Association of *ultraviolet radiation resistance-associated* gene polymorphisms with rheumatoid arthritis

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Abstract. The ultraviolet radiation resistance-associated gene (UVRAG) protein binds to the Beclin 1/PI3-kinase III complex and promotes autophagy. Autophagy may be upregulated by endoplasmic reticulum (ER) stress. Persistent and excessive ER stress may alter synovial fibroblast apoptosis and this alteration may affect the pathogenesis of rheumatoid arthritis (RA). In this study, we investigated whether *UVRAG* genetic polymorphisms are associated with RA. To determine the association between *UVRAG* polymorphisms and RA, we genotyped five *UVRAG* single-nucleotide polymorphisms (SNPs; rs7111334, intron C/T; rs7933235, intron A/G; rs1380075, intron T/A; rs1458836, near the 5' gene terminal G/A; and rs636420, exon 15 C/T) using a direct sequencing method in 243 RA patients and 417 control subjects. Among these, one SNP (rs7111334) exhibited significant genotypic/allelic differences between RA patients and the control group. Therefore, this study suggested a possible association between *UVRAG* polymorphisms and RA susceptibility.

Introduction

The *ultraviolet radiation resistance-associated gene (UVRAG)* partially complements the ultraviolet sensitivity of xeroderma pigmentosum cells (1). Liang *et al* (2) reported that UVRAG binds with the Beclin 1/PI3-kinase III complex and induces autophagy. They also reported that UVRAG plays an additional role in autophagy by promoting the fusion of autophagosomes with lysosomes (3). Recently, Yin *et al* (4) also suggested that UVRAG forms two different complexes (UVRAG-Beclin 1 and UVRAG-Bax), which promote the equilibrium between autophagy and cell death. Autophagy is involved in the trafficking events that regulate innate and adaptive immunity (5). As a central player in the immunological regulation of pathogen removal, autophagy may also carry antigens to major histocompatibility complex compartments, modulate lymphocyte survival and homeostasis and mediate cytokine production (6-9). In brief, autophagy is modulated to regulate its unbalanced activation. It may be upregulated in response to extra- or intracellular stress and signals such as starvation, growth factor deprivation, endoplasmic reticulum (ER) stress, accumulation of unfolded proteins and pathogen infection (10). Autophagy may also protect cells from ER stress-induced cell death (11).

Rheumatoid arthritis (RA) is a chronic, systemic autoimmune inflammatory joint disease characterized by a distinctive pattern of bone and joint destruction (12). Alterations in synovial cell apoptosis, which regulates tissue composition and homeostasis, affect the pathogenesis of RA (13,14). These alterations may lead to synovial cell activation and contribute to chronic inflammation and hyperplasia. The ER also plays an important role in secretory cells, including synovial fibroblasts (15). Adaptive responses to the accumulation of misfolded proteins in the ER (i.e., ER stress) provide protection from cell death induced by oxidative stress and Ca^{2+} disturbances (16). Continuous, excessive ER stress induces cell death (17,18) through the initiation of apoptosis (19,20). ER stress may also contribute to autoimmune diseases, such as RA (1).

Despite the potential importance of *UVRAG* in RA pathogenesis, there are no reports regarding the association between *UVRAG* genetic variants and RA. Our aim was to investigate whether *UVRAG* single-nucleotide polymorphisms (SNPs) are associated with RA, as well as the clinicopathological characteristics of RA in a Korean population.

Materials and methods

Patients and control subjects. A case-control study was conducted to determine the genetic association between *UVRAG* SNPs and RA. Unrelated RA patients (n=243) were enrolled from two rheumatic centers (Soonchunhyang and

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Kyung Hee University hospitals). Each patient was diagnosed by a rheumatologist, according to the 1987 American College of Rheumatology Rheumatoid Arthritis Diagnostic Criteria (21). Control subjects (n=417) were recruited among volunteers who were examined in the context of a general health check-up program. Participants with RA and concurrent osteoarthritis or other severe diseases were excluded. Clinical and demographic data were obtained from medical records or interviews at the time of enrollment. Biochemical data were measured, including erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and rheumatoid factor (RF). Patients with bone erosion were classified by radiographic findings.

This study was conducted according to the Declaration of Helsinki guidelines and 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, Republic of Korea.

SNP genotyping. We searched for *UVRAG* SNPs using National Center for Biotechnology Information (NCBI) websites (www.ensembl.org, www.ncbi.nlm.nih.gov/SNP and www.hapmap.org). We selected five *UVRAG* SNPs for analysis, as previously described (22). The five selected SNPs consisted of one synonymous SNP (rs636420), three intronic SNPs (rs7111334, rs7933235 and rs1380075) and a SNP near the 5' gene terminal (rs1458836). Finally, four SNPs were assessed in this study. DNA was isolated from peripheral blood using the GenEx™ B DNA purification kit (GeneAll Biotechnology, Seoul, Korea). The *UVRAG* gene SNP was genotyped by the method previously described by Jeong *et al* (22). The polymerase chain reaction products were sequenced using an ABI PRISM 3730xl DNA analyzer (PE Applied Biosystems, Foster City, CA, USA). The sequence data were analyzed using SeqManII software (DNASTAR, Inc., Madison, WI, USA).

Statistical analysis. Hardy-Weinberg equilibrium (HWE) was assessed by SNPStats software (http://bioinfo.iconcologia.net/index.php) and SPSS software, version 18.0 (SPSS, Inc., Chicago, IL, USA). The associations between the SNP genotypes and RA and between the SNP genotypes and RA subgroups were estimated by computing odds ratios (ORs) and 95% confidence intervals (CIs) with logistic regression analyses, controlling for age and gender as covariables. In the logistic regression analysis for each SNP, models assuming codominant, dominant, or recessive inheritance were used. The χ^2 test was used to compare allele frequencies between groups. To avoid chance findings due to multiple testing, Bonferroni correction was applied by decreasing the significance levels to P=0.01 (P=0.05/5) for each of the five SNPs.

Results

Subject characteristics. The clinical and demographic characteristics of the RA patients and control subjects are presented in Table I. The mean age [± standard deviation (SD)] of the RA patients and the control subjects was 50.45 (±12.11) and 44.18 (±12.08) years, respectively. There were 44 male and 199 female (n=243) RA patients and 184 male and 233 female (n=417) control subjects. RA patients were classified into clinical subgroups according to ESR level (≥30 vs. <30 mm/h), CRP levels (≥0.5 mg/dl or <0.5 mg/dl), RF (present or absent) and bone erosion (present or absent). There were 147 RA patients (60.5%) with an ESR level of ≥30 and 96 (39.5%) with an ESR level of <30 mm/h. A total of 169 RA patients (69.5%) had an CRP value of ≥0.5 mg/dl and 74 (30.5%) had an CRP value of <0.5 mg/dl. There were 213 RA patients (87.7%) with and 30 (12.3%) without RF. Bone erosion was present in 109 (44.9%) and absent in 134 RA patients (55.1%).

Table I. Clinical and demographic characteristics of RA patients and control subjects.[a]

Characteristics	RA (n=243)	Control (n=417)
Age (years, mean ± SD)	50.45±12.11	44.18±12.08
Gender (male:female)	44:199	184:233
ESR (mm/h, mean ± SD)	40.63±29.27	-
CRP (mg/dl, mean ± SD)	2.45±5.26	-
Subgroups		
ESR (n, ≥30:<30 mm/h)	147:96	-
CRP (n, ≥0.5:<0.5 mg/dl)	169:74	-
RF (n, +:-)	213:30	-
Bone erosion (n, +:-)	109:134	-

[a]RA patients with inappropriate clinical data were excluded. RA, rheumatoid arthritis; SD, standard deviation; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; RF, rheumatoid factor; +, present; -, absent.

SNP genotype distributions. The genotype distributions of two SNPs (rs7111334 and rs7933235) were in HWE (P>0.05), whereas three SNPs (rs1380075, rs1458836 and rs636420) were not in HWE (P<0.05). Therefore, rs1380075, rs1458836 and rs636420 were excluded from further analysis. As shown in Table II, rs7111334 genotype frequency was statistically associated with RA in codominant models 1 and 2 and in the recessive model (OR=0.25, 95% CI: 0.09-0.70, P=0.0029, P^c=0.0145) after Bonferroni correction. In the codominant model, the CC and TT genotype frequencies were 58.9 and 7.2% in the control group and 67.1 and 2.1% in the RA group, respectively. The CC genotype was associated with an increased risk of RA [OR=0.87 (0.24), 95% CI: 0.60-1.25 (0.09-0.67), P=0.009, P^c=0.044]. In the recessive model, genotype frequencies containing the C allele (CC/CT) and not containing the C allele (TT) were 98.0 and 2.0% in the control group and 92.8 and 7.2% in the RA group, respectively. The rs7111334 allele frequency was also associated with RA (OR=0.665, 95% CI: 0.50-0.88, P=0.005, P^c=0.025). The rs7111334 C allele frequency was higher in the RA (83.0%) than in the control group (76.0%). The other SNP (rs7933235) was not associated with the development of RA (Table II).

Association between SNPs and clinical characteristics of RA. We then assessed the association between the three investi-

Table II. Genotype and allele frequencies of *UVRAG* SNPs in RA patients and control subjects.[a]

SNP	Genotype/allele	RA Freq	RA %	Control Freq	Control %	Model	OR	95% CI LCL	95% CI UCL	P-value	P^C
rs7111334	C/C	163	67.1	245	58.9	Codominant 1	0.870	0.600	1.250	0.009	**0.044**
	C/T	75	30.9	141	33.9	Codominant 2	0.240	0.090	0.670		
	T/T	5	2.0	30	7.2	Dominant	0.760	0.530	1.070	0.120	0.600
						Recessive	0.250	0.090	0.700	0.003	**0.015**
						Over-dominant	0.950	0.660	1.360	0.760	1.000
						Log-additive	0.700	0.520	0.950	0.019	0.095
	C	401	83.0	631	76.0						
	T	85	17.0	201	24.0		0.665	0.502	0.883	0.005	**0.025**
rs7933235	A/A	130	53.7	241	58.1	Codominant 1	1.460	1.020	2.090	0.038	0.190
	A/G	98	40.5	140	33.7	Codominant 2	0.690	0.340	1.390		
	G/G	14	5.8	34	8.2	Dominant	1.290	0.920	1.810	0.140	0.700
						Recessive	0.600	0.300	1.180	0.130	0.650
						Over-dominant	1.520	1.070	2.160	0.020	0.100
						Log-additive	1.080	0.820	1.400	0.600	1.000
	A	358	74.0	622	75.0						
	G	126	26.0	208	25.0		1.052	0.814	1.360	0.696	1.000
rs1380075	T/T	240	98.8	403	96.6	Codominant 1	0.420	0.110	1.590	0.300	1.000
	T/A	3	1.2	13	3.1	Codominant 2	0.000	0.000	NA		
	A/A	0	0.0	1	0.2	Dominant	0.400	0.110	1.490	0.140	1.000
						Recessive	0.000	0.000	NA	0.470	1.000
						Over-dominant	0.420	0.110	1.590	0.170	1.000
						Log-additive	0.400	0.110	1.460	0.130	1.000
	T	483	99.0	819	98.0						
	A	3	1.0	15	2.0		0.339	0.098	1.177	0.089	0.445
rs1458836	G/G	131	55.7	227	57.9	Codominant 1	1.270	0.880	1.840	0.200	1.000
	G/A	90	38.3	134	34.2	Codominant 2	0.700	0.340	1.440		
	A/A	14	6.0	31	7.9	Dominant	1.150	0.810	1.640	0.430	1.000
						Recessive	0.640	0.310	1.290	0.200	1.000
						Over-dominant	1.320	0.920	1.900	0.140	0.700
						Log-additive	1.020	0.770	1.340	0.920	1.000
	G	352	75.0	588	75.0						
	A	118	25.0	196	25.0		1.006	0.772	1.309	0.966	1.000
rs636420	C/C	121	51.3	208	50.0	Codominant 1	1.020	0.710	1.460	0.990	1.000
	C/T	96	40.7	171	41.1	Codominant 2	1.030	0.540	1.960		
	T/T	37	8.9	19	8.9	Dominant	1.020	0.730	1.440	0.900	1.000
						Recessive	1.030	0.550	1.910	0.940	1.000
						Over-dominant	1.010	0.720	1.430	0.940	1.000
						Log-additive	1.020	0.780	1.330	0.900	1.000
	C	338	72.0	587	71.0						
	T	134	28.0	245	29.0		0.950	0.740	1.219	0.686	1.000

[a]The total numbers of genotypes and alleles in each SNP are different, due to the exclusion of unclear or missing genotype data. Bold print denotes statistical significance. *UVRAG, ultraviolet radiation resistance-associated gene*; SNPs, single-nucleotide polymorphisms; RA, rheumatoid arthritis; CI, confidence interval; Freq, frequency; OR, odds ratio; LCL, lower confidence limit; UCL, upper confidence limit; P^c, P-value corrected by the Bonferroni method; NA, not available.

gated SNPs and clinical characteristics of the RA patients, including ESR, CRP, RF (present vs. absent) and bone erosion (present vs. absent). However, no significant differences were found in these markers among the subgroups (data not shown).

Discussion

The purpose of the present study was to evaluate the association between the genetic polymorphisms of UVRAG and

Table III. Genotype frequencies of *UVRAG* SNPs in different populations.[a]

		Korean					
SNP	Genotype	RA	Control	European	Chinese	Japanese	Sub-Saharan African
rs7111334	C/C	0.671	0.589	0.823	0.558	0.593	0.250
	C/T	0.309	0.339	0.168	0.349	0.337	0.473
	T/T	0.021	0.072	0.009	0.093	0.070	0.277
P-value				0.001	0.843	1.000	0.000
rs7933235	A/A	0.537	0.581	0.788	0.651	0.523	0.205
	A/G	0.405	0.337	0.204	0.326	0.430	0.500
	G/G	0.058	0.082	0.009	0.023	0.047	0.295
P-value				0.002	0.134	0.355	0.000
rs1380075	T/T	0.988	0.966	0.900	0.889	0.932	0.800
	T/A	0.012	0.031	0.100	0.111	0.068	0.200
	A/A	0.000	0.002	0.000	0.000	0.000	0.000
P-value				0.133	0.086	0.431	0.001
rs1458836	G/G	0.557	0.579	0.786	0.643	0.523	0.381
	G/A	0.383	0.342	0.205	0.333	0.430	0.487
	A/A	0.060	0.079	0.009	0.024	0.047	0.133
P-value				0.003	0.142	0.355	0.018
rs636420	C/C	0.513	0.500	0.885	0.581	0.547	1.000
	C/T	0.407	0.411	0.106	0.233	0.372	0.000
	T/T	0.089	0.089	0.009	0.186	0.081	0.000
P-value				0.000	0.010	0.778	0.000

[a]Data were retrieved from database http://www.ncbi.nlm.nih.gov/SNP, dbSNP Build 137. P-values were estimated via comparisons between the control group of our sample and each population. *UVRAG, ultraviolet radiation resistance-associated gene*; SNP, single-nucleotide polymorphism; RA, rheumatoid arthritis.

susceptibility to RA. We observed an association between the *UVRAG* gene and RA. The *UVRAG* rs7111334 SNP was associated with RA, with the CC genotype contributing to an increased risk of RA.

Although the role of UVRAG in the pathogenesis of autoimmune diseases such as RA has not been fully elucidated, previous studies reported that UVRAG is directly or indirectly involved in the development of autoimmunity (23,24). A previous study from our group also identified a possible association between UVRAG polymorphisms and the autoimmune disease vitiligo (22).

In order to compare our Korean population genotype data with other populations, we used the human SNP database (www.ncbi.nlm.nih.gov/SNP, dbSNP Build 137). This database contains genotype frequencies for rs7111334 (C/C:C/T:T/T; European, 0.823:0.168:0.009; Chinese, 0.558:0.349:0.093; Japanese, 0.593:0.337:0.070; and Sub-Saharan African, 0.250:0.473:0.277), rs7933235 (A/A:A/G:G/G; European, 0.788:0.204:0.009; Chinese, 0.651:0.326:0.023; Japanese, 0.523:0.430:0.047; and Sub-Saharan African, 0.205:0.500:0.295), rs1380075 (T/T:T/A:A/A; European, 0.900:0.100:0.000; Chinese, 0.889:0.111:0.000; Japanese, 0.932:0.068:0.000; and Sub-Saharan African, 0.800:0.200:0.000), rs1458836 (G/G:G/A:A/A; European, 0.786:0.205:0.009; Chinese, 0.643:0.333:0.024; Japanese, 0.523:0.430:0.047; and Sub-Saharan African, 0.381:0.487:0.133) and rs636420 (C/C:C/T:T/T; European, 0.885:0.106:0.009; Chinese, 0.581:0.233:0.186; Japanese, 0.547:0.372:0.081; and Sub-Saharan African, 1.000:0.000:0.000) (Table III). In the control group, the SNP genotype distributions that were analyzed in our study were found to be similar to those in Asian populations, particularly the Japanese population, but not to those in the European population. Thus, our results may be valuable in such a case control study of a specific Asian population.

In conclusion, this study is, to the best of our knowledge, the first to investigate the potential effect of *UVRAG* gene polymorphisms on RA patients. The results of this study suggest that *UVRAG* polymorphisms may contribute to increased RA susceptibility in the Korean population. Furthermore, *UVRAG* may be one of several genes confirmed to play a role in polygenic susceptibility to RA. The rs7111334 CC genotype in particular was associated with RA development and the C alleles of rs7111334 were implicated as a risk factor for RA. Due to the relatively limited number of subjects, our findings must be validated by further studies using larger sample sizes.

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