Published in final edited form as:

Arthritis Rheum. 2009 February ; 60(2): 364–371. doi:10.1002/art.24245.

Lack of Association of Caucasian Rheumatoid Arthritis Susceptibility Loci in a Korean Population

Hye-Soon Lee^{1,2}, Benjamin D Korman³, Julie M Le³, Daniel L Kastner³, Elaine F Remmers³, Peter K Gregersen², and Sang-Cheol Bae¹

¹Hanyang University College of Medicine and the Hospital for Rheumatic Diseases, Seoul, South Korea ²The Feinstein Institute for Medical Research, North Shore Long Island Jewish Health System, Manhasset, New York ³The National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, Maryland

Abstract

Objective—Recent studies have identified a number of novel rheumatoid arthritis (RA) loci in Caucasian populations. In this study, we sought to determine whether the genetic variants at 4q27, 6q23, *CCL21*, *TRAF1/C5*, and *CD40* identified in Caucasians are also associated with RA in a Korean case-control collection. We also comprehensively evaluated the genetic variation within *PTPN22*, a well established autoimmune disease-associated gene.

Methods—We designed a Sequenom iPLEX experiment to thoroughly evaluate the *PTPN22* linkage disequilibrium region using tag SNPs and disease-associated SNPs at 5 other previously reported Caucasian RA-associated loci in 1123 RA Korean RA patients and 1008 ethnically matched controls. We also re-sequenced the *PTPN22* gene to look for novel coding variants that might be contributing to disease in this population.

Results—None of the Caucasian RA susceptibility loci contributed significantly to disease in Koreans. Tag SNPs covering the *PTPN22* linkage disequilibrium block, while polymorphic, did not reveal any disease association and re-sequencing did not identify any new common coding region variants in this population. The 6q23 and 4q27 SNPs assayed were non-polymorphic in this population and the *TRAF1/C5*, *CD40*, and *CCL21* SNPs did not show any evidence for association.

Conclusions—Caucasian and Korean rheumatoid arthritis have different genetic risk factors. While patients of different ethnic groups share the HLA region as a major genetic risk locus, most other genes shown to be significantly associated with disease in Caucasians appear not to play a role in Korean RA.

Rheumatoid arthritis (RA) is a chronic autoimmune arthritis characterized by progressive joint destruction. Both genetic and environmental factors have been shown to play a role in the development of RA. While the largest genetic risk factor predisposing to RA, a common set of alleles at *HLA-DRB1*, has been associated with RA in populations of both Caucasian and Asian ancestry (1,2), many other risk loci have been shown to confer RA susceptibility in only one ethnic group. For example, although *PTPN22* has been consistently shown to be associated with RA in Caucasians, the 620W risk allele in this gene is not found and thus not disease-associated in Asian populations (3–7). In contrast, *PADI4*, *SLC22A4*, and *FCRL3* have been associated with RA in studies of Asian patients, but have given weak or negative results in populations of European ancestry (6,8–14). While these results suggest that the genetic risk alleles that confer susceptibility to RA are heterogeneous across major ethnic groups, recent reports have identified a common *STAT4* haplotype that confers a similar degree of risk for RA in both Asian and Caucasian populations (15,16).

Outside of the HLA region, the R620W variant of the *PTPN22* gene is the most consistently and most strongly disease-associated variant identified in rheumatoid arthritis association studies performed in Caucasian populations. Although this variant is not found in Asians, it is possible that other variants within this important candidate gene could contribute to genetic susceptibility to this disease in Asian populations. In addition to *PTPN22*, several recent genetic studies have identified new RA susceptibility loci in Caucasian populations. The *TRAF1/C5* locus has been identified both by a genome-wide association study (GWAS) and as a candidate gene (7,17). Two independent GWAS follow-up studies have identified a susceptibility locus on 6q23 near *TNFAIP3* (18,19). A large meta-analysis using GWAS and replication collections has identified the *CD40* and *CCL21* loci (20), and a 4q27 region including *IL2/IL21* has been identified through a candidate gene approach after being shown to be associated with other autoimmune diseases (21). To date, there has been no study of SNPs in these new regions associated with RA in an Asian population. Therefore, the aim of this study was to determine whether *PTPN22* variants or any of five disease-associated SNPs recently identified in Caucasians also contribute to Korean RA.

PATIENTS AND METHODS

Study Population

We included 1128 Korean RA patients who were enrolled consecutively from the outpatient clinic of The Hospital for Rheumatic Diseases, Hanyang University, Seoul, South Korea and 1022 ethnically matched controls that had no history of RA and other autoimmune diseases in the self reported questionnaire at the enrollment. All RA patients met the American College of Rheumatology 1987 classification criteria for RA (22). The study was approved by the institutional review board of Hanyang University Hospital. Written informed consent was obtained from all participants. All subjects were typed for HLA-DRB1 subtypes using polymerase chain reaction and sequence-specific oligonucleotide probe hybridization.

Since the second most significant risk allele for RA was DRB1*0901 in the Korean population, we regarded DRB1*0901 as a member of the SE group in addition to the following alleles: DRB1*0101, *0102, *0401, *0404, *0405, *0408, *0413, *1001, and *1402. Among 1128 RA patients, 807 patients had CCP data that were quantitatively measured in duplicate by enzyme-linked immunosorbent assay using the DIASTAT Anti-CCP kit FCCP 200 (Axis-Shield Plc, Scotland, UK) according to the manufacturer's instructions. The staging system proposed by Steinbrocker et al (23) was used as a measure of the radiographic severity of RA.

PTPN22 Re-sequencing

Genomic DNAs from 48 Korean sero-positive (RF+) RA patients were sequenced over the regions representing the promoter region (up to -2000 bp), all 21 exons including exon-intron boundaries and 3'UTR region of *PTPN22* using Sanger dideoxy DNA sequencing technique (Polymorphic DNA Technologies, Alameda, California).

SNP selection and assay design

We used Tagger (24) to select SNPs polymorphic in the HapMap CHB/JPT population across the 400 kilobase linkage disequilibrium block that includes *PTPN22*. In addition, all variants identified from the *PTPN22* exon re-sequencing were selected. A total of 34 SNPs/variants were selected from the *PTPN22* linkage disequilibrium block. Lastly, we identified SNPs that have been reported as associated with RA in either a Caucasian (*TRAF1/C5*, 6q23, 4q27, *CD40*, and *CCL21* loci) or Asian (*PADI4*) population and selected one disease-associated SNP per region (total of 6 SNPs selected). The 40 selected SNPs were used to

design multiplex genotyping assays using Sequenom RealSNP (<http://www.realsnp.com>) and Assay Design software.

Genotyping

Multiplex PCR was used to amplify DNA products containing up to 25 SNPs in one reaction from 5 ng genomic DNA. Synthetic oligonucleotides that bind adjacent to the SNP site were then hybridized and extended with nucleotides complementary to the template SNP site using modified nucleotides that terminate the extension reaction at the interrogated SNP and generate alternate products of sufficiently different mass to be separated by mass spectrometry. The extended products were separated by MALDI-TOF mass spectrometry and the genotypes determined by SpectroTyper software (Sequenom, Inc, San Diego, California). Calls were evaluated and edited by cluster analysis performed with the SpectroTyper software version 4.0.

Data analysis

Association tests for SNPs and haplotypes in cases and controls and regional linkage disequilibrium structure for analyzed SNPs were determined using Haploview version 4.0 (25). We excluded SNPs with significant deviation from Hardy Weinberg equilibrium (P value less than 0.005) and SNPs with minor allele frequency (MAF) less than 0.01. We reanalyzed the association of all SNPs with the following specific subgroups of RA (CCP positive RA, severe erosive RA, or SE positive RA) using multivariate logistic regression with adjustments for age and sex. These subgroup analyses were performed using SAS software, version 9.1 (SAS Institute, Cary, NC).

RESULTS

Patient and control characteristics

Among the patients with RA, the mean $\pm$ SD age was 52.4 ± 11.9 years, the mean $\pm$ SD age at disease onset was 39.9 ± 11.5 years, 89.7% were female, 81.4% were positive for the SE, 17.0% showed no erosive changes (Steinbrocker stage I), and 83.0% demonstrated erosions (Steinbrocker stages II, III, and IV). Among RA patients with CCP data ($N=807$), 681 (84.4%) patients were CCP positive. The mean $\pm$ SD age of control subjects was 36.8 ± 12.5 years, and 86.3% were female.

Re-sequencing

Nineteen sequence variants (i.e., variants identified in one or more resequenced DNAs) were identified in the exonic (exons and intronic splice junction regions) and the promoter regions of *PTPN22* in the resequenced Korean DNA samples. Of these, 13 had previously been identified and 6 were novel. The novel variants were submitted to dbSNP (rs numbers to be added when available). Although the novel variants were all rare (found in only a single DNA of the 48 resequenced), they were included in the case-control genotyping. One of the novel variants was found in the promoter region, four were intronic, and one was a splice junction variant.

Genotyping

Of the 40 SNPs included in the assay design, assays for two SNPs failed (Table 1). The genotype call rate was greater than 95% for the remaining markers with the exception of rs6537798 which had a 77.7% call rate. No cases or controls were polymorphic at rs2476601 (R620W) while between 1 and 9 minor alleles were present out of 4178 alleles in the 6 novel *PTPN22* variants, the 6q23 SNP rs6920220, and the 4q27 SNP rs6822844 (Table

1 and Table 2). Additionally, the *PTPN22* tag SNP rs1018592 was not analyzed because it exhibited significant deviation from Hardy-Weinberg equilibrium.

RA association tests

No statistically significant association ($p < 0.05$) with RA was detected between any of the 24 polymorphic *PTPN22* tag SNPs (Table 1) from the linkage disequilibrium block covering the *AP4B1/C1orf178/PTPN22/RSBN1/PTHF1/MAG13* genes. In addition, Caucasian RA-associated SNPs at *TRAF1/C5*, *CD40*, and *CCL21* were found to be polymorphic in the Korean population but not to be associated with disease (Table 2). As noted above, the 4q27 and 6q23 variants were too rare in this population to be evaluated for association (Table 2). The *PADI4* SNP rs2240320 was noted to be strongly disease-associated with a p -value of 1.15×10^{-8} and with an odds ratio of 1.43 (95% confidence limits: 1.27 to 1.62) for the presence of the disease-associated T allele on case chromosomes compared with control chromosomes (Table 2). In the subgroup analyses, there were no significant genotypic or allelic associations of any of these SNPs in the radiographic severity subgroups (Steinbrocker stage 1 and stages 2–4), the SE status subgroups (SE positive and negative group), and the CCP subgroups (CCP positive and negative group) compared with controls, except for *PADI4* SNP rs2240310, which was significantly associated with RA irrespective of SE status (p -value < 0.01) as well as CCP positivity (p -value < 0.0001).

PTPN22 linkage disequilibrium

The linkage disequilibrium structure of the broad genomic region containing the *PTPN22* gene was similar in HapMap CEU and CHB+JPT individuals with a 400 kb region of strong linkage disequilibrium containing 6 genes (*MAG13*, *PTHF1*, *RSBN1*, *PTPN22*, *C1orf178*, *AP4B1*) identified in both populations (see Supplemental Figure 1). The 24 *PTPN22* tag SNPs, which passed quality control, captured with r^2 greater than 0.8, 90% of the variation (166 of 185 alleles with HapMap CHB+JPT minor allele frequency greater than 5%) in the 400 kb region. Although the linkage disequilibrium structure of the *PTPN22* gene region was similar in HapMap CEU and CHB+JPT individuals, the frequencies of the common haplotypes in these two populations were very different. Eleven of the 13 tag SNPs used for the haplotype association analysis of the 85kb *PTPN22* gene region (see below) were genotyped in the HapMap CHB+JPT and CEU samples. These 11 SNPs defined only four common haplotypes (those with greater than 5% frequency) in both populations. All four common haplotypes found in the Asian samples were also common haplotypes in the CEU samples, but the haplotype frequencies were strikingly different between the two populations (Table 3). Interestingly, the 620W autoimmune disease-associated variant is found only on a portion (approximately half) of the copies of Haplotype 1 in the CEU samples, but is not found on any of the Haplotype 1 copies in the CHB+JPT samples (Table 3).

Haplotype analysis

PTPN22 haplotypes were constructed using the 13 SNPs from Table 1 numbers 6 through 25 with minor allele frequency greater than 0.05 using Haploview software. These 13 SNPs covered the entire *PTPN22* gene region. The four common haplotypes (with frequency $> 5\%$) together accounted for 96.6% of the chromosomes of the individuals genotyped. Association tests were performed for each of these four haplotypes and none were found to be associated with disease (Table 4).

DISCUSSION

Recent whole genome association studies in European ancestry case-control collections have shown that after HLA variants, SNPs within the *PTPN22* genetic region are consistently the

second most significantly disease-associated variants in the genome (3,7). The proposed causative variant, a SNP encoding a structural variant R620W of the *PTPN22* protein, however, is not present in the Asian population. We therefore sought to determine whether other *PTPN22* alleles are associated with Asian RA. We also evaluated variants in five other loci that have strong evidence for association in Caucasian RA in a large Korean RA case-control series.

SNP variants within the *PTPN22* linkage disequilibrium block were evaluated using a tag SNP approach to interrogate the known common variants in the region. None of the 24 polymorphic SNPs covering the genomic region including *PTPN22* were associated with susceptibility to rheumatoid arthritis in this Korean population. Of note, we have confirmed that the R620W variant is non-polymorphic in this population and have shown that a promoter SNP, rs2488457, which has been reported as associated with RA in Caucasians and type 1 diabetes in Japanese (26), had no evidence for association in Korean RA. The genotyped SNPs cover 90% of the known polymorphic SNPs in the 400 kilobase *PTPN22* linkage disequilibrium block (with $r^2 > 0.8$), and none were associated with disease. Furthermore, none of the 4 common *PTPN22* haplotypes were associated with disease. Coupled with the fact that no common *PTPN22* structural variants were identified by re-sequencing, these data provide strong evidence that common *PTPN22* region genetic variants are not associated with RA in this population. This is in stark contrast to the very strong association signals seen in multiple Caucasian RA case-control studies. Although none of the common *PTPN22* variants were associated with disease, the novel rare variants are more difficult to evaluate. Interestingly, three of the novel variants identified (one promoter and two intronic) were found in a single RA case but in no controls. Further work will be required to determine whether any of the rare variants of the *PTPN22* gene might be private mutations that contribute to disease susceptibility.

In addition, this study has shown that SNPs in five additional regions (*TRAF1/C5*, 6q23, 4q27, *CD40*, and *CCL21*) that are strongly associated with RA in Caucasians are either non-polymorphic (6q23 and 4q27) or not associated with Korean RA (*TRAF1/C5*, *CD40*, and *CCL21*). For these SNPs, we can only say with certainty that the SNP associated with disease in Caucasians is not associated with disease in Koreans. Further investigation and fine mapping similar to what we have done with *PTPN22* would be needed to determine whether other polymorphic SNPs in these regions are associated with RA in Koreans. By comparison, we genotyped the *PADI4* SNP rs2240340 for which we have previously reported an association with a subset (545 cases and 392 controls) of this case-control series and found a striking association with a p-value for association of 1.15×10^{-8} in this larger collection (9).

These data make a convincing argument that the genetic risk factors for RA differ substantially between Asian and Caucasian populations. While the HLA region has been clearly demonstrated to be the most substantial genetic risk factor in populations of both ancestries, the other genetic contributions seem to be very different. *PTPN22* is consistently the second strongest genetic effect in Caucasian whole genome association studies and yet our data indicate that not only is the R620W variant not present, but no other common polymorphisms in the region are significant risk alleles in Koreans. The other Caucasian loci for which we assayed SNPs (*TRAF1/C5*, 6q23, 4q27, *CD40*, *CCL21*) were derived from rigorously interpreted and well-replicated studies and probably represent many of the next tier of susceptibility loci in European ancestry populations. The fact that none of these SNPs demonstrated association in this large Korean study reveals a large degree of genetic heterogeneity of RA across continental ancestry differences, especially when juxtaposed with the *PADI4* locus, which has been strongly associated with RA in this and other Asian populations, but which has shown little evidence for association in Caucasians (14).

In conclusion, we found no association between Korean RA and tag SNPs covering the entire *PTPN22* region or with individual SNPs shown to be strongly associated with disease in Caucasians at the *TRAF1/C5*, 6q23, 4q27, *CD40*, and *CCL21* loci. When contrasted with other loci such as *PADI4*, *FCRL3*, and *SLC22A4*, which have been shown to be associated with RA in Asian but not Caucasian populations, these data indicate that most of the disease-associated variants have arisen independently in different continental populations and that different points in disease-associated pathways are influenced by genetic risk factors in the different populations. Therefore, one would expect that whole genome association studies in Asian populations would reveal more and different susceptibility loci than those that have been described in Caucasian disease; identification of such loci should expand our understanding of the processes that lead to the development of rheumatoid arthritis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported in part by a grant of the Korea Health 21 R&D Project, Ministry of Health and Welfare, Republic of Korea (01-PJ3-PG6-01GN11-0002) and by the Intramural Program of the National Institute of Arthritis and Musculoskeletal and Skin Diseases at the National Institutes of Health, Bethesda, Maryland, USA. B.D.K. was supported by the NIH Clinical Research Training Program, a public-private partnership between the Foundation for the National Institutes of Health and Pfizer, Inc. This work was also supported by grants from the National Institutes of Health: R01 AR44422 and N01 AI95386 (P.K.G.)

REFERENCES

1. Jun KR, Choi SE, Cha CH, Oh HB, Heo YS, Ahn HY, et al. Meta-analysis of the association between HLA-DRB1 allele and rheumatoid arthritis susceptibility in Asian populations. *J Korean Med Sci.* 2007; 22(6):973–980. [PubMed: 18162709]
2. Silman AJ, Pearson JE. Epidemiology and genetics of rheumatoid arthritis. *Arthritis Res.* 2002; 4 Suppl 3:S265–S272. [PubMed: 12110146]
3. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature.* 2007; 447(7145):661–678. [PubMed: 17554300]
4. Begovich AB, Carlton VE, Honigberg LA, Schrod 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(2):330–337. [PubMed: 15208781]
5. Gregersen PK, Lee HS, Batliwalla F, Begovich AB. *PTPN22*: setting thresholds for autoimmunity. *Semin Immunol.* 2006; 18(4):214–223. [PubMed: 16731003]
6. Mori M, Yamada R, Kobayashi K, Kawaida R, Yamamoto K. Ethnic differences in allele frequency of autoimmune-disease-associated SNPs. *J Hum Genet.* 2005; 50(5):264–266. [PubMed: 15883854]
7. 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(12):1199–1209. [PubMed: 17804836]
8. Hu X, Chang M, Saiki RK, Cargill MA, Begovich AB, Ardlie KG, et al. The functional –169T→C single-nucleotide polymorphism in *FCRL3* is not associated with rheumatoid arthritis in white North Americans. *Arthritis Rheum.* 2006; 54(3):1022–1025. [PubMed: 16508985]
9. Kang CP, Lee HS, Ju H, Cho H, Kang C, Bae SC. A functional haplotype of the *PADI4* gene associated with increased rheumatoid arthritis susceptibility in Koreans. *Arthritis Rheum.* 2006; 54(1):90–96. [PubMed: 16385500]
10. Kochi Y, Yamada R, Suzuki A, Harley JB, Shirasawa S, Sawada T, et al. A functional variant in *FCRL3*, encoding Fc receptor-like 3, is associated with rheumatoid arthritis and several autoimmunities. *Nat Genet.* 2005; 37(5):478–485. [PubMed: 15838509]

11. Plenge RM, Padyukov L, Remmers EF, Purcell S, Lee AT, Karlson EW, et al. Replication of putative candidate-gene associations with rheumatoid arthritis in >4,000 samples from North America and Sweden: association of susceptibility with PTPN22, CTLA4, and PADI4. *Am J Hum Genet.* 2005; 77(6):1044–1060. [PubMed: 16380915]
12. Suzuki A, Yamada R, Chang X, Tokunishi 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(4):395–402. [PubMed: 12833157]
13. Tokunishi S, Yamada R, Chang X, Suzuki A, Kochi Y, Sawada T, et al. An intronic SNP in a RUNX1 binding site of SLC22A4, encoding an organic cation transporter, is associated with rheumatoid arthritis. *Nat Genet.* 2003; 35(4):341–348. [PubMed: 14608356]
14. Lee YH, Rho YH, Choi SJ, Ji JD, Song GG. PADI4 polymorphisms and rheumatoid arthritis susceptibility: a meta-analysis. *Rheumatol Int.* 2007; 27(9):827–833. [PubMed: 17265154]
15. Lee HS, Remmers EF, Le JM, Kastner DL, Bae SC, Gregersen PK. Association of STAT4 with rheumatoid arthritis in the Korean population. *Mol Med.* 2007; 13(9–10):455–460. [PubMed: 17932559]
16. Remmers EF, Plenge RM, Lee AT, Graham RR, Hom G, Behrens TW, et al. STAT4 and the risk of rheumatoid arthritis and systemic lupus erythematosus. *N Engl J Med.* 2007; 357(10):977–986. [PubMed: 17804842]
17. Kurreeman FA, Padyukov L, Marques RB, Schrodi SJ, Seddighzadeh M, Stoecken-Rijsbergen G, et al. A candidate gene approach identifies the TRAF1/C5 region as a risk factor for rheumatoid arthritis. *PLoS Med.* 2007; 4(9):e278. [PubMed: 17880261]
18. 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(12):1477–1482. [PubMed: 17982456]
19. Thomson W, Barton A, Ke X, Eyre S, Hinks A, Bowes J, et al. Rheumatoid arthritis association at 6q23. *Nat Genet.* 2007; 39(12):1431–1433. [PubMed: 17982455]
20. Raychudhuri S, et al. Common variants at CD40 and other loci confer risk of rheumatoid arthritis. *Nat Genet.* In press.
21. Zhernakova A, Alizadeh BZ, Bevoja M, van Leeuwen MA, Coenen MJ, Franke B, et al. Novel association in chromosome 4q27 region with rheumatoid arthritis and confirmation of type 1 diabetes point to a general risk locus for autoimmune diseases. *Am J Hum Genet.* 2007; 81(6):1284–1288. [PubMed: 17999365]
22. 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(3):315–324. [PubMed: 3358796]
23. Steinbrocker O, Traeger CH, Batterman RC. Therapeutic criteria in rheumatoid arthritis. *J Am Med Assoc.* 1994; 271(8):659–662. [PubMed: 18150288]
24. de Bakker PI, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D. Efficiency and power in genetic association studies. *Nat Genet.* 2005; 37(11):1217–1223. [PubMed: 16244653]
25. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics.* 2005; 21(2):263–265. [PubMed: 15297300]
26. Kawasaki E, Awata T, Ikegami H, Kobayashi T, Maruyama T, Nakanishi K, et al. Systematic search for single nucleotide polymorphisms in a lymphoid tyrosine phosphatase gene (PTPN22): association between a promoter polymorphism and type 1 diabetes in Asian populations. *Am J Med Genet A.* 2006; 140(6):586–593. [PubMed: 16470599]

Association tests of SNPs in the *PTPN22* region in 1123 Korean rheumatoid arthritis cases and 1008 controls.

Table 1

No.	SNP	Chr 1 position	Major allele : minor allele	Cases Allele counts (minor allele frequency)	Controls Allele counts (minor allele frequency)	Allele Chi-sq p-value	Odds ratio (95% CI)	Cases Hom-minor allele counts (freq)	Cases Het counts (freq)	Cases Hom-major allele counts (freq)	Controls Hom-minor allele counts (freq)
1	rs7554019	113887246	Assay failed								
2	rs17359281	113987482	A:G	1723:393 (0.19)	1589:349 (0.18)	0.64	1.04 (0.89–1.22)	33 (0.03)	327 (0.31)	698 (0.66)	34 (0.04)
3	rs1230673	113996036	T:C	1923:193 (0.09)	1750:192 (0.10)	0.41	1.09 (0.89–1.35)	5 (0.00)	183 (0.17)	870 (0.82)	13 (0.01)
4	rs6537792	114021124	G:C	1401:723 (0.34)	1267:671 (0.35)	0.70	1.02 (0.90–1.17)	112 (0.11)	499 (0.47)	451 (0.42)	114 (0.12)
5	rs3789597	114057662	C:T	1897:133 (0.07)	1761:139 (0.07)	0.35	1.13 (0.88–1.44)	4 (0.00)	125 (0.12)	886 (0.87)	9 (0.01)
6	rs6668829	114131976	C:T	1979:139 (0.07)	1802:132 (0.07)	0.74	1.04 (0.82–1.33)	4 (0.00)	131 (0.12)	924 (0.87)	8 (0.01)
7	rs6537798	114153229	C:A	1589:221 (0.12)	1291:195 (0.13)	0.43	1.09 (0.88–1.33)	15 (0.02)	191 (0.21)	699 (0.77)	20 (0.03)
8	rs3789604	114156465	T:G	1704:450 (0.21)	1592:408 (0.20)	0.70	1.03 (0.88–1.20)	45 (0.04)	360 (0.33)	672 (0.62)	49 (0.05)
9	rs3811021	114158186	T:C	1699:453 (0.21)	1599:407 (0.20)	0.54	1.05 (0.90–1.22)	46 (0.04)	361 (0.34)	669 (0.62)	48 (0.05)
10	rs1217412	114158734	G:A	1400:744 (0.35)	1280:698 (0.35)	0.69	1.03 (0.90–1.17)	120 (0.11)	504 (0.47)	448 (0.42)	128 (0.13)
11	rs1217413	114159273	G:A	1280:698 (0.35)	1266:730 (0.37)	0.45	1.05 (0.93–1.19)	124 (0.12)	514 (0.48)	437 (0.41)	135 (0.14)
12	rs3761935	114174051	T:G	1669:455 (0.21)	1581:399 (0.20)	0.32	1.08 (0.93–1.26)	48 (0.05)	359 (0.34)	655 (0.62)	47 (0.05)
13	rs2797415	114178616	T:C	1405:747 (0.35)	1289:715 (0.36)	0.51	1.04 (0.92–1.19)	120 (0.11)	507 (0.47)	449 (0.42)	130 (0.13)
14	rs2476601*	114179091	G:A	2156:0 (0.00)	2008:0 (0.00)	-	-	0 (0.00)	0 (0.00)	1078 (1.00)	0 (0.00)
15	Novel var6	114182653	T:C	2150:4 (0.00)	2008:2 (0.00)	-	-	0 (0.00)	4 (0.00)	1073 (1.00)	0 (0.00)
16	rs1746853	114184620	T:G	1548:602 (0.28)	1419:571 (0.29)	0.62	1.03 (0.90–1.18)	84 (0.08)	434 (0.40)	557 (0.52)	97 (0.10)
17	rs3827734	114192648	A:C	2126:30 (0.01)	1986:18 (0.01)	0.14	1.56 (0.87–2.80)	1 (0.00)	28 (0.03)	1049 (0.97)	0 (0.00)
18	Novel var5	114192772	G:T	2151:5 (0.00)	2004:4 (0.00)	-	-	0 (0.00)	5 (0.00)	1073 (1.00)	0 (0.00)
19	rs1217407	114195271	A:G	1371:753 (0.36)	1277:707 (0.36)	0.90	1.01 (0.89–1.15)	127 (0.12)	499 (0.47)	436 (0.41)	129 (0.13)
20	rs3765598	114195986	C:T	1668:448 (0.21)	1553:395 (0.20)	0.48	1.06 (0.91–1.23)	48 (0.05)	352 (0.33)	658 (0.62)	47 (0.05)
21	Novel var4	114202742	G:A	2131:1 (0.00)	1988:0 (0.00)	-	-	0 (0.00)	1 (0.00)	1065 (1.00)	0 (0.00)
22	rs1217418	114202754	A:G	1859:297 (0.14)	1700:306 (0.15)	0.18	1.13 (0.95–1.34)	16 (0.01)	265 (0.25)	797 (0.74)	24 (0.02)
23	Novel var3	114203459	C:A	2131:1 (0.00)	1988:0 (0.00)	-	-	0 (0.00)	1 (0.00)	1065 (1.00)	0 (0.00)
24	Novel var2	114203467	C:G	2150:2 (0.00)	2008:2 (0.00)	-	-	0 (0.00)	2 (0.00)	1074 (1.00)	0 (0.00)
25	rs2488457	114216891	C:G	1385:767 (0.36)	1267:735 (0.37)	0.47	1.05 (0.92–1.19)	124 (0.12)	519 (0.48)	433 (0.40)	133 (0.13)
26	Novel var1	114216892	C:T	2153:1 (0.00)	2010:0 (0.00)	-	-	0 (0.00)	1 (0.00)	1076 (1.00)	0 (0.00)
27	rs1235005	114218960	G:C	1811:305 (0.14)	1644:300 (0.15)	0.36	1.08 (0.91–1.29)	19 (0.02)	267 (0.25)	772 (0.73)	25 (0.03)

No.	SNP	Chr 1 position	Major allele : minor allele	Cases Allele counts (minor allele frequency)	Controls Allele counts (minor allele frequency)	Allele Chi-sq p-value	Odds ratio (95% CI)	Cases: Hom- minor allele counts (freq)	Cases: Het counts (freq)	Cases: Hom- major allele counts (freq)	Controls: Hom- minor allele counts (freq)
28	rs12566340	114221851	T:C	1357:763 (0.36)	1242:702 (0.36)	0.94	1.01 (0.88–1.14)	131 (0.12)	501 (0.47)	428 (0.40)	128 (0.13)
29	rs2358994	114230984	A:G	1325:787 (0.37)	1199:741 (0.38)	0.54	1.04 (0.92–1.18)	141 (0.13)	505 (0.48)	410 (0.39)	139 (0.14)
30	rs1018592	114232141	H-W**								
31	rs1217393	114235469	Assay failed								
32	rs10776775	114238005	A:G	1661:451 (0.21)	1520:422 (0.22)	0.77	1.02 (0.88–1.19)	39 (0.04)	373 (0.35)	644 (0.61)	43 (0.04)
33	rs1217401	114240474	A:G	1968:148 (0.07)	1786:156 (0.08)	0.21	1.16 (0.92–1.47)	5 (0.00)	138 (0.13)	915 (0.86)	7 (0.01)
34	rs1217398	114249299	C:G	1815:309 (0.15)	1678:268 (0.14)	0.48	1.07 (0.89–1.27)	20 (0.02)	269 (0.25)	773 (0.73)	19 (0.02)

* R620W variant.

** Hardy Weinberg disequilibrium.

Disease variants from recent studies in Caucasian populations are not associated with disease in Korean rheumatoid arthritis cases and controls.

Table 2

Gene or region	SNP	Major allele : minor allele	Cases: Allele counts (minor allele frequency)	Controls: Allele counts (minor allele frequency)	Allele Chi-sq p-value	Odds ratio (95% CI)	Cases: Hom-minor allele counts (freq)	Cases: Het counts (freq)	Controls: Hom-minor allele counts (freq)	Controls: Het counts (freq)	Controls: Hom-major allele counts (freq)	Genotype Chi-sq p-value
Associations reported in Caucasian populations:												
4q27	rs6822844	G:T	2229:1 (0.00)	1987:1 (0.00)	-	-	0 (0.00)	1 (0.00)	0 (0.00)	1 (0.00)	993 (1.00)	-
6q23	rs6920220	G:A	2223:3 (0.00)	1973:1 (0.00)	-	-	0 (0.00)	3 (0.00)	0 (0.00)	0 (0.00)	986 (1.00)	-
CCL21	rs2812378	T:C	2070:158 (0.07)	1856:120 (0.06)	0.18	1.18 (0.92–1.51)	8 (0.01)	142 (0.13)	7 (0.01)	106 (0.11)	875 (0.89)	0.358
TRAF1/C5	rs3761847	G:A	1139:1087 (0.51)	974:976 (0.50)	0.43	1.05 (0.93–1.19)	256 (0.23)	575 (0.52)	233 (0.24)	508 (0.52)	234 (0.24)	0.751
CD40	rs4810485	G:T	1483:743 (0.34)	1341:635 (0.32)	0.39	1.06 (0.93–1.20)	122 (0.11)	499 (0.45)	106 (0.11)	423 (0.43)	459 (0.46)	0.577
Association reported in Asian populations:												
PADI4	rs2240340	G:A	1168:1056 (0.48)	1207:763 (0.39)	1.15E-08	1.43 (1.26–1.62)	254 (0.23)	548 (0.49)	158 (0.16)	447 (0.45)	380 (0.39)	1.10E-07

Table 3

PTPN22 gene region haplotypes and frequencies in HapMap populations.

Haplotype	Allele present on the haplotype												Freq CEU	
	rs666829	rs6537798	rs3789604	rs3811021	rs1217413	rs2797415	rs1746853	rs2476601 (R620W)	rs2127407	rs3765598	rs1217418	rs2488457		Freq CHB+JPT
1A	C	C	T	T	G	T	T	A	A	C	A	C	0	0.142
1B	C	C	T	T	G	T	T	G	A	C	A	C	0.579	0.092
2	C	C	G	C	A	C	G	G	G	T	A	G	0.206	0.198
3	C	A	T	T	A	C	G	G	G	C	G	G	0.112	0.200
4	T	A	T	T	A	C	T	G	G	C	G	G	0.055	0.283

Table 4

PTPN22 haplotype association analysis in Korean RA cases and controls.

Haplotype *	Estimated hap freq (cases, controls)	p-value	Odds ratio (95% CI)
1B	0.633, 0.624	0.548	1.04 (0.92 – 1.18)
2	0.198, 0.192	0.609	1.04 (0.89 – 1.22)
3	0.070, 0.082	0.141	0.84 (0.67 – 1.06)
4	0.066, 0.068	0.820	0.97 (0.76 – 1.24)

* Haplotypes were as identified in Table 3 with the addition of two SNPs, rs1217412 and rs3761935. The A allele of rs1217412 was found only on Haplotype 1B and the G allele of rs3761935 was found only on Haplotype 2.