GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Association study of polymorphisms between the Radixin gene and rheumatoid arthritis in a Korean population

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Genet. Mol. Res. 13 (2): 3697-3703 (2014)
Received August 15, 2013
Accepted December 10, 2013
Published May 9, 2014
DOI http://dx.doi.org/10.4238/2014.May.9.14

ABSTRACT. Radixin (RDX) is part of the ezrin-radixin-moesin (ERM) protein family. It functions as a membrane-cytoskeletal linker in actin-rich cell surface structures and is thought to be essential for cortical cytoskeleton organization, cell motility, adhesion, and proliferation. An increase in phosphorylated ERM in fibroblast-like synoviocytes contributes to rheumatoid arthritis (RA) synovial hyperplasia. We examined the genetic association between the *RDX* gene and RA in a Korean population. To identify the relationship between *RDX* gene polymorphisms and RA, we genotyped 2 single nucleotide polymorphisms (SNPs; rs11213326 and rs12575162) of *RDX* using a

direct sequencing method in 296 RA patients and 493 control subjects. In this study, the 2 SNPs showed no association with RA disease susceptibility. However, further analysis based on clinical information of the RA patient group showed that the SNPs were associated with the erythrocyte sedimentation rate (ESR) in RA patients. These data suggest an association between *RDX* polymorphisms and the clinical features of RA patients, particularly the ESR.

Key words: Rheumatoid arthritis; Radixin; SNP

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic autoimmune disease of the joints that involves chronic inflammation, synovial hyperplasia, and bone and cartilage erosion. Evidence suggests that fibroblast-like synoviocytes (FLS) is a key component in the pathophysiological process of RA (Huber et al., 2006). Abnormal proliferation of resident FLS is thought to be a critical contributor to rheumatoid synovial hyperplasia, and ultimately results in the destructive phase of the disease, causing cartilage and bone damage. Therefore, targeting proliferating fibroblasts may promote the regeneration of synovial joints (Kramer et al., 2003).

Ezrin/radixin/moesin (ERM) is a family of proteins that act as cross-linkers between the plasma membrane and the cytoskeleton by binding to membrane proteins at their NH_2-terminal (N) domains and to filamentous actin (F-actin) at their COOH-terminal (C) domains (Bretscher et al., 2002). ERM proteins are critical modulators in the formation of microvilli, cell adhesion, cell motility, preservation of cell shape, and membrane trafficking (Paglini et al., 1998; Matsui et al., 1999). Recently, increasing evidence has shown that ERM proteins are not only involved in alteration of the cellular physique, but also in a number of cell signaling pathways (Louver-Vallee, 2000). These findings brought about the identification of other biological functions of ERM, such as cell proliferation and apoptosis, as well as their regulation by various stimuli in a cell type-specific manner (Louver-Vallee, 2000; Gautreau et al., 2002; Tsuda et al., 2004; Kishore et al., 2005). Increased phosphorylation of ERM proteins contributes to the proliferation of rheumatoid FLS (Huang et al., 2011). Phosphorylation of ERM is regulated by several types of stimuli, including tumor necrosis factor alpha (TNF-α) and interleukin-1β (IL-1β) (Chen et al., 2001; Rozenblatt-Rosen et al., 2002; Kishore et al., 2005). Furthermore, a previous study described a possible relationship between ERM proteins and the pathophysiology of RA. Wagatsuma et al. (1996) reported that ERM proteins may be novel auto-immune target antigens for RA.

Radixin (RDX) is a member of the cytoskeleton linker protein family, ERM, and can connect transmembrane proteins to the actin cytoskeleton, thus promoting cell functions involving a dynamic cytoskeleton, such as morphological changes, cell division, and migration (Paglini et al., 1998; Matsui et al., 1999). Based on several studies of the ERM protein family and RA-FLS, we hypothesized that the *RDX* gene, as a member of the ERM gene family, may be the susceptibility gene for RA. Therefore, we investigated the genetic association between *RDX* gene polymorphisms and RA in a Korean population.

MATERIAL AND METHODS

Subjects

A case-control study was conducted to determine the genetic association between *RDX* single nucleotide polymorphisms (SNPs) and RA. A total of 296 unrelated patients with RA were enrolled from rheumatic centers (Soonchunhyang University Hospital and Kyung Hee University Hospital). Each patient was diagnosed by a rheumatologist according to ACR 1987 Rheumatoid Arthritis diagnostic criteria (Arnett et al., 1988). A total of 493 control subjects were recruited from individuals who participated in a general health checkup program. Participants with RA, osteoarthritis (OA), or other severe diseases were excluded. This study was carried out 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.

Demographic data were obtained from patient medical records or by interviews at the time of enrollment. Biochemical parameters were measured, including the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) and rheumatoid factor (RF) levels. Patients with bone erosion were classified based on radiographic findings.

SNP genotyping

We searched *RDX* SNPs using the National Center for Biotechnology Information (NCBI) database (http://www.ensembl.org, http://www.ncbi.nlm.nih.gov/SNP and http://www.hapmap.org). SNPs with >5% minor allele frequency (MAF) and >10% heterozygous genotype frequencies in the Asian population were included. Two SNPs (rs11213326 and rs12575162) were added using the aggressive tagging option of the Tagger program (http://www.broad.mit.edu/mpg/tagger). DNA was isolated from peripheral blood using the GenEx™ B DNA purification kit (GeneAll Biotechnology; Seoul, Korea). Genomic DNA was amplified by polymerase chain reaction (PCR) using primers for each SNP. Primer sequences were as follows: rs11213326 (sense: 5'-TAA GTG ATT TTG TTT CTG CCT TTT C-3' and anti-sense: 5'-CCG TTT TAA GCA TGT TAC TTT GAC T-3'); rs12575162 (sense: 5'-CTG GTG AAA TCC CGA CTC TAC T-3' and anti-sense: 5'-CCA AAG CCT AAC CAA AGA GTC A-3'). PCR products were genotyped using the direct sequencing method employing an ABI Prism 3730XL automatic sequencer (PE Applied Biosystems; Foster City, CA, USA). Sequence data were analyzed using the SeqManII software (DNAStar Inc.; Madison, WI, USA).

Statistical analysis

Hardy-Weinberg equilibrium (HWE) was assessed using SNPStats (http://bioinfo.iconcologia.net/index.php). SNPStats and SNPAnalyzer Pro (Istech Inc.; Goyang, Korea) were also used to evaluate the odds ratios (ORs), 95% confidence intervals (CIs), and P values. Multiple logistic regression analysis, adjusted for age and gender as covariables, was also performed. In the logistic regression analysis for each SNP, the following models were used that

assumed either co-dominant inheritance (where the relative hazard differed between subjects with 1 minor allele and those with 2 minor alleles), dominant inheritance (subjects with 1 or 2 minor alleles had the same relative hazard for the disease), or recessive inheritance (subjects with 2 minor alleles were at increased risk of the disease). Bonferroni correction was applied by multiplying the P values by the number of SNPs (N = 2).

RESULTS

Clinical and demographic features of subjects

Table 1 presents the clinical and demographic features of RA and control subjects. The mean ages [± standard deviation (SD)] of RA patients and control subjects were 49.80 ± 12.96 years and 43.59 ± 12.81 years, respectively. There were 58 male and 238 female RA patients (N = 296) and 213 male and 280 female control subjects (N = 493). RA patients were classified into clinical subgroups according to ESR levels (≥30 and <30 mm/h), CRP levels (≥0.5 and <0.5 mg/dL), RF (present and absent, +/-), and bone erosion (+/-). The numbers of RA patients with an ESR level ≥30 and <30 mm/h was 180 (60.81%) and 116 (39.19%), respectively. The number of patients with a CRP value ≥0.5 and <0.5 mg/dL was 209 (70.61%) and 87 (29.39%), respectively. The number of patients with RF present and absent was 258 (87.16%) and 38 (12.84%), respectively. The number of patients with bone erosion present and absent was 139 (46.96%) and 157 (53.04 %), respectively.

Table 1. Clinical and demographic features of the rheumatoid arthritis (RA) and control subjects.

	RA (N = 296)	Control (N = 493)
Age (years, means ± SD)	49.80 ± 12.96	43.59 ± 12.81
Gender (male/female)	58/238	213/280
ESR (mm/h, means ± SD)	41.55 ± 29.65	
CRP (mg/dL, means ± SD)	2.27 ± 4.86	
Subgroups		
ESR (N, ≥30/<30 mm/h)	180/116	
CRP (N, ≥0.5/<0.5 mg/dL)	209/87	
RF (N, +/-)	258/38	
Bone erosion (N, +/-)	139/157	

SD = standard deviation; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; RF = rheumatoid factor. RA patients with inappropriate clinical data were excluded.

Genetic association of *RDX* SNPs in RA

Two SNPs (rs11213326 and rs12575162) of the *RDX* gene were polymorphic, and the genotype distributions of the SNPs were in HWE (P > 0.05; data not shown). The power of the sample size was calculated using a genetic power calculator (http://pngu.mgh.harvard.edu/~purcell/gpc/cc2.html). The sample powers of the SNPs were 0.996 (rs2228261, number of effective samples for 80% power = 103) and 0.996 (rs2292305, number of effective samples for 80% power = 104), respectively ($\alpha = 0.05$, genotype relative risk = 2-fold). Thus, we had statistical confidence in our results. We first accessed the relationship between the 2 examined SNPs and case-control samples. However, no significant differences were observed in genotypic and allelic frequencies between the 2 SNPs and RA susceptibility after Bonferroni correction (Table 2).

Table 2. Genotype and allele frequencies of *RDX* SNPs in rheumatoid arthritis (RA) and control' subjects.

SNP	Genotype/Allele	RA		Control		Model	OR	95%CI		P	P^c
		Freq.	%	Freq.	%			LCL	UCL		
rs11213326	CC	72	25.5	146	31.5	Co-dominant	1.34	0.92	1.94	0.25	0.5
	CT	150	53.2	230	49.6		1.38	0.87	2.19	0.14	0.28
	TT	60	21.3	88	19	Dominant	1.35	0.95	1.93	0.1	0.19
						Recessive	1.14	0.77	1.7	0.5	1
	C	294	52	522	56						
	T	270	48	406	44		1.18	0.96	1.46	0.12	0.24
rs12575162	AA	71	24.9	156	32.3	Co-dominant	1.43	0.99	2.07	0.1	0.2
	AG	153	53.7	237	49.1		1.5	0.95	2.36	0.06	0.12
	GG	61	21.4	90	18.6	Dominant	1.45	1.03	2.06	0.03	0.07
						Recessive	1.19	0.81	1.75	0.38	0.76
	A	295	52	549	57						
	G	275	48	417	43		1.23	1	1.51	0.05	0.11

SNP = singe nucleotide polymorphism; Freq. = frequency; OR = odds ratio; CI = confidence intervals; LCL = lower confidence limit; UCL = upper confidence limit; P^c = P value corrected by the Bonferroni's method. Total numbers of genotypes and alleles in each SNP are different, because the unclear or missing genotype data are excluded.

Next, we analyzed the association between the examined SNPs and clinical features of RA patients, including ESR, CRP, RF, and bone erosion. As shown in Table 3, the genotype frequency of rs11213326 was significantly associated with ESR in the co-dominant model (OR = 1.19, 95%CI = 0.66-2.14, P^c = 0.004; OR = 3.70, 95%CI = 1.62-8.45, P^c = 0.002) and recessive model (OR = 3.29, 95%CI = 1.61-6.73, P^c = 0.001). The allele frequency of rs11213326 was also associated with the ESR value (OR = 1.60, 95%CI = 1.13-2.25, P^c = 0.016). The T allele frequency of rs12575162 was higher in the ESR ≥30 group of RA patients (52.3%) than in the ESR level <30 group of RA patients (40.7%). In addition, in the analysis of rs12575162, the SNP was significantly associated with the ESR level in the co-dominant model (OR = 1.11, 95%CI = 0.61-2, P = 0.002; OR = 3.76, 95%CI = 1.66-8.56, P^c = 0.002) and recessive model (OR = 3.50, 95% CI = 1.72-7.12, P^c = 0.004). The allele frequency of rs12575162 was also associated with the ESR level (OR = 1.595, 95%CI = 1.13-2.25, P^c = 0.016). The G allele frequency of rs12575162 was higher in the ESR ≥30 group of RA patients (53.6%) than in the ESR <30 group of RA patients (41.1%). We also investigated the relationship between SNPs and other clinical features of RA patients, including CRP level, RF (+/-) and bone erosion (+/-). However, no significant differences in these markers were observed (data not shown).

We compared our genotype frequencies with the human SNP database (http://www.ncbi.nlm.nih.gov/SNP, dbSNP Build 137) to identify ethnic similarities and differences.

This database shows the following genotype frequencies: for rs11213326, C/C:C/T:T/T, European 0.142:0.558:0.301; Chinese 0.442:0.279:0.279; Japanese 0.346:0.488:0.167; Sub-Saharan African 0.107:0.455:0.438, and for rs12575162, A/A:A/G:G/G, European 0.142:0.566:0.292; Chinese 0.452:0.310:0.238; Japanese 0.353:0.518:0.129; Sub-Saharan African 0.301:0.513:0.186 (Table 4). The rs11213326 genotype distributions of the control group were similar to those of Asian populations, particularly those of Japan. In addition, the rs12575162 genotype frequency was similar to those of sub-Saharan African populations.

Table 3. Genotype and allele frequencies of SNP between erythrocyte sedimentation rate in rheumatoid arthritis (RA) group.

SNP	Genotype/allele	ESR				Model	OR	95%CI		P	P^c
		<30 mm/h		≥30 mm/h				LCL	UCL		
		Freq.	%	Freq.	%						
rs11213326	CC	32	29.6	40	23.0	Co-dominant	1.19	0.66	2.14	0.002	0.004
	CT	64	59.3	86	49.4		3.70	1.62	8.45	0.001	0.002
	TT	12	11.1	48	27.6	Dominant	1.56	0.88	2.75	0.13	0.26
						Recessive	3.29	1.61	6.73	0.001	0.001
	C	128	59.3	166	47.7						
	T	88	40.7	182	52.3		1.60	1.13	2.25	0.008	0.016
rs12575162	AA	32	28.6	39	22.5	Co-dominant	1.11	0.61	2	0.001	0.002
	AG	68	60.7	85	49.1		3.76	1.66	8.56	0.001	0.002
	GG	12	10.7	49	28.3	Dominant	1.49	0.84	2.63	0.17	0.340
						Recessive	3.50	1.72	7.12	0.002	0.004
	A	132	58.9	163	46.4						
	G	92	41.1	188	53.6		1.61	1.15	2.26	0.006	0.012

ESR = erythrocyte sedimentation rate; SNP = singe nucleotide polymorphism; Freq. = frequency; OR = odds ratio; CI = confidence intervals; LCL = lower confidence limit; UCL = upper confidence limit; P^c = P value corrected by the Bonferroni's method. Total numbers of genotypes and alleles in each SNP are different, because the unclear or missing genotype data are excluded.

Table 4. Genotype frequency of *RDX* SNPs in each population.

SNP	Genotype	RA	Control	European	Chinese	Japanese	Sub-Saharan African
rs11213326	CC	0.255	0.315	0.142	0.442	0.345	0.107
	CT	0.532	0.496	0.558	0.279	0.488	0.455
	TT	0.213	0.190	0.301	0.279	0.167	0.438
P				<0.001	<0.001	0.233	<0.001
rs12575162	AA	0.249	0.323	0.142	0.452	0.353	0.301
	AG	0.537	0.491	0.566	0.310	0.518	0.513
	GG	0.214	0.186	0.292	0.238	0.129	0.186
P				<0.001	<0.001	0.002	0.533

RA = rheumatoid arthritis; SNP = single nucleotide polymorphism.

DISCUSSION

Increased phosphorylation of ERM proteins in synovial tissues and FLS from RA patients are involved in the modulation of TNF-α- or IL-1β-induced proliferation of RA-FLS (Huang et al., 2011). Although the mechanisms of ERM protein phosphorylation in RA-FLS in other cell lines, including endothelial cells and epithelial cells, have not yet been described, phosphorylation of ERM proteins is regulated by many stimuli, including TNF-α and IL-1β (Chen et al., 2001; Rozenblatt-Rosen et al., 2002; Kishore et al., 2005). In RA-FLS, ERM proteins are activated in a dose- and time-dependent manner by TNF-α, IL-1β, or platelet-derived growth factor (PDGF), which are critical cytokines in RA pathogenesis, suggesting that phosphorylation of ERM mediates various cytokine-induced signal transducing events in RA-FLS (Huang et al., 2011). RDX is part of a cytoskeleton linker protein family, ERM, which can connect transmembrane proteins to the actin cytoskeleton, thus promoting cell functions involving a dynamic cytoskeleton such as morphological changes, cell division, and migration (Paglini et al., 1998; Matsui et al., 1999). Thus, we hypothesized that RDX is involved in the pathophysiology of RA.

In our case-control association study, SNPs of the *RDX* gene were not associated with susceptibility to RA in a Korean population. However, our analysis of clinical features showed that 2 SNPs of the *RDX* gene were significantly associated with ESR levels. In particular, the TT genotype and the T allele of rs11213326 were associated with a relatively high ESR level (≥30). The GG genotype and the G allele of rs12575162 were also associated with a relatively high ESR level (≥30). The ESR is an indicator of inflammation; high levels of activity are more often present in patients with severe inflammation. Therefore, RA patients with the T allele of rs11213326 and the G allele of rs12575162 may show more severe inflammation than patients without these alleles. Based on our results, we hypothesize that the genotype of *RDX* SNPs should be further examined for developing novel targets for the treatment of RA.

We identified an association between the *RDX* gene and RA susceptibility and its clinical features; however, replication studies are needed to verify the association between the *RDX* gene and RA. In addition, because of the relatively small number of subjects, our findings are preliminary, and thus should be validated in further studies with larger sample sizes such as a cohort study. Nevertheless, our results support the hypothesis that the *RDX* gene plays a role in the pathophysiology of RA.

ACKNOWLEDGMENTS

Research supported by the Soonchunhyang University Research Fund.

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