Cyclooxygenase-2 Genotype and Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To determine the association between cyclooxygenase-2 (COX-2) genotypes and risk and severity of rheumatoid arthritis (RA) in a Korean population.

Methods. A total of 258 Korean patients with RA and 400 control subjects were recruited from Hanyang University Hospital. Subjects were genotyped for the –765G/C polymorphism of the COX-2 gene by RFLP-PCR analysis. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to estimate risk. Severity of RA was assessed by anatomical stage according to Steinbrocker, et al.

Results. No association was observed between COX-2 genotype and risk or severity of RA. However, among those without the shared epitope (SE), carriers of the low activity C allele had a lower risk of RA and less severe form of RA than subjects with the G/G genotype. The OR (95% CI) was 0.36 (0.14–0.95) for risk of RA and 0.04 (0.01–0.41) for severity.

Conclusion. These results suggest that COX-2 genotyping might be useful in predicting the risk and severity of RA in individuals without the SE. (J Rheumatol 2006;33:1231–4)

Key Indexing Terms:
POLYMORPHISM CYCLOOXYGENASE-2 SHARED EPITOPE
KOREA RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease that results from both genetic and environmental factors. Genetic susceptibility to RA has been studied extensively, with genetic factors accounting for an estimated 60% of the disease risk[1]. Among the genetic markers studied to date, the HLA-DRB1 shared epitope (SE) is one of the most important determinants of risk and severity of RA[2,3]. Certain combinations of the DRB1 alleles have been associated with more severe and milder forms of RA[2–5].

Cyclooxygenase (COX) is the key enzyme in prostaglandin (PG) biosynthesis and is a target for inhibition by nonsteroidal antiinflammatory drugs (NSAID). COX-1 and COX-2 are the major isoforms. In general, the COX-1 isoform is constitutively expressed in most tissues and acts as a housekeeping enzyme to regulate vascular homeostasis, protect the gastric mucosa, and maintain renal integrity. COX-2, however, is less widely distributed, but is highly inducible by growth factors, cytokines, and tumor promoters, suggesting a possible role for this enzyme in the development of inflammatory diseases[6,7]. The expression of COX-2, also called PG-endoperoxide synthetase 2, was found to be higher in synovial tissue from patients with RA than in normal synovial samples[8–11]. Moreover, inhibition of COX-2 by NSAID, including selective COX-2 inhibitors, reduces joint inflammation and pain in patients with RA[12,13].

A single nucleotide polymorphism (–765G/C) in the promoter region of the COX-2 gene (rs20417) was identified and shown to be in a putative binding site for the Sp1 transcription factor[14,15]. Subjects carrying the C allele had lower promoter activity and lower plasma levels of C-reactive protein (CRP), a marker of low-grade inflammation, compared to individuals who were homozygous for the G allele.

Although COX-2 is a common target for drugs used to treat RA, no studies have reported whether genetic differences in COX-2 are associated with risk or severity of the disease. We examined whether the –765G/C polymorphism of COX-2 is associated with risk and severity of RA using a case-control study design. We also investigated whether COX-2 genotypes interact with the SE.

MATERIALS AND METHODS

Study participants. Two hundred fifty-eight patients meeting the 1987 American College of Rheumatology (ACR) classification criteria for RA[16] were recruited from the Hospital for Rheumatic Diseases, Hanyang University, Seoul, Korea. Four hundred Korean disease-free controls that were ethnically identical to the patients were also enrolled from the same hospital (nurses, paramedics, and laboratory workers). Written informed consent was obtained from each subject, and the protocol was approved by the

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Institutional Review Board of Hanyang University Hospital. 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 interviews at the time of enrollment.

Clinical variables. The functional class in patients with RA was determined based on ACR criteria for classification of global functional status in RA[16]. Subjects completed the Korean-language version of the Health Assessment Questionnaire[17]. We used the staging system proposed by Steinbrocker, *et al*[18] as a radiographic marker of RA severity, and classified patients as having mild RA (stage I) or severe RA (stages II, III, and IV). Titers for serum rheumatoid factor (RF) were measured by nephelometry, with a positive RF titer defined as the level occurring in < 5% of normal people (i.e., < 20IU/ml)[19].

Genotyping. The COX-2 (–765G/C) polymorphism was detected as described by Papafili, *et al*[14] using restriction–fragment length polymorphism polymerase chain reaction (RFLP-PCR), without knowledge of the case–control status. About 10 ng of DNA was amplified by thermal cycling using the HotStar™ (Qiagen, Mississauga, ON, Canada) DNA polymerase kit with PCR buffer containing 1.5 mM $MgCl_2$, each dNTP at 0.2 mM, 0.5 U of Taq, and 8 pmol of each primer set. The following primers were synthesized by ACGT (Toronto, ON, Canada): 5′CCG CTT CCT TTG TCC ATC AG′3 (forward) and 5′GGC TGT ATA TCT GCT CTA TAT GC′3 (reverse). PCR conditions comprised an initial denaturation at 95°C for 15 min followed by 35 cycles of 94°C for 30 s, 68°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 10 min. PCR products were digested with 2 U of the *Aci*I restriction enzyme, resolved by 2% agarose gel electrophoresis, and stained with ethidium bromide. Bands were visualized using the FluorChem™ UV imaging system. The primers amplify a 306-bp fragment that is cut into 188 bp and 118 bp fragments only with the G allele.

HLA-DRB1 typing. The HLA-DRB1 typing and further subtyping of all alleles were performed by PCR, sequence-specific oligonucleotide probe hybridization[20], and direct DNA sequencing analysis[21]. We defined the SE as having HLA-DRB1*0101, *0401, *0404, *0405, *0410, *1001, and *1406 alleles.

Statistical analyses. All data were analyzed using standard statistical software (SAS v8.2, SAS Institute, Cary, NC, USA). Independent t tests were used to assess differences in the general characteristics between patients with RA and normal controls as well as differences between patients with mild and severe RA. Odds ratios (OR) and 95% confidence intervals (CI) were used to estimate the risk of RA associated with the C allele at position –765 of the COX-2 gene with reference to the G/G genotype after adjusting for age, sex, and disease duration. A probability of less than 0.05 was used as the criterion of significance.

RESULTS

Table 1 lists the general characteristics of RA cases and normal controls. Controls were younger and consisted of fewer females compared to cases. Patients with RA were distributed among the 4 functional classes based on ACR criteria, with 9.3% and 90.7% having mild RA (anatomical stage I) and severe RA (stage II, III, and IV), respectively. Those with mild RA had shorter duration of disease and comprised fewer females compared to patients with severe RA.

Carriers with one or 2 copies of SE demonstrated an increased risk for developing RA (OR 3.48, 95% CI 2.47–4.88; OR 12.0, 95% CI 5.07-28.40, respectively) compared to those without SE. However, SE was not associated with RA severity (Table 2).

The genotype distributions among cases and controls are summarized in Table 3. A total of 9.7% of RA cases and 13.3% of controls were carriers of the low activity C allele at position –765 in the COX-2 gene. Only 6 subjects (one case and 5 controls) were homozygous for the C allele. The COX-2 genotypes were not significantly associated with either risk or severity of RA (Table 3). The C allele was not associated with altered risk of RA in subjects with the SE (OR 1.01, 95% CI 0.49–2.09; Table 4). However, in those without the SE, the risk of RA associated with carriers of the C allele was significantly lower (OR 0.36, 95% CI 0.14–0.36; Table 4). Severity was also not associated with the C allele in subjects with the SE (OR 1.84, 95% CI 0.23–14.9; Table 4), but was associated with less severe RA in those without the SE (OR 0.06, 95% CI 0.01–0.41; Table 4).

DISCUSSION

We investigated the effects of COX-2 genotypes on risk and severity of RA. To our knowledge, ours is the first study to examine the association between COX-2 genotypes and RA. The results demonstrate that the low activity C allele is associated with a lower risk and milder RA only in subjects without the SE. It is possible that the presence of the SE, which is a strong genetic risk factor[2,3], may have masked an effect of COX-2 in those individuals. Papafili, *et al*[14] reported that a variant in the COX-2 promoter, the –765C allele, has significantly lower (30%) promoter activity compared with the –765G allele. They found that among patients undergoing elective coronary artery bypass graft surgery, carriers of the –765C allele had significantly lower plasma levels of CRP compared to patients who were homozygous for the G allele. Cipollone, *et al*[15] also reported that the –765C polymorphism of the COX-2 gene was associated with a decreased risk of myocardial infarction and stroke. Our findings that the –765C allele is significantly associated with risk and severity in RA patients without the SE are compatible with these reports[14,15] in terms of reducing inflammation.

The COX-2 inhibitors, which selectively inhibit production of prostaglandin E_2 and prostacyclin without inhibiting platelet production of thromboxane A_2, might increase the risk of prothrombotic activity[22]. In the Vioxx Gastrointestinal Outcomes Research (VIGOR) trial[23], there was a 4-fold increase in the rate of myocardial infarction in the rofecoxib group. However, a growing concern is that accelerated atherosclerosis is driven by inflammatory mechanisms similar to those responsible for RA. Therefore, antiinflammatory properties of the COX-2 inhibitor might provoke anti-atherogenic effects[24]. Chenevard, *et al*[25] reported that COX-2 inhibitor improves endothelium-dependent vasodilation and reduces low-grade chronic inflammation and oxidative stress in coronary artery disease. Therefore, the COX-2 inhibitor has both advantages and disadvantages. Ulrich, *et al*[26] reported that regular aspirin and NSAID use was associated with reduced colorectal adenoma risk. However, the –765 CC genotype was not associated with a reduced adenoma risk among regular users of aspirin or other NSAID. It was suggested that NSAID

Table 1. Characteristics of Korean cases with RA and controls.

	Controls, n = 400	Cases, n = 258	p	Mild RA*, n = 24	Severe RA**, n = 234	p
Age, yrs, mean ± SEM (range)	41.8 ± 14.1 (19–76)	49.3 ± 11.2 (24–72)	0.001	48.7 ± 8.4 (38–71)	49.3 ± 11.4 (24–72)	NS
Sex, female/male (ratio)	351/49 (7:1)	249/9 (28:1)	0.001	21/3 (7:1)	228/6 (38:1)	0.011*
Age at onset, yrs, mean ± SEM (range)	—	41.7 ± 11.1 (13–68)		42.0 ± 9.1 (29–67)	41.5 ± 11.0 (13–68)	NS
Disease duration, yrs, mean ± SEM (range)	—	12.9 ± 7.0 (1–40)		9.1 ± 5.0 (2–20)	13.2 ± 7.0 (2–40)	0.0005*
Treatment duration, yrs, mean ± SEM (range)	—	7.6 ± 3.6 (1–18)		6.8 ± 4.0 (2–14)	7.8 ± 3.6 (2–18)	NS
RF-positive, %	—	80.1		75.9	80.7	NS
Functional class [n = 247] no.)%)						
I	—	55 (22)		7 (29)	48 (22)	
II	—	64 (26)		7 (29)	57 (25)	
III	—	81 (33)		7 (29)	74 (33)	
IV	—	47 (19)		3 (12)	44 (20)	
Anatomical class, stage						
I	—	24 (9)		—	—	
II	—	64 (25)		—	—	
III	—	121 (47)		—	—	
IV	—	49 (19)		—	—	

* Stage I according to radiologic criteria of Steinbrocker, *et al*[18]. ** Stages II, III, and IV according to radiologic criteria of Steinbrocker, et *al*. SEM: standard error of the mean. NS: nonsignificant.

Table 2. Frequency of shared epitope (SE) and risk and severity of RA.

	Controls, n = 400	Cases, n = 258	OR (95% CI)	p	Mild RA, n = 24	Severe RA, n = 234	OR (95% CI)	p
SE, n (%)								
+/+	7 (2)	28 (11)	12.0 (5.07–28.40)	< 0.001	4 (16)	24 (10)	0.74 (0.21–2.57)	0.22
+/–	120 (30)	139 (54)	3.48 (2.47–4.88)	< 0.001	10 (42)	129 (55)	1.59 (0.64–3.99)	0.32
–/–	273 (68)	91 (35)	1		10 (42)	81 (35)	1	

SE: HLA-DRB1*0101, *0401, *0404, *0405, *0410, *1001, and *1406.

Table 3. Frequencies of –765G/C COX-2 genotypes and risk and severity of RA.

	Controls, n = 400, n (%)	Cases, n = 258, n (%)		OR (95% CI)	p***
Genotype					
G/G	347 (86.8)	233 (90.3)		1	
G/C + C/C*	53 (13.3)	25 (9.7)		0.70 (0.42–1.16)	0.16
		Mild RA, n = 24	Severe RA, n = 234		
G/G		20 (83.3)	213 (91.0)	1	
G/C + C/C**		4 (16.7)	21 (9.0)	0.49 (0.15–1.58)	0.26

* Six subjects (5 controls and one RA patient) were homozygous for the variant C allele. ** One patient with severe RA was homozygous for the variant C allele. *** Adjusted for age, sex, and duration of disease.

use may not be beneficial for colorectal polyp chemoprevention among a genetically defined subgroup of individuals with already lowered COX-2 levels[26]. Thus, the interaction between COX-2 genotypes and use of COX-2 inhibitors in RA patients warrants further investigation.

In summary, the –765 C allele of COX-2 was associated with a lower risk and milder RA in subjects without the SE. COX-2 genotyping may, therefore, be useful in predicting risk and severity of RA in individuals without the SE.

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Table 4. Interaction between the COX-2 genotype and the shared epitope (SE) on risk and severity of RA. Two subjects (one control and one RA) were homozygous for the variant C allele with the SE, and 4 controls were homozygous for the variant C allele without the SE.

HLA-RB1*SE/SE or *SE/X	COX-2	Controls, n = 400, n (%)	Cases, n = 258, n (%)		OR (95% CI)	p*
+	G/G	104 (88.1)	147 (88.0)		1	
+	G/C+C/C	14 (11.9)	20 (12.0)		1.01 (0.49–2.09)	0.98
–	G/G	243 (86.2)	86 (94.5)		1	
–	G/C+C/C	39 (13.8)	5 (5.5)		0.36 (0.14–0.95)	0.02
			Mild RA, n = 24	Severe RA, n = 234		
+	G/G		13 (92.9)	134 (87.6)	1	
+	G/C+C/C		1 (7.1)	19 (12.4)	1.84 (0.23–14.9)	0.54
–	G/G		7 (70.0)	79 (97.5)	1	
–	G/C+C/C		3 (30.0)	2 (2.5)	0.06 (0.01–0.41)	0.005

* Adjusted for age, sex, and disease duration. X: other than SE.

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