ARTHRITIS & RHEUMATISM
Vol. 54, No. 1, January 2006, pp 90–96
DOI 10.1002/art.21536

A Functional Haplotype of the *PADI4* Gene Associated With Increased Rheumatoid Arthritis Susceptibility in Koreans

Changsoo Paul Kang,[1] Hye-Soon Lee,[2] Hyoungseok Ju,[1] Hyunmi Cho,[1] Changwon Kang,[1] and Sang-Cheol Bae[2]

Objective. **Anticitrullinating autoantibodies are specific markers for rheumatoid arthritis (RA). A functional haplotype of 4 exonic single-nucleotide polymorphisms (SNPs) in a citrullinating enzyme, peptidylarginine deiminase 4 (PADI4), was shown to be associated with susceptibility to RA in a Japanese population and was shown to increase the stability of *PADI4* messenger RNA. However, the association was not confirmed in 4 subsequent studies involving Caucasian RA patients living in the UK, a French Caucasian population, and a Spanish population. The aim of the current study was to investigate the association of SNPs in the *PADI4* gene with RA in a Korean population.**

Methods. **Four exonic SNPs of the *PADI4* gene (padi4_89, padi4_90, padi4_92, and padi4_104) were genotyped in 545 unrelated patients with RA and 392 controls, using the MassArray SNP genotyping system. Allelic, genotypic, and haplotypic associations of the SNPs with RA susceptibility were examined using the chi-square test and multivariate logistic regression analyses.**

Results. **Increased RA susceptibility was significantly associated with the minor alleles of padi4_89 ($P = 2.3 \times 10^{-5}$), padi4_90 ($P = 2.3 \times 10^{-5}$), padi4_92 ($P = 2.1 \times 10^{-5}$), and padi4_104 ($P = 1.1 \times 10^{-3}$) and the haplotype carrying the 4 minor alleles ($P = 1.0 \times 10^{-4}$). Genotypes carrying the minor alleles and HLA–DRB1 shared epitope (SE) alleles ($P = 9.4 \times 10^{-21}$) were also associated with increased RA susceptibility. The genotypic associations were sustained among individuals who did not carry any SE alleles, except in the case of padi4_104. Individuals carrying the risk SNPs and/or SE alleles were more susceptible to RA than were individuals carrying neither risk SNPs nor SE alleles.**

Conclusion. **The *PADI4* SNPs and haplotypes are associated with RA susceptibility in Koreans. Thus, the association of *PADI4* with RA may depend on genetic heterogeneity between Asians and Europeans.**

Rheumatoid arthritis (RA) is a chronic inflammatory disease of unknown etiology that leads to progressive joint destruction. Epidemiologic genetic data suggest that the heritability of RA is 53–60% (1). The relative risk for siblings of patients with RA ranges from 2 to 17 (2), which suggests that both genetic and environmental factors contribute to RA susceptibility. According to family-based whole-genome linkage analysis data, the RA susceptibility loci are scattered across several chromosomes (3–6), among which HLA–DRB1 is consistently implicated as an RA susceptibility locus. Because this locus accounts for only approximately one-third of total genetic effects (7), other RA susceptibility genes or loci remain to be elucidated (8).

The peptidylarginine deiminase 4 (*PADI4*) gene was recently suggested to be an additional RA susceptibility gene in a Japanese population, based on the results of high-resolution linkage disequilibrium (LD) mapping for a potential RA susceptibility locus at 1p36 (9). A haplotype consisting of 17 *PADI4* single-nucleotide poly-

Dr. C. P. Kang, Dr. C. Kang, and Mr. Ju were participants in the Brain Korea 21 Program. Dr. C. Kang's work was supported by the Molecular and Cellular BioDiscovery Research Program (grant 2004-01861). Dr. Bae's work was supported by the Korea Health 21 R&D Project (grant 01-PJ3-PG6-01GN11-0002).

[1]Changsoo Paul Kang, PhD, Hyoungseok Ju, Hyunmi Cho, Changwon Kang, PhD: Korea Advanced Institute of Science and Technology, Daejeon, Korea; [2]Hye-Soon Lee, MD, PhD, Sang-Cheol Bae, MD, PhD, MPH: Hanyang University College of Medicine and the Hospital for Rheumatic Diseases, Seoul, South Korea.

Drs. C. P. Kang and Lee contributed equally to this work.

Address correspondence and reprint requests to Changwon Kang, PhD, Department of Biological Sciences, Korea Advanced Institute of Science and Technology (KAIST), 373-1 Guseong-dong, Yuseong-gu, Daejeon 305-701, South Korea. E-mail: ckang@kaist.ac.kr; or Sang-Cheol Bae, MD, PhD, Department of Internal Medicine, Division of Rheumatology, The Hospital for Rheumatic Diseases, Hanyang University Medical Center, Seoul 133-792, South Korea. E-mail: scbae@hanyang.ac.kr.

Submitted for publication April 12, 2005; accepted in revised form September 20, 2005.

morphisms (SNPs), 4 of which are located in exons, was reported to be associated with RA susceptibility in a Japanese population, and *PADI4* messenger RNA (mRNA) carrying a susceptibility haplotype of the 4 exonic SNPs is significantly more stable than its mirror haplotype mRNA. The susceptibility haplotype stabilizing *PADI4* mRNA would increase the cellular levels of PADI4 protein and citrullinated peptides because PADI4 posttranslationally converts arginine residues to citrulline. In fact, the detection of anti–citrullinated peptide (anti-CCP) autoantibodies by various methods has been used as a diagnostic test for RA for decades since the discovery of the crucial role of citrullination in RA (10,11). Although in the Japanese study, anticitrullinating autoantibodies were detected more frequently in a group of individuals homozygous for the susceptibility haplotype than in the other group of the remaining individuals (9), an association of the *PADI4* haplotypes with levels of anti-CCP autoantibodies has not yet been detected (12,13).

When the SNPs in the *PADI4* gene were later tested for association with RA in 2 populations of Caucasian subjects living in the UK (12,14), French Caucasian families (15), and a Spanish population (16), no association was observed. This type of replication failure has been reported in many association studies (17) and may arise from genetic heterogeneity between study groups or ethnic populations or from incorrect genotyping in the initial or replication study (18). Bias regarding the proportions of different stages of disease severity among the patients may also contribute to the inconsistency when alleles or haplotypes affect disease severity rather than susceptibility.

Because results of the initial association study involving Japanese patients were not reproduced in the 4 subsequent association studies with Europeans, the present study was designed to test whether the 4 exonic SNPs of *PADI4* and their haplotypes are associated with RA susceptibility in a Korean population.

PATIENTS AND METHODS

Patients. A total of 545 Korean patients with RA (92% of whom were women) and 392 controls (88% of whom were women) were recruited from the Hospital for Rheumatic Diseases, Hanyang University, Seoul, South Korea. The study was approved by the Institutional Review Board of Hanyang University Medical Center, and all subjects provided written informed consent. The mean ± SD age of the patients was 50.3 ± 11.5 years, and that of the controls was 41.9 ± 14.1 years. The mean ± SD age of the patients at the onset of RA was 37.9 ± 11.9 years. All patients satisfied the American College of Rheumatology (formerly, the American Rheumatism Association) 1987 revised criteria for a diagnosis of RA (19). Genomic DNA was extracted from blood leukocytes from all subjects, using standard protocols.

Genotyping of HLA–DRB1 alleles and *PADI4* SNPs. All of the patient and control samples were genotyped for the HLA–DRB1 alleles, using polymerase chain reaction and sequence-specific oligonucleotide probe hybridization, according to the reference protocol of the 12th International Histocompatibility Workshop (20), followed by direct DNA sequencing (21).

Three nonsynonymous *PADI4* SNPs (padi4_89 [rs11203366], padi4_90 [rs11203367], and padi4_92 [rs874881]) and 1 synonymous *PADI4* SNP (padi4_104 [rs1748033]) were genotyped using the MassArray system (Sequenom, San Diego, CA) according to the manufacturer's instructions. SpectroTyper software (Sequenom) was used to characterize the alleles. The accuracy of genotyping was controlled by testing in a blinded manner, using 12 samples for each SNP.

Statistical analysis. Allele associations between RA and individual *PADI4* SNPs were assessed using the chi-square test and 2×2 contingency tables. For genotype association tests, multivariate logistic regression analysis was performed using SPSS software (version 11.5; SPSS, Chicago, IL), with adjustment for age, because the mean ages of patients and controls were significantly different ($P = 1.6 \times 10^{-23}$). Individual samples from patients and controls who carried none of the minor risk alleles of the *PADI4* SNPs (padi4_89G, padi4_90T, padi4_92G, and padi4_104T) and no shared epitope (SE) alleles (*0101, *0401, *0404, *0405, *0410, *1001, *1402, and *1406) served as the reference group. The combined effects of *PADI4* SNPs with HLA–DRB1 SE alleles in RA susceptibility were evaluated by logistic regression analyses. Haplotypes were reconstructed using the Haplotyper program (22), which is based on a Bayesian algorithm.

RESULTS

Findings of allele and genotype association tests and LD estimation. Samples obtained from 545 patients with RA and 392 controls were genotyped individually for 4 exonic SNPs. Every sample was successfully genotyped for all 4 SNPs, and the SNPs were in Hardy-Weinberg equilibrium ($P > 0.05$) in both the patient and control groups. The padi4_89 (163A>G; 55Ser>Gly), padi4_90 (245C>T; 82Ala>Val), and padi4_92 (335C>G; 112Ala>Gly) alleles are nonsynonymous, and padi4_104 (349C>T; 117Leu) is synonymous (numbers within the parentheses indicate either nucleotide or amino acid positions; major alleles are shown first, followed by minor alleles).

Each of the 4 SNPs was significantly associated with RA (Table 1). The minor alleles of the 3 nonsynonymous SNPs were strongly associated with increased RA susceptibility (odds ratio [OR] 1.5 [95% confidence interval (95% CI) 1.2–1.8], $P = 2.3 \times 10^{-5}$ for padi4_89

Table 1. Association of the minor alleles of 4 exonic *PADI4* SNPs with RA susceptibility*

		Allele 2 frequency										
		Korean population (current study)				Japanese population (9)†			UK Caucasian population (14)			
SNP	Allele 1/2	Patients (n = 545)	Controls (n = 392)	OR (95% CI)	*P*	Patients (n = 830)	Controls (n = 658)	*P*	Patients (n = 839)	Controls (n = 481)	OR (95% CI)	*P*
padi4_89	A/G	0.48	0.38	1.5 (1.2–1.8)	2.3×10^{-5}	0.45	0.40‡	0.069	0.43	0.40	1.2 (1.0–1.4)	0.083
padi4_90	C/T	0.48	0.38	1.5 (1.2–1.8)	2.3×10^{-5}	0.50‡	0.40‡	6.9×10^{-3}	0.43	0.40	1.1 (1.0–1.4)	0.12
padi4_92	C/G	0.48	0.38	1.5 (1.2–1.8)	2.1×10^{-5}	0.45	0.39	4.4×10^{-4}	0.45	0.42	1.1 (0.9–1.3)	0.31
padi4_104	C/T	0.41	0.34	1.4 (1.1–1.7)	1.1×10^{-3}	0.39	0.33§	5.1×10^{-4}	0.34	0.31	1.1 (1.0–1.4)	0.12

* The major alleles are referred to as allele 1, and the minor alleles as allele 2. SNP = single-nucleotide polymorphism; RA = rheumatoid arthritis; OR = odds ratio; 95% CI = 95% confidence interval.
† Data were retrieved from Supplementary Table 1 in reference 9, which does not show ORs or 95% CIs.
‡ A total of 188 samples were genotyped.
§ A total of 736 samples were genotyped.

and padi4_90; OR 1.5 [95% CI 1.2–1.8], $P = 2.1 \times 10^{-5}$ for padi4_92), while the minor allele of synonymous SNP padi4_104 was moderately associated with increased RA susceptibility (OR 1.4 [95% CI 1.1–1.7], $P = 1.1 \times 10^{-3}$).

The padi4_89 and padi4_90 SNPs, both of which are located in exon 2, were in complete LD with each other, with no breakage detected, and the padi4_92 SNP in exon 3 was in almost complete LD with them, given that breakage was detected in samples from only 3 individuals. The padi4_104 SNP in exon 4 also was in very high LD ($|D'| > 0.99$) with the other SNPs. Thus, the 4 SNPs constitute a single haplotype block. The association test results for padi4_89 and padi4_90 would be exactly the same, and those for padi4_92 would be very similar to them, whereas the results for padi4_104 would be somewhat different from those for the other SNPs.

All 4 minor alleles exhibited significant associations with RA in Koreans; these results are similar to the data on Japanese patients previously reported by Suzuki et al (9), with the exception of padi4_89 (Table 1). When the allele frequencies were calculated using the genotype frequency data for a UK Caucasian population reported by Barton et al (14), no allelic association was observed for any SNP (Table 1). In another study involving Caucasian patients with RA living in the UK, Harney et al (12) reported no allelic association of padi4_90 and padi4_104, as well as some other *PADI4* SNPs, with RA. Martinez et al (16) reported that padi_94 and padi_104 were not associated with RA in the Spanish population.

Genotypic associations of all 4 SNPs with RA susceptibility were also significant by logistic regression analysis, as shown in Table 2. Age-adjusted ORs for the SNPs were 1.7 or 1.8, with *P* values on the order of 10^{-4} for padi4_89, padi4_90 ($P = 1.5 \times 10^{-4}$), and padi4_92 ($P = 1.4 \times 10^{-4}$) and was $P = 1.0 \times 10^{-2}$ for padi4_104.

Combined effects of *PADI4* risk SNPs and HLA–DRB1 SE alleles. HLA–DRB1 SE alleles have been shown to be strongly associated with RA in Korean patients as well as in other populations (7,23,24). When SE allele carriers were compared with noncarriers in this study, SE alleles were strongly associated with RA susceptibility (OR 4.0 [95% CI 3.0–5.3], $P = 9.4 \times 10^{-21}$), as shown in Table 2.

Table 2. Association of *PADI4* SNP genotypes and SE alleles with RA susceptibility*

Polymorphism/ genotype	Patients (n = 545)	Controls (n = 392)	OR (95% CI)	*P*
padi4_89				
AG + GG	397 (72.8)	240 (61.2)	1.7 (1.3–2.2)	1.5×10^{-4}
AA	148 (27.2)	152 (38.8)	1	–
padi4_90				
CT + TT	397 (72.8)	240 (61.2)	1.7 (1.3–2.2)	1.5×10^{-4}
CC	148 (27.2)	152 (38.8)	1	–
padi4_92				
CG + GG	399 (73.2)	241 (61.5)	1.7 (1.2–2.4)	1.4×10^{-4}
CC	146 (26.8)	151 (38.5)	1	–
padi4_104				
CT + TT	358 (65.7)	226 (57.7)	1.8 (1.2–1.9)	1.0×10^{-2}
CC	187 (34.3)	166 (42.3)	1	–
SE†				
SE+	369 (67.7)	136 (34.7)	4.0 (3.0–5.3)	9.4×10^{-21}
SE−	176 (32.3)	256 (65.3)	1	–

* Values are the number (%). Age-adjusted odds ratios (ORs) and *P* values for carriers of minor susceptibility alleles versus noncarriers were calculated by multivariate logistic regression. SNP = single-nucleotide polymorphism; SE = shared epitope; RA = rheumatoid arthritis; 95% CI = 95% confidence interval.
† HLA–DRB1 SE alleles include *0101, *0401, *0404, *0405, *0410, *1001, *1402, and *1406.

Table 3. Combined effects of *PADI4* SNPs and HLA–DRB1 SE alleles*

Genotype	Patients (n = 545)	Controls (n = 392)	OR (95% CI)	*P*
SE+/padi4_89G+	265	87	8.0 (5.0–13)	2.4×10^{-18}
SE+/padi4_89G−	104	49	5.4 (3.2–9.1)	3.1×10^{-10}
SE−/padi4_89G+	132	153	2.1 (1.4–3.3)	6.5×10^{-4}
SE−/padi4_89G−	44	103	1	–
SE+/padi4_90T+	265	87	8.0 (5.0–13)	2.4×10^{-18}
SE+/padi4_90T−	104	49	5.4 (3.2–9.1)	3.1×10^{-10}
SE−/padi4_90T+	132	153	2.1 (1.4–3.3)	6.5×10^{-4}
SE−/padi4_90T−	44	103	1	–
SE+/padi4_92G+	267	87	7.9 (5.0–13)	3.0×10^{-18}
SE+/padi4_92G−	102	49	5.3 (3.1–8.9)	5.9×10^{-10}
SE−/padi4_92G+	132	154	2.1 (1.4–3.3)	8.3×10^{-4}
SE−/padi4_92G−	44	102	1	–
SE+/padi4_104T+	244	80	5.7 (3.7–8.8)	1.1×10^{-15}
SE+/padi4_104T−	125	56	4.1 (2.6–6.6)	2.7×10^{-9}
SE−/padi4_104T+	114	146	1.5 (1.0–2.2)	8.0×10^{-2}
SE−/padi4_104T−	62	110	1	–

* G and T represent risk alleles. Age-adjusted odds ratios (ORs), 95% confidence intervals (95% CIs), and *P* values were calculated by logistic regression analysis in comparison with the reference genotype groups carrying neither the risk single-nucleotide polymorphisms (SNPs) nor the shared epitope (SE) alleles.

We then divided the samples into 4 groups according to the presence of the risk allele in each SNP and of any HLA–DRB1 SE allele (Table 3). For analysis of the padi4_89 SNP, for example, both patient and control samples were divided into SE+/padi4_89G+, SE+/padi4_89G−, SE−/padi4_89G+, and SE−/padi4_89G− groups (with G being the risk allele). Individuals carrying the risk allele padi4_89 but no SE allele (SE−/padi4_89G+) had a 2.1-fold higher risk than the reference individuals carrying neither padi4_89 nor an SE allele (SE−/padi4_89G−), with the genotypic association being significant (OR 2.1 [95% CI 1.4–3.3], $P = 6.5 \times 10^{-4}$) in the absence of SE alleles. Also, individuals carrying SE+/padi4_89G− had a 5.4-fold increased risk compared with the reference subjects, with the association of SE alleles being significant (OR 5.4 [95% CI 3.2–9.1], $P = 3.1 \times 10^{-10}$) in the absence of the padi4_89 risk allele. Moreover, individuals carrying both the padi4_89 risk allele and an SE allele (SE+/padi4_89G+) had the highest risk (8.0-fold versus the reference group; OR 8.0 [95% CI 5.0–13], $P = 2.4 \times 10^{-18}$).

The results were the same for the padi4_90 SNP, which was in complete LD with padi4_89 and was very similar to padi4_92 (Table 3). A similar pattern was also observed for padi4_104, but a genotypic association was not observed for padi4_104 ($P = 0.08$) in SE allele–negative individuals (Table 3). Thus, persons carrying a risk SNP and/or an SE allele are more susceptible to RA than are those carrying neither a risk SNP nor an SE allele.

The interaction between each *PADI4* risk allele and SE alleles was examined by linear logistic model analysis. The interaction effect was not significant for any of the 4 SNPs ($P = 0.26$, $P = 0.26$, $P = 0.33$, and $P = 0.96$ for padi4_89, padi4_90, padi4_92, and padi4_104, respectively).

Findings of haplotype analysis and association tests. When haplotypes were reconstructed with respect to the 4 *PADI4* SNPs using the Bayesian algorithm-based Haplotyper program, 5 haplotypes were found in our samples. Haplotypes ACCT and ACGT were omitted from the association tests because only 1 and 3 copies, respectively, were found (e.g., haplotype ACGT consists of padi4_89A, padi4_90C, padi4_92G, and padi4_104T). Among the 3 common haplotypes shown in Table 4, 2 mirror haplotypes, ACCC and GTGT, accounted for 96% of the controls and 94% of the patients. The most frequent haplotype (ACCC) carries only the major nonrisk alleles of the SNPs, and its frequency was lower in patients (52%) than in controls (62%). The second most frequent haplotype (GTGT) carries only the minor risk alleles and was associated with increased RA susceptibility (OR 1.5 [95% CI 1.2–1.8], $P = 1.0 \times 10^{-4}$) in comparison with the reference haplotype ACCC. The RA-susceptibility hap-

Table 4. Association of *PADI4* haplotypes with rheumatoid arthritis susceptibility*

	Korean population (current study)				UK Caucasian population (14)			
Haplotype	Cases (n = 1,090)	Controls (n = 784)	OR (95% CI)	*P*	Cases (n = 645)	Controls (n = 373)	OR (95% CI)	*P*
ACCC	563 (0.52)	483 (0.62)	1	–	372 (0.56)	224 (0.59)	1	–
GTGT	452 (0.42)	265 (0.34)	1.5 (1.2–1.8)	1.0×10^{-4}	215 (0.32)	113 (0.30)	1.1 (0.9–1.5)	0.34
GTGC	73 (0.067)	34 (0.043)	1.8 (1.2–2.8)	4.8×10^{-3}	58 (0.087)	36 (0.094)	1.0 (0.6–1.5)	0.89

* Haplotypes ACCT and ACGT (frequencies <0.2%) were not included. Nucleotides are listed in the order of padi4_89, padi4_90, padi4_92, and padi4_104. Odds ratios (ORs), 95% confidence intervals (95% CIs), and *P* values for each haplotype were calculated by logistic regression analysis in comparison with the reference haplotype ACCC.

lotype (GTGT) was more frequent in patients (42%) than in controls (34%). The third haplotype (GTGC), which carries all of the risk alleles except padi4_104, also appeared to be associated with increased RA susceptibility (OR 1.8 [95% CI 1.2–2.8], $P = 4.8 \times 10^{-3}$).

When haplotypes were reconstructed with only 3 SNPs (omitting padi4_104), only 2 haplotypes, ACC and GTG, constituted virtually all of the samples (99.8%). The frequency of haplotype GTG was higher in patients (48%) than in controls (37%), whereas the frequency of haplotype ACC was lower in patients (52%) than in controls (62%). Haplotype GTG was significantly associated with increased RA susceptibility, as compared with haplotype ACC (OR 1.5 [95% CI 1.3–1.8], $P = 1.2 \times 10^{-5}$).

When the same tests were performed using data from the UK Caucasian population described by Barton et al (14), however, no association of either 3- or 4-SNP haplotypes with RA was detected (Table 4). Haplotypes for a Japanese population were previously constructed with 17 *PADI4* SNPs (9), but a lack of information on their copy numbers made it impossible to obtain 4-SNP haplotype data for these Japanese patients for comparison with our data.

DISCUSSION

The present study showed that the minor alleles of 4 exonic SNPs in the *PADI4* gene and the haplotype comprising them were associated with ~1.5-fold increased susceptibility to RA in a Korean population (Tables 1 and 4). The minor allele–carrying genotypes were also associated with a 1.7- or 1.8-fold increase in RA susceptibility (Table 2). Although the SE allele–carrying individuals showed 4.0-fold increased susceptibility, the genotypic associations were sustained (OR 2.1) among individuals who did not carry any SE alleles, except in the case of padi4_104 (Table 3). Therefore, *PADI4* was shown to be associated with RA susceptibility in Koreans.

Recently, a haplotype consisting of 17 *PADI4* SNPs (comprising 4 exonic and 13 nonexonic SNPs) was reported to be associated with RA susceptibility in a Japanese population. The *PADI4* mRNA carrying the haplotype (GTGT) of the minor alleles in the 4 exonic SNPs was significantly more stable than the major allele haplotype (ACCC) mRNA, suggesting that haplotype GTGT increases RA susceptibility (9). A subsequent study in a UK Caucasian population, however, revealed no association with RA of haplotype GTGT or any of its constituent SNP alleles (12,14,16). In contrast, haplotype GTGT was significantly associated with increased RA susceptibility in the present study of a Korean population ($P = 1.0 \times 10^{-4}$). This finding is consistent with the implications of the Japanese study (9), although in the Japanese study, the association tests were performed with 17-SNP haplotypes rather than with 4-SNP haplotypes.

Despite the consistency of the haplotype association between Korean and Japanese populations, the allele association test results differed slightly between the 2 populations. Although all 4 constituent SNPs showed significant association with RA in Koreans, the padi4_89 SNP was not associated with RA ($P = 0.069$) in that Japanese population (Table 1).

The discrepancy may be associated with genetic heterogeneity of RA susceptibility–increasing variants in different ethnic groups. Although *PADI4* is likely to be an RA-susceptibility gene in Koreans and Japanese, in UK Caucasian, French, and Spanish populations, the *PADI4* gene might not carry variants that increase RA susceptibility. The minor allele frequency for each SNP was similar among the control populations in the Korean, Japanese, and UK studies, with the largest differences being 2%, 2%, 4%, and 3% in padi4_89, padi4_90, padi4_92, and padi4_104, respectively (Table 1). The estimated frequencies of the haplotypes in these control populations were also similar (Table 4). If the susceptibility haplotype of the 4 exonic SNPs also increases RA susceptibility in Europeans, it is very likely to be attributable to an increase in *PADI4* mRNA stability. Because this is not the case, however, it is still possible that in Koreans and Japanese, the susceptibility haplotype is linked with an as-yet-unknown functional genetic variant(s) of *PADI4* (or nearby) that is absent in Europeans. It is possible that such a variant(s) was missed in the sequencing of DNA from the 48 Japanese RA patients in the previous study (9).

Another possible explanation is a variation in the effects of other genetic factors. RA susceptibility due to the *PADI4* functional haplotype might be modified by the presence of a certain allele(s) in other genes. Recently, Hill et al (25) studied the interaction of arginine- or citrulline-harboring peptides with 8 variants of HLA–DRB1 (*0101, *0401, *0404, *0301, *0701, *0802, *1101, and *1302 alleles) and showed that the *0401 variant had the highest affinity for citrullinated peptide, leading to activation of CD4+ T cells. Thus, one possible hypothesis is that the pathogenesis of RA mediated by *PADI4* is influenced by the status of HLA–DRB1 variants, which is different between Asians and Europeans. For example, DRB1*0401 is the most common risk

allele among European patients with RA (7) but is rare (<2%) in Asian patients (23,24). In contrast, DRB1*0405 is the variant that is most significantly associated with RA in Asian patients (23,24). The binding affinity of the *0405 variant to citrullinated peptide was not measured by Hill et al (25). If the binding affinity of the *0405 variant is significantly different from that of the *0401 variant, this may explain the genetic heterogeneity of the *PADI4* association with RA susceptibility in Asians and Europeans.

The percentages of female patients with RA in the present study of Koreans (92%) and in the Japanese study (84%) were substantially higher than those in the UK (74%) and Spanish (75%) studies (9,14,16). However, the discrepancy between the Asian and European association test results cannot be explained by the differences in the percentage of female patients, because Barton et al (14) showed no association in the subanalysis using samples from only women from the UK, whereas in the subanalysis with Korean female patients (n = 499) and controls (n = 344), a significant association was detected (e.g., for padi4_89 risk allele–carrying genotypes, age-adjusted OR 1.9 [95% CI 1.4–2.5], $P = 7.0 \times 10^{-5}$).

The average age at disease onset in our Korean patients (37.9 years) was lower than that in UK Caucasian patients (43 years) and Spanish RA patients (53 years) (14,16). The Korean patients were divided into 2 subgroups according to their age at disease onset. One group (n = 272) comprised patients whose age at disease onset was higher than the median (38.3 years), and the other group (n = 273) comprised the remaining patients. When samples from these 2 subgroups were separately compared with total control samples, for example, the genotypes with the padi4_89 risk allele were associated with 2.1-fold increased susceptibility in the subgroup of patients whose average age at disease onset was 48.2 years (OR adjusted for age at diagnosis and sex 2.1 [95% CI 1.4–3.1], $P = 3.7 \times 10^{-4}$), and with 1.6-fold increased susceptibility in the subgroup of patients whose average age at disease onset was 28.6 years (OR adjusted for age at diagnosis and sex 1.6 [95% CI 1.1–2.2], $P = 6.2 \times 10^{-3}$). Thus, the differences in the distribution of age at disease onset between the study populations do not appear to have caused the replication discrepancies.

Although a functional haplotype of *PADI4* has been demonstrated to be associated with RA susceptibility in Korean and Japanese populations, the functional haplotype has not yet been demonstrated to be associated with increased levels of anti-CCP autoantibodies, which have been useful in the diagnosis of RA. Because no such association was observed in Europeans (12,13), the haplotype association with antibody levels needs to be tested in Asians. Also, citrullinating genes other than *PADI4* and downstream or upstream genes in the citrullination pathway should be investigated to determine whether combined effects with the *PADI4* haplotype are present.

ACKNOWLEDGMENTS

We thank Jung-Ah Kim, Young-Hi Lee, and Eun-Kyoung Ju (Hospital for Rheumatic Diseases) for assistance with sample preparation and data collection, and Professor Sung-Ho Kim and Geon Youp Noh (Department of Applied Mathematics, KAIST) for assistance with statistical analyses.

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