplification and sequencing analysis are listed in Table 1.

COX-2 genotyping with fluorescence polarization detection. Polymorphic sites of the *COX-2* gene were genotyped by amplifying primers and probes designed for TaqMan (Livak 1999)[26] (Table 2). Primer Express (Applied Biosystems) was used to design the polymerase chain reaction (PCR) primers and the TaqMan MGB probes. One allelic probe was labeled with FAM™ dye and the other with the fluorescent VIC® dye. PCR were run in TaqMan Universal Master mix without UNG (Applied Biosystems) with PCR primers at 900 nM and TaqMan MGB probe at 200 nM. Reactions were performed in a 384-well format in a total reaction volume of 5 µl using 20 ng of genomic DNA. The plates then were placed in a thermal cycler (PE 9700, Applied Biosystems) and heated at 50°C for 2 min, then 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min with a final soak at 25°C. The TaqMan assay plates were transferred from the thermal cycler to a sequence detection system (Prism 7900HT, Applied Biosystems) where the fluorescence intensity in each well of the plate was read. Fluorescence data files from each plate were analyzed by automated allele-calling software (SDS 2.1).

Table 1. Primer sequences for *COX-2* sequence variants screening.

Name		Sequence (5' → 3')
COX-2_1F	Forward	CCG TGT CTC ATG AAG AAT CA
	Reverse	GGC GAT GGC CAG AAT TT
COX-2_2F	Forward	GGA CAT TTA GCG TCC CTG C
	Reverse	GGT TTC CGC CAG ATG TCT T
COX-2_3F	Forward	GCA AAG ACT GCG AAG AAG AA
	Reverse	AGC TCT TTC CCA AGT CAC G
COX-2_4F	Forward	TCC ATT CTA AGG CAG GTT AAA AA
	Reverse	TTG GCG ATT AAG ATG GAA GG
COX-2_5F	Forward	CCT GAA AAA TCA ATA TTG CCA
	Reverse	CAA GAA AGG AGA TGG TGA CTG
COX-2_6F	Forward	GCA AAT GAG CGT CTT GGT AT
	Reverse	GCG GCA TAA TCA TGG TAC A
COX-2_7F	Forward	TCA GTT TGT AGC TTT GGT GGA
	Reverse	GCA ACT GGA ATG CAA TTT TTA
COX-2_8F	Forward	TGA CAA GGA AGA AAA CAG AAA TGA
	Reverse	AAA TTC AAT GGG ACA CCA GC
COX-2_9F	Forward	CTG GTG TCC CAT TGA ATT TT
	Reverse	CCA TCT CGA AAA GAA AAC CA
COX-2_10F	Forward	CTG GCC CCT AAA CTT CTT AAA
	Reverse	CGC AAC AGG AGT ACT GAC TTC
COX-2_11F	Forward	ATC AAT GCA AGT TCT TCC CG
	Reverse	TCC AAG ACA GCT TCT TTT TGG T
COX-2_12F	Forward	TCA CCT GTA AAA GCT TGT TTG ATT
	Reverse	AGG AAC AGC ATG CAG GTA GC
COX-2_13F	Forward	TTG CAA AAG TAG CAA TGA CCT C
	Reverse	TCA GTG ACA ATG AGA TGT GGA AAA
COX-2_14F	Forward	TTC TTT TCC ACA TCT CAT TGT CA
	Reverse	ACA TTC GCA TAC ACA ACC CA
COX-2_15F	Forward	TTC AGT GCC TCA GAC AAA TG
	Reverse	AAG ATT TTG AAA GTG GTG CTG

HLA-DRB1 genotyping. HLA-DRB1 was genotyped by PCR and sequence-specific oligonucleotide probe hybridization techniques using the reference protocol of the Twelfth International Histocompatibility Workshop[27], followed by direct DNA sequencing[28]. We defined the SE as having the following alleles: HLA-DRB1*0101, *0102, *0401, *0404, *0405, *0408, *0410, *1001, *1402, and *1406.

Statistical analyses. Independent t tests were used to assess differences in the general characteristics between patients with RA and healthy controls as well as differences between patients with mild and severe RA. Logistic regression models were used to calculate odds ratios (OR) and 95% confidence intervals (95% CI) and the corresponding probability values for each SNP site and haplotype. Results of codominant, dominant, and recessive

Table 2. The TaqMan probe for *COX-2* SNP genotyping.

Loci	RS	ABI Assay on Demand (sequence)	
–1329A > G	rs689466		C__2517145_20
–899G > C	rs20417	Forward	TGC TTA GGA CCA GTA TTA TGA GGA GAA
		Reverse	CCC CCT CCT TGT TTC TTG GAA
		VIC	CCT TTC CCC CCT CTC T
		FAM	CTT TCC CGC CTC TCT
+6365T > C	rs5275		C__7550203_10

models are given. Chi-square tests were used to determine whether individual polymorphisms were in Hardy-Weinberg equilibrium. We examined Lewontin's D' (|D'|) and the linkage disequilibrium (LD) coefficient (r^2) between all pairs of biallelic loci[29]. Haplotype structures were visualized by Haploview software[30]. The haplotypes of each individual were inferred using the PHASE algorithm developed by Stephens, *et al*[31], which uses a Bayesian approach incorporating *a priori* expectations of haplotypic structure based on population genetics and coalescent theory. To achieve the optimal correction for multiple testing of SNP in LD with each other, the effective number of independent marker loci (2.7071) in COX-2 was calculated using SNPSpD software (http://genepi.qimr.edu.au/general/daleN/SNPSpD/), on the basis of the spectral decomposition of matrices of pairwise LD between SNP.

RESULTS

Characteristics of study subjects. Clinical characteristics of the study subjects are summarized in Table 3. Controls were younger, but the female-to-male ratio did not differ significantly between cases and controls. Patients with RA were distributed among the 4 functional classes based on ACR criteria, with 80.4% and 19.6% having mild RA (anatomical stage I) and severe RA (stage II, III, or IV), respectively. Those with severe RA were younger than those with mild RA, and those with mild RA had a later onset and shorter duration of disease. The SE status is also listed in Table 3. Carriers with one or 2 copies of the SE were at an increased risk of developing RA compared to those without the SE (OR 3.72, 95% CI 2.98–4.64, and OR 9.87, 95% CI 6.35–15.32, respectively). However, the SE was not associated with RA severity.

COX-2 gene polymorphisms and haplotypes. Direct sequencing identified 23 SNP. The *COX-2* gene is located on

Table 3. Characteristics of Korean cases with rheumatoid arthritis (RA) and controls.

Characteristic	Cases, n = 1201	Controls, n = 973	p	Severe RA*, n = 963	Mild RA**, n = 235	p
Age, yrs, mean ± SEM (range)	52.5 ± 12.3 (20–82)	37.3 ± 12.6 (17–79)	< 0.001	53.1 ± 12.3 (21–82)	50.3 ± 11.8 (20–80)	0.002
Sex, female/male (ratio)	1064/137 (8:1)	833/140 (6:1)	NS	857/106 (8:1)	203/32 (6:1)	NS
Age at onset, yrs, mean ± SEM (range)	40.9 ± 12.5 (6–78)	—		39.8 ± 12.5 (6–73)	45.0 ± 12.0 (18–78)	< 0.001
Disease duration, yrs, mean ± SEM (range)	11.7 ± 8.5 (1–50)	—		13.2 ± 8.4 (1–50)	5.3 ± 5.2 (1–30)	< 0.001
Treatment duration, yrs, mean ± SEM (range)	2.31 ± 5.10 (0–37)	—		2.66 ± 5.51 (0–37)	0.87 ± 2.38 (0–25.9)	< 0.001
Functional class, n (%)						
I	269 (23.1)	—		186 (19.8)	82 (36.1)	
II	301 (25.8)	—		239 (25.5)	62 (27.3)	
III	279 (24.0)	—		231 (24.6)	48 (21.2)	
IV	316 (27.1)	—		282 (30.1)	35 (15.4)	
Anatomical class, stage						
I	235 (19.6)	—		—	—	
II	415 (34.6)	—		—	—	
III	364 (30.4)	—		—	—	
IV	184 (15.4)	—		—	—	

SE	Cases, n (%)	Controls, n (%)	OR (95% CI)	p	Severe RA*, n (%)	Mild RA**, n (%)	OR (95% CI)	p
–/–	384 (32.0)	626 (64.3)	1		303 (31.4)	80 (34.0)	1	
+/–	639 (53.2)	314 (32.3)	3.72 (2.98–4.64)	< 0.0001	512 (53.1)	125 (53.2)	1.26 (0.88–1.79)	0.20
+/+	178 (14.8)	33 (3.4)	9.87 (6.35–15.32)	< 0.0001	149 (15.5)	30 (12.8)	1.54 (0.93–2.54)	0.09

* Stages II, III, and IV according to radiologic criteria of Steinbrocker, *et al*[25]. ** Stage I according to radiologic criteria of Steinbrocker, *et al*[25]. The shared epitope (SE) was defined as having the following alleles: HLA-DRB1*0101, *0102, *0401, *0404, *0405, *0408, *0410, *1001, *1402, and *1406. SEM: standard error of the mean; NS: not significant.

chromosome 1q25.2–q25.3, is less than 8 kb long, and includes 10 exons. The location of the SNP relative to the genomic structure of the *COX-2* gene is indicated in Figure 1A. We selected 2 common SNP [*–1329A→G* (*rs689466*) and *6365T→C* (*rs5275*)] for study. The minor allele frequencies of these 2 SNP were higher than 5%. Another SNP used in this study was *–899G→C (rs20417),* which is not common in Koreans but was significantly associated with the risk of RA in a previous study[21].

Three of the 6 haplotypes in *COX-2* showed frequencies higher than 5% (Figure 1B). All 3 SNP were in strong LD with one another (D' = 0.95–1.0; Figure 1C).

Genotypic and allelic frequencies for COX-2 polymorphisms. We genotyped 3 *COX-2* SNP; their allele and genotype frequencies are summarized in Table 4. In our study population, the minor allele frequencies of the *–1329A→G* and *6365T→C* SNP were high (0.467 and 0.201, respectively), and that of *–899G→C* was lower (0.048). Genotype distributions of all loci were in Hardy–Weinberg equilibrium ($p > 0.05$).

COX-2 polymorphisms and risk of RA (Table 5). Samples obtained from 1201 patients with RA and 973 controls were genotyped individually for 3 *COX-2* SNP (*–1329A→G*, *–899G→C*, and *6365T→C*). The *–1329A→G* SNP was not significantly associated with the risk of RA among the 3 groups in codominant, dominant, and recessive models. But the risk of RA was significantly lower for the *C* allele for *6365T→C* (OR 0.50, 95% CI 0.29–0.85, in the recessive model, and OR 0.80, 95% CI 0.67–0.97, in the codominant model). The *C* allele for *–899G→C* also showed a signifi-

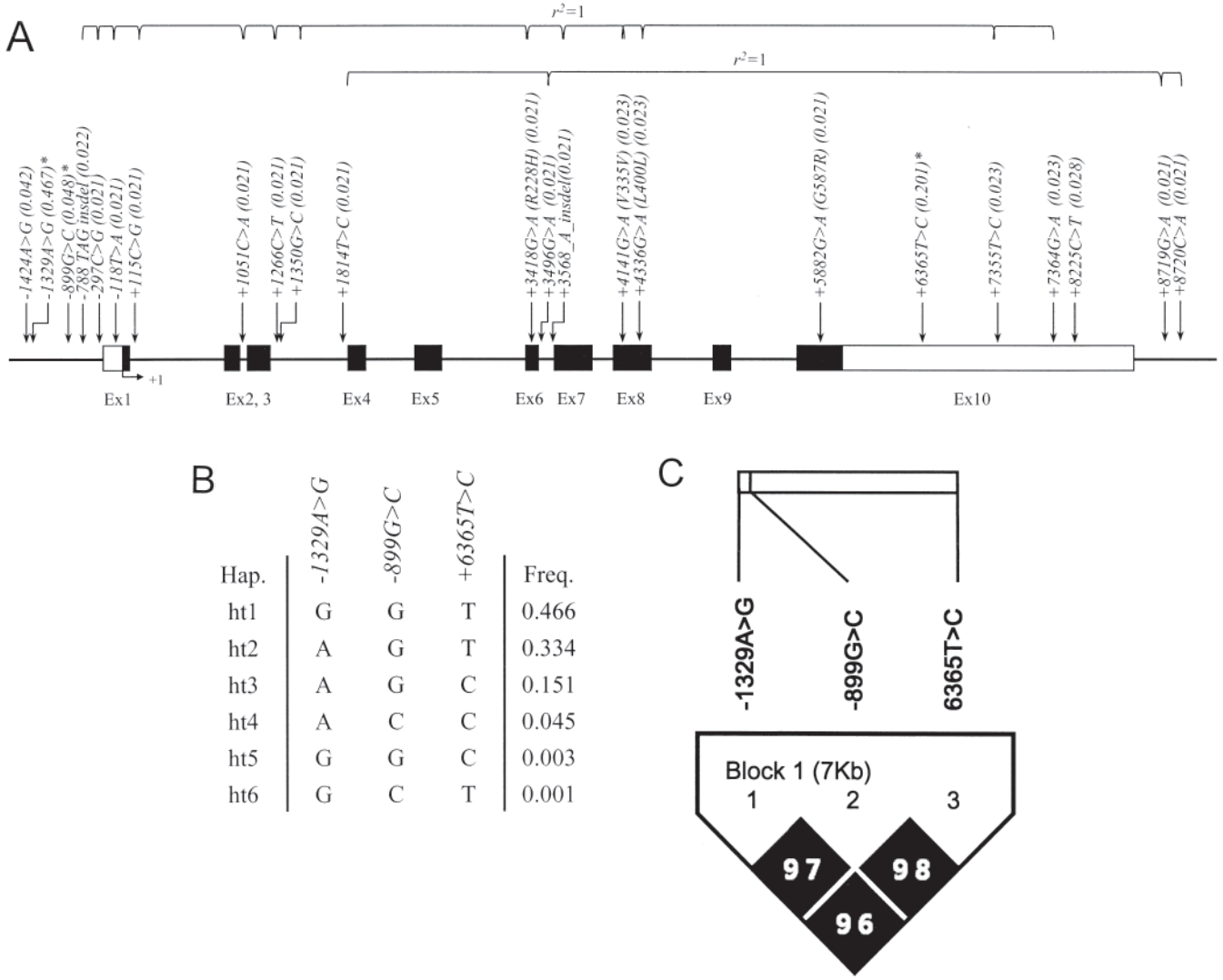

Figure 1. Gene maps and haplotypes of cyclooxygenase-2 (*COX-2*). Coding exons are marked by black blocks, and 5'- and 3'-untranslated regions by white blocks. The first base of the translational start site is denoted as nucleotide +1. *Polymorphisms genotyped in the larger group of Korean subjects (n = 2174). The frequencies of polymorphisms without larger-scale genotyping are based on sequencing data (n = 24). A linkage disequilibrium (LD) coefficient (r^2) of 1 indicates absolute LD among polymorphisms. A. Polymorphisms identified in *COX-2* on chromosome 1q25.2-q25.3 (GenBank no. NT_004487.18). B. Haplotypes of *COX-2*. C. LD coefficients (|D'|) among *COX-2* polymorphisms.

Table 4. Frequencies of *COX-2* polymorphisms, deviation from Hardy-Weinberg equilibrium (HWE) in a Korean population.

Gene	Loci	Position	AA Change	rs	Genotype			Frequency	Heterozygosity	p*
COX-2	*–1329A > G*	Promoter	—	rs689466	A	AG	G	0.467	0.498	0.481
					617	1049	474			
	–899G > C	Promoter	—	rs20417	G	CG	C	0.048	0.091	0.669
					1943	197	4			
	6365T > C	Exon 10	—	rs5275	T	CT	C	0.201	0.322	0.974
					1374	693	87			

* p for deviation from Hardy-Weinberg equilibrium in this population.

Table 5. Logistic analysis of RA susceptibility with cyclooxygenase-2 (*COX-2*) polymorphisms and haplotypes controlling for age, sex, and the shared epitope (SE) in a Korean population. Logistic regression models were used to calculate OR and 95% CI and the corresponding probability values for each SNP site and haplotype. Results of codominant, dominant, and recessive models are given. Age (continuous value), sex (female = 0, male = 1), and the SE (negative = 0, positive = 1) were adjusted by inclusion in logistic analysis as covariates.

Locus	rs	Position	Genotype	Frequency (%)		Codominant Model			Dominant Model			Recessive Model		
				Cases	Controls	OR (95% CI)	p	p^{corr*}	OR (95% CI)	p	p^{corr*}	OR (95% CI)	p	p^{corr*}
–1329A>G	rs689466	Promoter	AA	340 (28.7)	277 (29.0)									
			AG	592 (50.0)	457 (47.8)	0.97 (0.83–1.12)	0.66	NS	1.02 (0.81–1.29)	0.87	NS	0.89 (0.69–1.14)	0.35	0.95
			GG	252 (21.3)	222 (23.2)									
–899G>C	rs20417	Promoter	GG	1090 (91.9)	853 (89.0)									
			CG	96 (8.1)	101 (10.5)	0.67 (0.48–0.95)	0.02	0.05	0.71 (0.50–1.01)	0.05	NS	—	—	—
			CC	0 (0.0)	4 (0.4)									
6365T>C	rs5275	Exon 10	TT	772 (64.9)	602 (62.5)									
			CT	382 (32.1)	311 (32.3)	0.80 (0.67–0.97)	0.02	0.05	0.83 (0.67–1.03)	0.10	NS	0.50 (0.29–0.85)	0.010	0.03
			CC	36 (3.0)	51 (5.3)									
ht1	ht1 (G-G-T)	—	–/–	336 (28.6)	280 (29.4)									
			–/ht1	592 (50.3)	453 (47.5)	0.98 (0.85–1.14)	0.78	NS	1.04 (0.82–1.31)	0.76	NS	0.90 (0.70–1.16)	0.42	NS
			ht1/ht1	249 (21.2)	220 (23.1)									
ht2	ht2 (A-G-T)	—	–/–	511 (43.4)	447 (46.9)									
			–/ht2	522 (44.4)	405 (42.5)	1.19 (1.02–1.39)	0.03	NS	1.20 (0.97–1.49)	0.09	NS	1.39 (0.99–1.94)	0.05	NS
			ht2/ht2	144 (12.2)	101 (10.6)									
ht3	ht3 (A-G-C)	—	–/–	847 (72.0)	691 (72.5)									
			–/ht3	306 (26.0)	232 (24.3)	0.90 (0.74–1.11)	0.34	NS	0.96 (0.75–1.21)	0.70	NS	0.50 (0.26–0.98)	0.04	NS
			ht3/ht3	24 (2.0)	30 (3.2)									

* To achieve optimal correction for multiple testing of single-nucleotide polymorphisms (SNP) in linkage disequilibrium (LD) with each other, the effective number of independent marker loci (2.7071) in *COX-2* was calculated using SNPSpD software, on the basis of the spectral decomposition (SpD) of matrices of pairwise LD between SNP. NS: nonsignificant.

cant lower risk of RA (OR 0.67, 95% CI 0.48–0.95, in the codominant model). Haplotypic analysis revealed no significant association between *COX-2* haplotypes and the risk of RA.

COX-2 polymorphisms and risk of RA in subjects with or without the SE (Table 6). The *C* allele for *6365T→C* did not affect the risk of RA in subjects with the SE. However, the risk of RA was significantly lower for the homologous *C* allele in those without the SE (OR 0.32, 95% CI 0.15–0.71).

COX-2 polymorphisms and radiologic severity of RA. The radiologic severity of RA was not significantly associated with *COX-2* polymorphisms and haplotypes.

DISCUSSION

Our study showed that the *C* allele for *6365T→C* and the *C* allele for *–899G→C* in the *COX-2* gene were associated with a protective effect against RA in a Korean population. The *C* allele for *6365T→C* in the *COX-2* gene also produced a decreased risk of RA in subjects without the SE, although the SE-carrying individuals did not show a genotypic association with risk of RA.

The *6365T→C* SNP in exon 10 of the *COX-2* gene is located in the 3'-untranslated region (UTR) of the gene at 427 nucleotides downstream from the stop codon. Little is known about the functionality of the *COX-2 6365T→C* polymorphism, but the 3'-UTR of the murine gene for *COX-2* contains several regulatory elements that influence mRNA stability and translational efficiency[32]. Therefore, polymorphisms in the corresponding region of the human gene for *COX-2* could similarly influence COX-2 expression. In view of the potential role of *COX-2* polymorphisms in *COX-2* expression and susceptibility to RA, future studies should

Table 6. Logistic analysis of RA susceptibility with *COX-2* polymorphisms and haplotypes controlling for age and sex in the shared epitope (SE) population. Logistic regression models were used to calculate OR and 95% CI and corresponding probability values for each SNP site and haplotype. Results of codominant, dominant, and recessive models are given. Age (continuous value), sex (female = 0, male = 1), and the SE (negative = 0, positive = 1) were adjusted by inclusion in logistic analysis as covariates.

SE	Locus	Frequency		Codominant Model			Dominant Model			Recessive Model		
				OR (95% CI)	p	p^{corr*}	OR (95% CI)	p	p^{corr*}	OR (95% CI)	p	p^{corr*}
Positive		Cases, n = 811	Controls, n = 341									
	–1329A>G	0.460	0.454	0.96 (0.78–1.19)	0.72	NS	0.98 (0.70–1.35)	0.88	NS	0.92 (0.64–1.32)	0.65	NS
	–899G>C	0.039	0.060	0.69 (0.43–1.12)	0.13	NS	0.70 (0.43–1.15)	0.16	NS	—	—	—
	6365T>C	0.195	0.227	0.81 (0.63–1.06)	0.12	NS	0.79 (0.58–1.07)	0.12	NS	0.78 (0.35–1.73)	0.54	NS
	ht1	0.460	0.454	0.96 (0.78–1.19)	0.73	NS	0.97 (0.70–1.35)	0.86	NS	0.93 (0.64–1.34)	0.70	NS
	ht2	0.344	0.319	1.22 (0.97–1.53)	0.09	NS	1.21 (0.90–1.63)	0.21	NS	1.53 (0.92–2.52)	0.10	NS
	ht3	0.153	0.165	0.86 (0.65–1.15)	0.32	NS	0.85 (0.61–1.18)	0.32	NS	0.80 (0.30–2.17)	0.67	NS
Negative		Cases, n = 379	Controls, n = 623									
	–1329A>G	0.468	0.481	0.97 (0.79–1.19)	0.76	NS	1.06 (0.76–1.48)	0.73	NS	0.86 (0.60–1.22)	0.38	NS
	–899G>C	0.044	0.055	0.67 (0.42–1.09)	0.11	NS	0.73 (0.44–1.21)	0.22	NS	—	—	—
	6365T>C	0.181	0.207	0.80 (0.62–1.04)	0.09	NS	0.89 (0.65–1.21)	0.45	NS	0.32 (0.15–0.71)	0.005	0.01
	ht1	0.469	0.476	0.99 (0.81–1.22)	0.93	NS	1.10 (0.79–1.53)	0.57	NS	0.87 (0.61–1.24)	0.44	NS
	ht2	0.345	0.318	1.16 (0.94–1.45)	0.17	NS	1.20 (0.88–1.61)	0.25	NS	1.29 (0.82–2.03)	0.28	NS
	ht3	0.144	0.147	0.95 (0.71–1.27)	0.72	NS	1.08 (0.77–1.52)	0.65	NS	0.32 (0.12–0.86)	0.02	NS

* To achieve optimal correction for multiple testing of SNP in LD with each other, the effective number of independent marker loci (2.7071) in *COX-2* was calculated using SNPSpD software on the basis of the SpD of matrices of pairwise LD between SNP. NS: nonsignificant.

investigate their role for determining the pharmacogenetics of *COX-2* inhibitors. Several studies have reported an association between the *6365T→C* SNP and various diseases, but the results have varied regarding which alleles are "susceptive" and "protective." The *C* allele of the *6365T→C* SNP has been associated with an increased risk for non-small-cell lung cancer in Caucasians[33], and for colorectal cancer[34] and breast cancer[35]. In contrast, other studies found a protective effect of the same genetic polymorphism against lung cancer in Asians[36,37] and for prostate cancer[38] and Parkinson's disease[39]. The reasons for this discrepancy are unclear, but may reflect the different etiologies of each disease. Further, the effects of *COX-2* gene polymorphisms on cancer risk are likely to be influenced by gene–environment interactions, such as smoking, which is known to be the most important risk factor for lung cancer that induces the expression of *COX-2*[40]. Unfortunately, in our study, no data were available on environmental factors such as nonsteroidal antiinflammatory drug use and diet that could potentially interact with the *COX-2* genotype. Another explanation for the discrepant associations between the *COX-2 6365T→C* polymorphism and different diseases could be ethnicity differences. The *COX-2 6365T→C* polymorphism could be in LD with other causal genetic variants, and this LD would likely differ across different ethnic populations.

Lee, *et al*[21] reported that the *–899G→C* SNP did not alter the risk of RA, but in subjects without the SE, the *C* allele was associated with a lower risk of RA (OR 0.36, 95% CI 0.14–0.36). That study involved 658 subjects: 258 cases and 400 controls. Our investigation involving 1201 RA patients and 973 controls revealed a weak association between the *C* allele of the *–899G→C* SNP and a lower risk of RA. However, when subjects were divided by SE-carrying status, this SNP was not associated with susceptibility of RA in each group. In Koreans, the minor allele frequency of the *–899G→C* SNP was very low, so our results need to be confirmed in studies in other ethnic groups.

Our study showed a possible protective influence of the *C* allele for *6365T→C* and the *C* allele for *–899G→C* in RA, and is the first to investigate the possible influence of the *COX-2 6365G→C* SNP on the susceptibility of RA (the association of this SNP with other autoimmune diseases or chronic inflammatory diseases has also not been investigated previously). However, the polymorphisms analyzed here reflect only part of the variability of the *COX-2* gene, and our results should be interpreted with caution until they are confirmed by others.

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