Replication of European Rheumatoid Arthritis Loci in a Pakistani Population

SYED FAZAL JALIL, ATTYA BHATTI, F. YESIM DEMIRCI, XINGBIN WANG, ILTAF AHMED, MUSHTAQ AHMED, M. MICHAEL BARMADA, JAVAID M. MALIK, PETER JOHN, and M. ILYAS KAMBOH

ABSTRACT. ***Objective.*** Genetic studies have identified several rheumatoid arthritis (RA) susceptibility loci in European-derived populations. The same biological pathways may be involved in determining the RA risk in different population groups. We sought to replicate the association of 33 single-nucleotide polymorphisms (SNP) from 31 RA susceptibility loci confirmed among Europeans in a unique Pakistani population.

Methods. We genotyped 33 SNP in a sample of 366 Pakistanis that comprised related and unrelated cases and controls. Genotyping was performed using TaqMan assays and the results were analyzed with family case-control software.

Results. Twelve of the 33 SNP were replicated in this sample with significant p values ranging from 7.05E-06 to 3.72E-02, the most significant being the KIF5A-PIP4K2C/rs1678542 SNP.

Conclusion. Our observations suggest that a number of RA susceptibility loci and related pathways are shared across different populations. (J Rheumatol First Release Feb 1 2013; doi:10.3899/jrheum.121050)

Key Indexing Terms:
RHEUMATOID ARTHRITIS — SUSCEPTIBILITY
RELATED-UNRELATED ASSOCIATION STUDY — AUTOIMMUNE DISEASE

Rheumatoid arthritis (RA) is a chronic, systemic, inflammatory autoimmune disease affecting about 0.5%–1% of European-derived populations and 0.2%–0.3% of Asian populations[1,2]. RA is characterized by inflammation of the synovial fluid, which leads to severe pain and bone destruction. The etiology of RA is complex and is suggested to be the outcome of various environmental, genetic, and hormonal factors[3,4]. RA has a strong genetic basis with estimated heritability ranging from 50% to 60%[5,6,7].

Since 2000, many studies have been conducted toward understanding genetic susceptibility to RA. Genome-wide association studies (GWAS) and their metaanalyses have confirmed more than 30 risk loci for RA, including HLA-DRB1, PTPN22, CD40, STAT4, OLIG3, TNFAIP3, TNFRSF14, CTLA4, CCL2, PADI4, CD2, CD58, FCGR2A, PTPRC, REL, SPRED2, AFF3, CD28, IL2, IL21, C5orf30, IRF5, CCL21, TRAF6, CCR6, CD40, and TRAF1/C5[8,9,10,11,12,13,14,15,16,17,18]. Many initial genome-wide significant associations have been replicated in subsequent studies, with few exceptions[19], the latter probably due to the complex etiology of RA.

We hypothesized that genetic background for RA susceptibility is shared, at least in part, across different populations. To examine this hypothesis, we genotyped 33 SNP from 31 RA loci confirmed in a recent European GWAS metaanalysis[18], within a unique Pakistani population that previously had not been examined with respect to genetic risk for RA.

MATERIALS AND METHODS

Subjects. Two groups of subjects, a case-control sample of unrelated individuals (n = 214) and a family-based sample (n = 152), were included in our study. Blood samples and relevant information were collected from subjects recruited from different rheumatology centers located in 2 adjacent cities in Pakistan: the Military and Fauji Foundation Hospitals in Rawalpindi and the Kahota Research Laboratories Hospital in Islamabad. A total of 239 patients with RA satisfying the American College of Rheumatology (ACR) 1987 criteria[20] were enrolled. The mean age at onset of disease was 39.1 ± 13.0 years in RA cases (63% females). The control group (n = 127; 54% females) had no history of autoimmune diseases and their mean age was 41.2 ± 12.0 years. The main characteristics of RA cases and controls are given in Table 1. All subjects were recruited after providing informed consent. The study was approved by the National University of Science and Technology Review Board in Pakistan and the University of Pittsburgh Institutional Review Board in the USA.

Clinical diagnosis of patients was made through standard methods of

From the Atta-Ur-Rahman School of Applied Biosciences (ASAB), National University of Science and Technology (NUST), Islamabad, Pakistan; and Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania, USA.

Supported by the Higher Education Commission (HEC) of Pakistan and by US National Institutes of Health grant HL092397.

S.F. Jalil, MS, PhD Student; A. Bhatti, PhD; I. Ahmed, MS, PhD Student; P. John, PhD, Atta-Ur-Rahman School of Applied Biosciences, National University of Science and Technology; F.Y. Demirci, MD; X. Wang, PhD; M.M. Barmada, PhD; M.I. Kamboh, PhD, Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh; M. Ahmed, MD, Rheumatology Unit, Rawalpindi Military Hospital; J.M. Malik, MD, Rahmat Noor Clinic and Arthritis Research Center, Rawalpindi, Pakistan.

Address correspondence to Prof. M.I. Kamboh, Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA 15261, USA. E-mail: kamboh@pitt.edu

Accepted for publication December 4, 2012.

Table 1. Sample characteristics of patients with rheumatoid arthritis and controls.

Characteristics	Cases, n = 239	Controls, n = 127
Related individuals, n	69	83
Unrelated individuals, n	170	44
Mean age, yrs (± SD)	39.1 ± 13.0	41.2 ± 12.0
Female, %	63	54
Rheumatoid factor seropositive, %	100	0

physical examination and review of medical screening test results. All participants were interviewed and a screening questionnaire for each was filled under the supervision of a certified rheumatologist. Subjects having multiple affected individuals in their family were classified as having familial RA. The pedigrees of families with multiple patients with RA were constructed using a standard method[21].

Genomic DNA was extracted from whole blood using either the standard phenol/chloroform method or the Qiagen genomic DNA extraction kit, following the manufacturer's instructions (Qiagen Inc.). DNA was quantified using the Quant-iT^{TM} PicoGreen® dsDNA assay kit (Life Technologies).

Genotyping. A total of 33 single nucleotide polymorphisms (SNP) from 31 confirmed RA risk loci (26 previously known and 7 newly identified) were selected from a previous GWAS metaanalysis performed in individuals of European ancestry[18]. Detailed information about these SNP is given in Table 2. All SNP were genotyped using the TaqMan SNP genotyping assays (Applied Biosystems) following the manufacturer's protocols. TaqMan assay was not available for one of the SNP initially selected (HLA-DRB1/rs6910071), therefore another significant SNP (rs660895)[22] was evaluated from this locus for association analysis. PCR amplification was performed in 384-well plates on dual-block GeneAmp® PCR System 9700 (Applied Biosystems) and plates were read and analyzed on ABI-Prism 7900HT sequence detection systems.

Statistical analysis. Alleles and genotype frequencies were calculated through allele-counting methods, and deviations from Hardy-Weinberg equilibrium (HWE) were tested using the chi-square goodness-of-fit test. The family-based samples were examined for Mendelian inconsistencies in the pedigree data using the PedChek program[23] (Website: http://watson.hgen.pitt.edu). Associations of all SNP with RA were tested in the combined set of both unrelated case-control and family-based samples using the family case-control (FamCC) software Ver 1.0[24], which is a unified analysis approach based on principal component for family and unrelated samples. Specifically, the FamCC consists of 3 sequential steps: (1) principal components are generated from the genotype data; (2) multiple linear regression on the top 10 principal components is performed for both the phenotypes and markers for the unrelated individuals, respectively; and (3) the residuals of the phenotypes and the markers are calculated based on the estimated coefficients in the linear mode in the second step, and then association between the phenotype and genotype is assessed by testing the correlations between these residuals using the following statistics:

$$S^2 = \frac{T^2}{Var(T)}$$

where $T = \sum_{i=1}^{N} T_i = \sum_{i=1}^{N_f} T_i + \sum_{i=1}^{N_c+N_d} T_i$ and N_f is the number of nuclear families; N_c and N_d are the number of unrelated controls and cases, respectively. T_i, the statistic of the ith family, is defined by $T_i = \frac{1}{k_i} \sum_{j=1}^{k_i} g_{ij}^* y_{ij}^*$, k_i is the number of individuals of ith family. For unrelated individuals it is 1. The variance of T is defined by $Var(T) = \frac{N_f}{N_f-1} \sum_{i=1}^{N_f} (T_i - \overline{T}_2)^2 + \frac{N_c+N_d}{N_c+N_d-1} \sum_{i=1}^{N_c+N_d} (T_i - \overline{T}_1)^2$. $\overline{T}_1$ and $\overline{T}_2$ are the mean of T_i of unrelated and family datasets, respectively. A nominal p value < 0.05 was considered statistically significant.

RESULTS

A total of 33 successfully genotyped SNP corresponding to 31 confirmed RA susceptibility loci in Europeans were analyzed in a Pakistani sample comprising 366 RA cases and controls that included 214 unrelated and 152 related individuals. The genotyping call rate was > 98% for all SNP. Genotyping error was estimated by repeating 10% of the samples and the discrepancy rate was found to be 0% for all but 2 SNP (RBPJ/rs874040 and SPRED2/rs934734) for which the error rate was 0.031%. All SNP were found to be in HWE in the unrelated case-control sample; similarly, no Mendelian inconsistency was found in the family-based sample. We combined both unrelated case-control and family-based data to obtain a more effective population size. FamCC, a unified method for unrelated case-controls and family-based samples, was used for the association analysis. The association results and p values for 33 evaluated SNP are shown in Table 3. Of the 33 SNP confirmed among Europeans, we successfully replicated 12 SNP in our combined sample ($p < 0.05$) with the same allele and same direction of association. The most significant SNP was KIF5A-PIP4K2C/rs1678542 (p = 7.05E-06), followed by CD2-CD58/rs11586238 (p = 4.56E-05), TNFAIP3/rs10499194 (p = 2.51E-04), and HLA-DRB1/rs660895 (p = 3.78E-04). Marginal associations with 4 additional SNP (p = 5.70E-02 to 9.61E-02) and possible associations with 7 SNP (p = 1.06E-01 to 2.91E-01) were also observed showing the same trend for association as previously reported.

DISCUSSION

Recent GWAS conducted mainly in European and American populations have identified or confirmed a number of risk loci for RA. Replication studies of known loci across various ethnic groups can indicate future directions by the identification of population-specific RA susceptibility loci/genes. Stahl, *et al*[18] conducted a metaanalysis of 6 GWAS involving a total of 5539 RA cases and 20,169 controls of European descent, followed by replication in 6768 RA cases and 8806 controls (totaling 41,282 samples). They not only confirmed the 24 known RA loci (26 SNP) but also identified 7 new risk loci (genome-wide significance in the combined sample), 4 of which were previously implicated in other autoimmune diseases, while 3 were new RA and autoimmune risk loci.

To our knowledge, the genetic factors responsible for RA in Pakistanis have not been examined before. Pakistanis, along with North Indians, are believed to have considerable white ancestry[25,26,27]. Thus, we hypothesized that most, if not all, RA loci identified in white or white-derived populations might also be responsible for RA in Pakistanis. To test this hypothesis, we genotyped 33 SNP from 31 loci

Table 2. Thirty-one rheumatoid arthritis (RA) risk loci previously confirmed in individuals of European ancestry and the TaqMan assays used for genotyping in our sample (all single-nucleotide polymorphisms were selected from Stahl, *et al*[18] except for one* derived from Arya, *et al*[22]).

Sample	Ref SNP ID	Assay ID	Assay Type	Locus	Gene	Position	Alleles VIC/ FAM	Location
1	rs3890745	C__26678889_10	Premade	1p36	*TNFRSF14*	2553624	T/C	Intergenic/ unknown
2	rs2476601	C__16021387_20	Premade	1p13	*PTPN22*	114377568	A/G	Intergenic/ unknown
3	rs11586238	C__2820555_10	Premade	1p13	*CD2-CD58*	117263138	C/G	Intergenic/ unknown
4	rs12746613	C__1118952_10	Premade	1q23	*FCGR2A*	161467042	C/T	Intergenic/ unknown
5	rs10919563	C__31565763_10	Premade	1q31	*PTPRC*	198700442	A/G	Intron
6	rs13031237	C__3219755_10	Premade	2p16	*REL*	61136129	G/T	Intron
7	rs934734	C__3227346_10	Premade	2p14	*SPRED2*	65595586	A/G	Intron
8	rs10865035	C__2099360_10	Premade	2q11	*AFF3*	100835734	A/G	Intergenic/ unknown
9	rs7574865	C__29882391_10	Premade	2q32	*STAT4*	191964633	G/T	Intron
10	rs1980422	C__11459546_10	Premade	2q33	*CD28*	204610396	C/T	Intergenic/unknown
11	rs3087243	C__3296043_10	Premade	2q33	*CTLA4*	204738919	A/G	Intergenic/unknown
12	rs13315591	C__32002440_10	Premade	3p14	*PXK*	58556841	A/C	Intron
13	rs874040	C__8285908_10	Premade	4p15	*RBPJ*	26108197	C/G	Intergenic/unknown
14	rs6822844	C__28983601_10	Premade	4q27	*IL2-IL21*	123509421	G/T	Intergenic/unknown
15	rs26232	C__2388799_20	Premade	5q21	*C5orf30*	102596720	C/T	Intron
16	rs6859219	C__3215520_10	Premade	5q11	*ANKRD55-IL6ST*	55438580	A/C	Intron
17	rs660895*	C_26546458-30	Premade	6p21	*HLA-DRB1*	3277380	A/G	Intergenic/unknown
18	rs6910071**	NA	NA	6p21	*HLA-DRB1*	32282855	A/G	Intron
19	rs548234	C__14436_10	Premade	6q21	*PRDM1*	106568034	C/T	Intergenic/unknown
20	rs10499194	C__1575581_10	Premade	6q23	*TNFAIP3*	138002637	C/T	Intergenic/unknown
21	rs6920220	C__29431952_10	Premade	6q23	*TNFAIP3*	138006504	A/G	Intergenic/unknown
22	rs5029937	C__27440189_10	Premade	6q23	*TNFAIP3*	138195151	G/T	Intron
23	rs394581	C__2966112_10	Premade	6q25	*TAGAP*	159482521	C/T	Intergenic/unknown
24	rs3093023	C__27496690_10	Premade	6q27	*CCR6*	167534290	A/G	Intron
25	rs10488631	C__2691242_10	Premade	7q32	*IRF5*	128594183	C/T	Intergenic/unknown
26	rs2736340	C__1886931_10	Premade	8p23	*BLK*	11343973	C/T	Intergenic/unknown
27	rs2812378	C__16113556_10	Premade	9p13	*CCL21*	34710260	A/G	Intergenic/unknown
28	rs3761847	C__2783640_10	Premade	9q33	*TRAF1-C5*	123690239	A/G	Intergenic/unknown
29	rs2104286	C__16095542_10	Premade	10p15	*IL2RA*	6099045	C/T	Intergenic/unknown
30	rs4750316	C__32117918_10	Premade	10p15	*PRKCQ*	6393260	C/G	Intergenic/unknown
31	rs540386	C__2408956_10	Premade	11p12	*TRAF6*	36525293	C/T	Intron
32	rs1678542	C__7529251_30	Premade	12q13	*KIF5A-PIP4K2C*	57968715	C/G	Intron
33	rs4810485	C__1260190_10	Premade	20q13	*CD40*	44747947	G/T	Intron
34	rs3218253	C__27917605_10	Premade	22q12	*IL2RB*	37544810	A/G	Intron

** No premade assay was available.

confirmed among Europeans in a Pakistani case-control and family-based sample.

When we analyzed our case-control and family-based samples separately we found limited associations in each group (data not shown), which were most likely due to relatively small sample sizes in each group. Therefore, to achieve a more effective sample size and increase the power, we combined the 2 groups (unrelated case-control and family-based samples) and analyzed the data using the family case-control (FamCC) method[24]. Unlike other statistical approaches, FamCC is a unified method that relies on principal components generated from genotype data, utilizing both unrelated case-control and family-based data simultaneously without any population stratification to test an alternative hypothesis of association only[24,28]. This method revealed robust replications for 12 of the 33 SNP examined, with p values ranging from 7.05E-06 to 3.72E-02. Because associations in these 12 genes

Table 3. Replication of RA risk loci, previously confirmed in Europeans, among Pakistanis. Values in bold type identify significance at p ≤ 0.05.

Ref SNP ID	Locus	Gene	Chromosome Position	MAF	p
rs1678542	12q13	*KIF5A- PIP4K2C*	57968715	0.42	**7.05E-06**
rs11586238	1p13	*CD2- CD58*	117263138	0.28	**4.56E-05**
rs10499194	6q23	*TNFAIP3*	138002637	0.21	**2.51E-04**
rs660895	6p21	*HLA-DRB1*	32577380	0.08	**3.78E-04**
rs7574865	2q32	*STAT4*	191964633	0.31	**4.25E-04**
rs10488631	7q32	*IRF5*	128594183	0.18	**3.76E-03**
rs13031237	2p16	*REL*	61136129	0.10	**5.67E-03**
rs6859219	5q11	*ANKRD55- IL6ST*	55438580	0.11	**6.34E-03**
rs540386	11p12	*TRAF6*	36525293	0.14	**1.07E-02**
rs394581	6q25	*TAGAP*	159482521	0.19	**1.53E-02**
rs26232	5q21	*C5orf30*	102596720	0.17	**2.90E-02**
rs2736340	8p23	*BLK*	11343973	0.35	**3.72E-02**
rs6920220	6q23	*TNFAIP3*	138006504	0.14	5.70E-02
rs548234	6q21	*PRDM1*	106568034	0.21	6.95E-02
rs2812378	9p13	*CCL21*	34710260	0.35	7.35E-02
rs1980422	2q33	*CD28*	204610396	0.12	9.61E-02
rs4750316	10p15	*PRKCQ*	6393260	0.17	1.06E-01
rs10865035	2q11	*AFF3*	100835734	0.50	1.17E-01
rs874040	4p15	*RBPJ*	26108197	0.18	2.19E-01
rs3761847	9q33	*TRAF1- C5*	123690239	0.29	2.53E-01
rs6822844	4q27	*IL2-IL21*	123509421	0.10	2.61E-01
rs2476601	1p13	*PTPN22*	114377568	0.01	2.75E-01
rs3087243	2q33	*CTLA4*	204738919	0.47	2.91E-01
rs5029937	6q23	*TNFAIP3*	138195151	0.03	3.31E-01
rs3890745	1p36	*TNFRSF14*	2553624	0.42	4.12E-01
rs3093023	6q27	*CCR6*	167534290	0.35	4.48E-01
rs2104286	10p15	*IL2RA*	6099045	0.13	7.89E-01
rs10919563	1q31	*PTPRC*	198700442	0.21	8.08E-01
rs934734	2p14	*SPRED2*	65595586	0.36	8.35E-01
rs12746613	1q23	*FCGR2A*	161467042	0.14	8.44E-01
rs4810485	20q13	*CD40*	44747947	0.20	8.52E-01
rs3218253	22q12	*IL2RB*	37544810	0.17	9.38E-01
rs13315591	3p14	*PXK*	58556841	0.07	9.98E-01

MAF: minor allele frequency.

(KIF5A-PIP4K2C, CD2-CD58, TNFAIP3, HLA-DRB1, STAT4, IRF5, REL, ANKRD55-IL6ST, TRAF6, TAGAP, C5orf30, BLK) have been reported in multiple studies, it is acceptable to consider $p < 0.05$ statistically significant in followup studies[29]. In addition to these 12 SNP, several others showed the same trend of association, albeit not significant at the 5% level.

Recently, Prasad, *et al*[30] performed a replication study of white RA loci in about 2000 North Indian RA cases and controls, and reported replication of only 7 loci (7 SNP) of the 32 loci (35 SNP) tested, by using either index or surrogate SNP. They were able to identify additional associations with 10 more loci after including additional SNP in the analysis. A comparison of the replication of the 31 loci (33 SNP) examined in our study versus Prasad's study is given in Table 4. Of the 31 loci examined in our study, 29

Table 4. Comparison of association results for 31 loci/33 single-nucleotide polymorphisms (SNP) genotyped in our study with results from the same loci in North Indian subjects[30]. Values in bold type identify significance at p ≤0.05.

Gene	SNP	p Values, Current Study	Prasad, *et al* Study Data[30]				
			Status and p Value	Surrogate SNP	p	Additional SNP	p
CD28	rs1980422	9.61E-02	NA	rs3116496	0.63	rs4675367	**0.041**
SPRED2	rs934734	8.35E-01	NA	NS			
HLA-DRB1	rs660895	**3.78E-04**	**2.56E-05**	NS			
C5orf30	rs26232	**2.90E-02**	0.79	NS			
TNFRSF14	rs3890745	4.12E-01	0.161	NS			
ANKRD55-IL6T	rs6859219	**6.34E-03**	NA	NS		rs6877664, rs10214316, rs149140, rs32498	**0.016** **0.005** **0.008** **0.006**
PRKCQ	rs4750316	1.06E-01	NA	rs10796045	0.09		
CCL21	rs2812378	7.35E-02	NA	rs13293020	**0.01**		
TAGAP	rs394581	**1.53E-02**	NA	rs169858	0.15	rs926657, rs9295089, rs212402	**0.003** **0.009** **0.009**
IL2RB	rs3218253	9.38E-01	NA	NS		rs228942	**0.04**
STAT4	rs7574865	**4.25E-04**	0.33	NS			
PRDM1	rs548234	6.95E-02	0.33	NS			
PTPRC	rs10919563	8.08E-01	NA	rs1011338	0.60	rs9803750, rs2359952	**4.3 ×10-4** **1.6 ×10-4**
BLK	rs2736340	**3.72E-02**	0.12	NS		rs4841548, rs17806523	**0.03** **0.009**
AFF3	rs1086505	1.17E-01	NA	NS		rs17023158, rs6706188, rs1437377	**0.005** **0.005** **0.003**
PTPN22	rs2476601	2.75E-01	0.76	rs1217407	**3.0 ×10-3**		
IRF5	rs10488631	**3.76E-03**	0.79	NS			
CD40	rs4810485	8.52E-01	NA	rs6074022	0.06	rs6065925	**0.005**
KIF5A-PIP4K2C	rs1678542	**7.05E-06**	NA	rs11172254	0.47		
RBPJ	rs874040	2.19E-01	NA	NS			
TNFAIP3	rs5029937	3.31E-01	NA	NS			
TNFAIP3	rs6920220	5.70E-02	NA	rs9321637	**0.04**	rs5029936	**0.03**
TNFAIP3	rs10499194	**2.51E-04**	NA	NS		rs3757173	**0.03**
IL2RA	rs2104286	7.89E-01	**1.9 ×10-4**	NS		rs12722589 rs3118470	**7.4 ×10-5** **2.0 ×10-3**
REL	rs13031237	**5.67E-03**	0.42	NS			
PXK	rs13315591	9.98E-01	NA	NS		rs7622074 rs6767498	**0.011** **0.005**
FCGR2A	rs12746613	8.44E-01	NA	rs12722986	0.84		
TRAF1-C5	rs3761847	2.53E-01	0.95	NS			
CCR6	rs3093023	4.48E-01	0.07	NA		rs1331301 rs1556413	**0.01** **0.02**
IL2-IL21	rs6822844	2.61E-01	NA	rs13119723	**0.008**		
CTLA4	rs3087243	2.91E-01	NA	rs231804	0.18	rs231726 rs10197319	**0.03** **0.03**
CD2- CD58	rs11586238	**4.56E-05**	NA	rs12405671	0.21		
TRAF6	rs540386	**1.07E-02**	NA	rs1046864	0.3		

NA: index SNP not included in the North Indian study SNP array; NS: no surrogate SNP included.

were also tested by Prasad, *et al*[30] that included either the index or a surrogate SNP or additional SNP in a given gene region. Based on this comparison, 17 of the 29 regions (58%) were replicated in the North Indian sample, slightly higher than the 38% replication rate (12/31) in our study, probably because additional SNP at certain loci were examined in the North Indian study, and their sample size was also larger than ours. Although many of the most closely associated signals in the North Indian sample are different from the index SNP reported in whites, the presence of other significant SNP in the same regions suggests the relevance of these gene regions in RA in multiple ethnicities. This is further confirmed in a recent multiancestry comparative analysis of GWAS data, in which 22 of the 40 European SNP (55%) were replicated in Japanese[31]. These data show that many of the RA loci identified to date among European or European-derived populations also confer RA risk in other population groups, including Pakistanis. It is also very likely that Pakistanis may have novel RA variants and/or loci, which could be detected only by whole-genome studies.

We have replicated 12 of the 31 European RA loci in Pakistanis, and our findings provide evidence that diverse populations share genetic susceptibility to RA. A limitation of our study was the relatively small size of our RA sample, and future studies using larger samples are needed to determine the genetic basis of RA in the Pakistani population. Despite the small sample size, however, we were able to replicate a number of reported significant signals in this unique population, which previously has not been characterized regarding genetic susceptibility to RA.

ACKNOWLEDGMENT

We thank all the physicians and rheumatologists for referring patients, and their supporting staff for collection of blood samples. We thank all the patients and family members for their participation and cooperation. We also acknowledge the control individuals who provided blood samples. We appreciate Dr. X. Zhu and Dr. T. Feng for their kindness in providing the FamCC software.

REFERENCES

1. Silman AJ, Pearson JE. Epidemiology and genetics of rheumatoid arthritis. Arthritis Res 2002;4:S265-7.
2. Teng E, Leong KP, Li HH, Thong B, Koh ET, Loi PL, et al; TTSH RA Study Group. Analysis of a genome-wide association study-linked locus (CCR6) in Asian rheumatoid arthritis. DNA Cell Biol 2012;31:607-10.
3. Firestein GS. Evolving concepts of rheumatoid arthritis. Nature 2003;423:356–61.
4. Klareskog L, Stolt P, Lundberg K, Källberg H, Bengtsson C, Grunewald J, et al. A new model for an etiology of rheumatoid arthritis: Smoking may trigger HLADR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination. Arthritis Rheum 2006;54:38–46.
5. Seldin MF, Amos CI, Ward R, Gregersen PK. The genetics revolution and the assault on rheumatoid arthritis. Arthritis Rheum 1999;42:1071–9.
6. MacGregor AJ, Snieder H, Rigby AS, Koskenvuo M, Kaprio J, Aho K, et al. Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. Arthritis Rheum 2000;43:30–37.
7. Bax M, Heemst JV, Huizinga TWJ, Toes REM. Genetics of rheumatoid arthritis: what have we learned. Immunogenetics 2011;63:459–66.
8. Suzuki A, Yamada R, Chang X, Tokuhiro S, Sawada T, Suzuki M, et al. Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. Nat Genet 2003;34:395–402.
9. Begovich AB, Carlton VE, Honigberg LA, Schrodi SJ, Chokkalingam AP, Alexander HC, et al. A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. Am J Hum Genet 2004;75:330–7.
10. Kurreeman FA, Padyukov L, Marques RB, Schrodi SJ, Seddighzadeh M, Stoeken-Rijsbergen G, et al. A candidate gene approach identifies the TRAF1/C5 region as a risk factor for rheumatoid arthritis. PLoS Med 2007;4:e278.
11. Plenge RM, Cotsapas C, Davies L, Price AL, de Bakker PI, Maller J, et al. Two independent alleles at 6q23 associated with risk of rheumatoid arthritis. Nat Genet 2007;39:1477-82.
12. Plenge RM, Seielstad M, Padyukov L, Lee AT, Remmers EF, Ding B, et al. TRAF1-C5 as a risk locus for rheumatoid arthritis — A genomewide study. N Engl J Med 2007;357:1199-209.
13. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;447:661-78.
14. Julià A, Ballina J, Cañete J, Balsa A, Tornero Molina J, Naranjo A, et al. Genome wide association study of rheumatoid arthritis in the Spanish population: KLF12 as a risk locus for rheumatoid arthritis susceptibility. Arthritis Rheum 2008;58:2275-86.
15. Raychaudhuri S, Remmers E, Lee A, Hackett R, Guiducci C, Burtt N, et al. Common variants at CD40 and other loci confer risk of rheumatoid arthritis. Nat Genet 2008;40:1216-23.
16. Gregersen PK, Amos CI, Lee AT, Lu Y, Remmers EF, Kastner DL, et al. REL: Encoding a member of the NF-kappa B family of transcription factors, is a newly defined risk locus for rheumatoid arthritis. Nat Genet 2009;41:820-3.
17. Kochi Y, Okada Y, Suzuki A, Ikari K, Terao C, Takahashi A, et al. A regulatory variant in CCR6 is associated with rheumatoid arthritis susceptibility. Nat Genet 2010;42:515-9.
18. Stahl EA, Raychaudhuri S, Remmers EF, Xie G, Eyre S, Thomson BP, et al. Genome-wide association study meta-analysis identifies seven new rheumatoid arthritis risk loci. Nat Genet 2010; 42:508-14.
19. Hu HJ, Jin EH, Yim SH, Yang SY, Jung SH, Shin SH, et al. Common variants at the promoter region of the APOM confer a risk of rheumatoid arthritis. Exp Mol Med 2011;43:613-21.
20. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
21. Bennet RL, Steinhaus KA, Uhrich SB, O'Sullivan CK, Resta RG, Lochner-Doyle D, et al. Recommendations for standardized human pedigree nomenclature: Pedigree Standardization Task Force of the National Society of Genetic Counselors. Am J Hum Genet 1995;56:745-52.
22. Arya R, Hare E, Del Rincon I, Jenkison CP, Duggirala R, Almasy LE. Effects of covariates and interactions on a genome-wide association analysis of rheumatoid arthritis. BMC Proc 2009;3:S84.
23. O'Connell JR, Weeks DE. PedCheck: A program for identifying genotype incompatibilities in linkage analysis. Am J Hum Genet 1998;63:259-66.
24. Zhu X, Li S, Cooper RS, Elston RC. A unified association analysis

approach for family and unrelated samples correcting for stratification. Am J Hum Genet 2008;82:352–65.
25. Quintana-Murci L, Chaix R, Wells RS, Behar DM, Sayar H, Scozzari R, et al. Where west meets east: The complex mtDNA landscape of the southwest and central Asian corridor. Am J Hum Genet 2004;74:827–45.
26. Rosenberg NA, Mahajan S, Ramachandran S, Zhao C, Pritchard JK, Feldman MW. Clines, clusters, and the effect of study design on the inference of human population structure. PLoS Genet 2005;1:e70.
27. Indian Genome Variation Consortium. Genetic landscape of the people of India: a canvas for disease gene exploration. J Genet 2008;87:3–20.
28. Sun X, Feng T, Song Y, Elston RC, Zhu X. Comparison of a unified analysis approach for family and unrelated samples with the transmission-disequilibrium test to study associations of hypertension in the Framingham Heart Study. BMC Proc 2009;3:S22.
29. Plenge RM, Bridges SL Jr, Huizinga TW, Criswell LA, Gregersen PK. Recommendations for publication of genetic association studies in Arthritis & Rheumatism. Arthritis Rheum 2011; 63:2839-47.
30. Prasad P, Kumar A, Gupta R, Juyal RC, Thelma BK. Caucasian and Asian specific Rheumatoid Arthritis risk loci reveal limited replication and apparent allelic heterogeneity in North Indians. PLoS One 2012;7:e31584.
31. Okada Y, Terao C, Ikari K, Kochi Y, Ohmura K, Suzuki A, et al. Meta analysis identifies nine new loci associated with rheumatoid arthritis in the Japanese population. Nat Genetics 2012;25:511-6.