***PTPN22* polymorphisms, but not R620W, were associated with the genetic susceptibility of systemic lupus erythematosus and rheumatoid arthritis in a Chinese Han population**

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Abstract

Objectives: The present study aimed to detect a possible association between *PTPN22* gene polymorphisms and rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) in a Chinese Han population.

Methods: 7 *PTPN22* SNPs were genotyped in 358 patients with RA and 713 patients with SLE, as well as 564 RA controls and 672 SLE controls by Restriction Fragment Length Polymorphism (RFLP). Association analyses were conducted on the whole data set. Significant relationships were also examined between clinical features and SNPs for both RA and SLE.

Results: Rs2476601 was lack of polymorphism with a $\leq 0.1\%$ frequency in both SLE and RA patients and healthy controls in our study. The two SNPs rs1217414 and rs3811021 of *PTPN22* shown strong association with both SLE (rs1217414T: $p_{adj} = 6.07e-004$, OR = 0.57; rs3811021C: $p_{adj} = 4.68e-005$, OR = 0.65) and RA (rs1217414T: $p_{adj} = 2.01e-008$, OR = 0.26; rs3811021C: $p_{adj} = 0.028$, OR = 0.70). And the rs3765598 revealed a strong risk factor for SLE ($p = 9.38e-009$, $p_{adj} = 6.57e-008$, OR=1.93), but not for RA ($p = 0.48$, OR=1.12). Moreover, protective haplotype ACTTC in RA ($p = 7.73e-016$, $p_{adj} = 5.51e-015$, OR[95%CI] = 0.02 [0.002-0.10]) and SLE ($p = 8.29e-018$, $p_{adj} = 5.80e-017$, OR[95%CI] = 0.11[0.06-0.21]) were observed. In addition, the distribution of risk haplotypes ACGTC and GCTTT in RA (ACGTC: $p = 0.0006$, $p_{adj} = 0.004$, OR[95%CI] = 1.85 [1.29-2.63]; GCTTT: $p = 2.62e-005$, $p_{adj} = 1.85e-004$, OR[95%CI] = 2.40 [1.57-3.65]) and SLE (ACGTC: $p = 0.0006$, $p_{adj} = 0.004$, OR[95%CI] = 1.85 [1.29-2.63]; ACGTC: $p = 7.74e-011$, $p_{adj} = 6.81e-010$, OR[95%CI] = 2.21 [1.12-3.34]; GCTTT: $p = 2.40$ [1.57-3.65], $p_{adj} = 2.26e-006$, OR[95%CI] = 2.64 [1.79-3.87]) were significant different from that in controls. Furthermore, significant association was observed between the *PTPN22* rs3765598 and antinuclear antibodies 1(ANA1) in SLE.

Conclusions: Our data provide strong evidence that the rs1217414 and rs3811021 in *PTPN22* gene might be common protective factors contributed to SLE and RA susceptibility in the Chinese Han population. While, the rs3765598 might increase the genetic susceptibility of SLE, but not RA.

Keywords: Protein tyrosine phosphatase nonreceptor 22 (*PTPN22*); Rheumatoid arthritis (RA), Systemic lupus erythematosus (SLE); single nucleotide polymorphisms (SNPs); Chinese Han

Introduction

Rheumatoid arthritis (RA [MIM 180300]) and systemic lupus erythematosus (SLE [MIM 152700]) are common complex autoimmune disorders that were thought to be caused by complex interaction of multiple susceptibility genes and environmental factors and affects approximately 0.32% (1% in Caucasian) and 0.1% (0.05% in Caucasian) of the Chinese population respectively [1]. Familiar and twins studies have provided robust evidence for the role of genetic factors in these diseases [2,3]. It is becoming evident that systemic autoimmune diseases, including RA and SLE, share common genetic causative factors in populations of different ethnic or racial origin. The involvement of major histocompatibility complex (MHC) alleles is a piece of evidence that supports the above hypothesis. However, non-MHC alleles were also required. Recently, a functional polymorphism (rs2476601, Arginine 620 Tryptophan (R620W)) in protein tyrosine phosphatase non-receptor type 22 (*PTPN22*), which encodes the lymphoid PTP (Lyp), was reported to be significantly associated with multiple autoimmune disorders including type I diabetes (T1D) [4], rheumatoid arthritis (RA) [5], systemic lupus erythematosus (SLE) [6] and Graves' disease [7]. This variant (R620W) results in a substitution in Arg620Trp that disrupts Lyp binding to Csk [8] and would thereby result in loss of negative regulation of T cell receptor (TCR) signaling, and thus autoimmune disorders. The association between R620W variant of the *PTPN22* gene and Type 1 diabetes (T1D) in North American and Sardinian populations was first described by Bottini et al. [8]. Subsequently, Begovich et al. observed a significant association between the same *PTPN22* R620W variant and rheumatoid arthritis (RA) [9]. Furthermore, Kyogoku et al. showed an association between the *PTPN22* R620W and systemic lupus erythematosus (SLE) in a North American white population [10]. Together, these data provide compelling evidence for involvement of *PTPN22* R620W in susceptibility to both systemic and organ-specific autoimmune diseases.

Interestingly, the R620W was not polymorphic in the Asian populations including Chinese Han, Korean and Japanese populations. Considering the important role of *PTPN22* in the pathogenesis of autoimmune diseases, more variants except for R620W should be taken into account in the development of autoimmune diseases such as RA and SLE in these populations. As reported in our previous study [11], an intron SNP (rs1217414) was significantly associated with ankylosing spondylitis (AS) in the Chinese Han population, which might suggest a new susceptible locus of *PTPN22* gene in autoimmune diseases in this population. Moreover, there have been limited available studies exploring the association

between the possible implications of *PTPN22* polymorphisms for susceptibility to both RA and SLE [12-14]. And none was conducted in Chinese Han population. In the present study, we aim to ascertain whether the *PTPN22* polymorphisms were etiologically relevant to RA and SLE in the Chinese Han population.

Subjects and methods

Sample collection

The study was approved by the Ethical Committee at the Changsha Medical University (CMUEC-12C0523). Written informed consents for genetic analysis were obtained from all subjects or their guardians. For the RA patient cohorts, 358 unrelated patients (Male/female: 94/264; age: 41.7 ± 11.6 years) who met the American College of Rheumatology (The American Rheumatism Association) 1987 revised criteria for RA [15] were recruited from the first affiliated hospital, Changsha Medical University. Rheumatoid factor (RF) status was determined for all the patients. Clinical features such as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), IgA, IgG, IgM were shown in Table 1. For SLE, a total of 713 Patients (Male/female: 102/611; age: 36.7 ± 13.4 years) who fulfilled the American College of Rheumatology 1982 criteria for SLE [16] were recruited from the first affiliated hospital, Changsha Medical University. Clinical features such as anti-Sm antibody (anti-Sm), anti-double-stranded DNA (anti-dsDNA), antinuclear antibody (ANA), ribonucleoprotein (RNP), single strand DNA-binding protein (SSB) were also listed in Table 1. In addition, 1236 unrelated control subjects who without the history of multiple autoimmune diseases (matched for ethnicity, gender and age) for this study was also enrolled (RA control : 564 ; SLE control: 672). All participants were Chinese Han in origin.

Genotyping

A combination of 7 informative *PTPN22* functional or tagging SNPs (selected from previous study [11]) was genotyped in both RA and SLE and healthy controls. Genomic DNA was extracted from peripheral leukocytes using the standard phenol–chloroform method. The multiplex PCR were carried out on the ABI Veriti Thermal Cycler (Applied Biosystems, USA) in a total volume of 10 μ l, including 1 μ l genomic DNA (contain 5-10 ng DNA), 1 μ l 1 $\times$ PCR Buffer(Takara) , 3.0 mM Mg²⁺, 0.3 mM dNTPs, 1 μ l primer mix(1 μ M each primer) , and 1 U Hotstar Taq polymerase (Qiagen Inc.). Cycling parameters were as follows: 95°C for 2 min; 10 cycles at 95°C for 20 s, 67°C -0.5°C /cycle for 40 s, 72°C for 30 s; 27 cycles at 95°C for 20 s, 55°C for 30 s, 72°C for 1 min; then 4°C forever. Genotyping was conducted using Restriction

Fragment Length Polymorphism (RFLP) and 10% of the samples were selected at random to perform repeat assays as genotyping quality control. Direct sequencing was analyzed by the ABI 3730XL DNA Sequencer (Applied Biosystems). The restriction enzymes and sequencing primers for SNPs were listed in Table 1s. Genotype assignment was successful in > 98% of samples tested.

Statistical analysis

Hardy-Weinberg equilibrium (HWE) was tested in the cases and controls using a classic chi-square test with 1 degree of freedom. Calculation power was obtained at the 0.05 level of significance, assuming an odds ratio (OR) of 1.5 (small effect size) using Quanto (<http://hydra.usc.edu/gxe>). The statistical analysis was performed using the PLINK 1.9 (<http://pngu.mgh.harvard.edu/~purcell/plink/>) [17]. Individual analyses of associations between *PTPN22* polymorphisms and RA and SLE, as well as autoantibody status were performed by comparing alleles in cases and controls using Fisher's exact test. The corresponding ORs and 95% confidence intervals (CI) were assessed using a standard logistic regression analysis. Bonferroni correction was applied to adjust the p value (P_{adj}) in multiple comparisons. Linkage disequilibrium (LD) analysis was performed in RA and SLE patients using Haploview program [18]. Analysis of haplotype diversity was performed using the expectation-maximization algorithm (EM). Specific P values and ORs and 95% confidence intervals (CI) were obtained by comparing each haplotype with the more common haplotype in the population using Fisher's exact test. Statistical significance was set at $p < 0.05$.

Results

All variants in cases and controls were in Hardy-Weinberg equilibrium (HWE) ($p > 0.05$). Moreover, the statistical power of the study to detect association with those minor allele frequencies (MAFs) ranged from 48.9 to 85 in RA population and from 41 to 93.6 in SLE population (Table 2).

Single-locus association

Genotype data for the 7 *PTPN22* SNPs successfully typed in the Chinese Han cases (RA and SLE) and controls were examined by single-marker analysis (Table 2, 3). The allele frequency of rs2476601 in both RA and SLE cases and controls (RA vs. controls: 0.1% vs. 0.08%; SLE vs. controls: 0% vs. 0%) was much lower than that in Caucasian population (>8%) [19] and was similar with the previous reports in Asians[11, 20]. The results showed no association between the missense SNP (rs2476601, R620W) and neither RA nor SLE in the

Chinese Han population (RA: $p = 0.74$, OR[95% CI] = 1.57[0.09-5.24]; SLE: $p > 0.05$). Thus, rs2476601 was omitted from further analysis. Genotype analysis showed that the distribution of dominant model of rs1217414 were significant different in SLE, RA and controls, even after the Bonferroni's correction(RA: $p_{\text{dom}} < 0.0001$, $p_{\text{adj}} < 0.0001$; SLE: $p_{\text{dom}} = 0.0002$, $p_{\text{adj}} = 0.0014$). While, the distribution of dominant and recessive models of rs3811021 were significant different in SLE and control(SLE: $p_{\text{dom}} < 0.0001$, $p_{\text{adj}} < 0.0001$; $p_{\text{rec}} = 0.006$, $p_{\text{adj}} = 0.042$), but not in RA($p > 0.05$). Significant associations between rs1217414 and rs3811021 and RA (rs1217414: $p = 2.86 \times 10^{-9}$, $p_{\text{adj}} = 2.01 \times 10^{-8}$, OR [95%CI] = 0.26[0.17-0.42]; rs3811021: $p = 0.004$, $p_{\text{adj}} = 0.028$, OR [95%CI] = 0.70[0.55-0.89]) and SLE (rs1217414: $p = 8.67 \times 10^{-5}$, $p_{\text{adj}} = 6.07 \times 10^{-4}$, OR [95%CI] = 0.57[0.43-0.76]; rs3811021: $p = 6.68 \times 10^{-6}$, $p_{\text{adj}} = 4.68 \times 10^{-5}$, OR [95%CI] = 0.65[0.54-0.78]) were observed in further allelic analysis.

In addition, the distributions of additive, dominant and recessive models of rs3765598 were significant different in SLE and control(SLE: $p_{\text{add}} < 0.0001$, $p_{\text{adj}} < 0.0001$; $p_{\text{dom}} < 0.0001$, $p_{\text{adj}} < 0.0001$; $p_{\text{rec}} = 0.0008$, $p_{\text{adj}} = 0.007$), but not in RA($p > 0.05$). Allelic analysis of the SLE cohort revealed that the frequency of the rs3765598T was significantly higher in patients (17%) compared with controls (9.5%) ($p = 9.38 \times 10^{-9}$, $p_{\text{adj}} = 6.57 \times 10^{-8}$, OR [95%CI] = 1.93[1.54-2.43]), but did not differ significantly from that in RA cohort (11%) and controls (9%) ($p = 0.48$, OR [95%CI] = 1.12[0.81-1.52]), which indicated that T allele in rs3765598 might revealed a strong risk factor for SLE, but not for RA (Table 3).

Haplotype analysis

Pairwise LD for the 6 *PTPN22* SNPs (except for rs2476601) was defined using the genotype data from RA and SLE cases separately, and the summary statistics r^2 was calculated using the HaploView program. Five SNPs(rs1217418, rs3765598, rs1746853, rs1970559, rs3811021) were in one block in SLE and RA populations (Supplementary figure 1 and 2). Haplotypes were predicted for these 5 markers based on the haplotype block using PLINK 1.9. Seven haplotypes in RA and SLE separately, each with a frequency $> 1\%$, were predicted in both cases and controls accounting for $> 95\%$ of all the haplotypes. The haplotype ACTTT was the most common haplotype with a frequency of approximately 65% in RA and SLE. Additionally, we observed marginally significant increase in the distribution of haplotype ACGTC and GCTTT in RA (ACGTC: $p = 0.0006$, $p_{\text{adj}} = 0.004$, OR[95%CI] = 1.85 [1.29-2.63]; GCTTT: $p = 2.62 \times 10^{-5}$, $p_{\text{adj}} = 1.85 \times 10^{-4}$, OR[95%CI] = 2.40 [1.57-3.65]) and SLE (ACGTC: $p = 7.74 \times 10^{-11}$, $p_{\text{adj}} = 6.81 \times 10^{-10}$, OR[95%CI] = 2.21 [1.12-3.34]; GCTTT: $p = 3.23 \times 10^{-7}$, $p_{\text{adj}} = 2.26 \times 10^{-6}$, OR[95%CI] = 2.64 [1.79-3.87]), even after a Stringent Bonferroni correction. Moreover,

haplotype ACTTC might define protective effect to RA($p = 7.73\text{e-}016$, $p_{\text{adj}} = 5.51\text{-}015$, OR[95%CI] = 0.02 [0.002-0.10]) and SLE($p = 8.29\text{e-}018$, $p_{\text{adj}} = 5.80\text{e-}017$, OR[95%CI] = 0.11[0.06-0.21]) (Table 4).

Association of the PTPN22 polymorphisms with clinical features in RA and SLE

Regression analysis was also used to evaluate possible correlation between the *PTPN22* risk alleles and the specific clinical and serological features in RA and SLE shown in Table 5.

ACPA-status was not known for RA patients in previous study. Among 358 patients with successful genotyping, 308 patients with RF information available were divided into two groups by RF status: RF-positive RA group and RF-negative RA group. Comparison of allele frequencies of RF-positive and RF-negative revealed no significant differences between the two groups ($p > 0.05$). As to stratification for ANA1 status in SLE, significant association was detected between rs3765598T and ANA1 status in ANA1-positive and ANA1-negative groups ($p = 0.006$, OR [95%CI] = 1.63[1.15-2.31]). No significant evidence of association between the *PTPN22* polymorphisms and other clinical features, either clinical features -positive SLE group or clinical features -negative SLE group, was detected.

Discussion

In the current study, 358 unrelated patients with RA and 713 patients with SLE were genotyped for polymorphisms in the *PTPN22* gene that was previously reported to be associated with autoimmune diseases. The data showed that two of the *PTPN22* polymorphisms examined (rs1217414 and rs3811021) were significantly associated with RA and SLE in our material. But no association between the functional rs2476601 polymorphism of the *PTPN22* gene and susceptibility to RA and SLE was observed, confirming the evidence that the *PTPN22* rs2476601 may not predispose individuals to the progress of RA and SLE in the Chinese Han cohorts. In addition, rs3765598 might revealed a strong risk factor for SLE, but not for RA. Furthermore, no association was found between the seven *PTPN22* SNPs and most of the clinical and laboratory parameters analyzed (except for rs3765598 and ANA 1 status in SLE).

Recently, both Genome Wide Association Studies (GWAS) and case-control studies indicated that *PTPN22* is second in importance in autoimmune disorders, following the MHC. The *PTPN22* gene, located at chromosome 1 (1p13.1-1p13.3) region which shows linkage to RA and SLE, codes for the lymphoid specific tyrosine phosphatase protein, Lyp. LYP was

shown to interact with the negative regulatory kinase, Csk, thereby inhibiting T lymphocyte activation [21-23]. A mutation (rs2476601, 1858C > T) in the *PTPN22* gene encodes the amino acid substitution of arginine to tryptophan, which disrupts the Lyp-Csk interaction and therefore results in autoimmune disorders.

Associations between this polymorphism and SLE susceptibility have been identified in different populations from European such as Sweden [24], Spanish [25], Crete [12] and Polish [26], as well as from north American [10] and Colombia [13]. Furthermore, growing bodies of studies have also shown significant association between this SNP and RA in UK [27] and Western Algerian [28]. Thus, *PTPN22* C1858T has been now unanimously accepted as a RA and SLE susceptibility locus. The frequencies of the 1858T in SLE (0%) and RA (0.1%) patients and controls (SLE controls: 0%; RA controls: 0.08%) in the present study were much lower than those previously reported for a US-based Caucasian RA and SLE cohorts [9]. And, no association was observed between *PTPN22* 1858T and neither SLE nor RA in our samples, which supports the evidence that the *PTPN22* 1858T might not be the susceptible locus for SLE and RA in the Chinese Han population. The result in present study was contrary to that in Caucasian in UK [27], African American [25], Western Algerian [28] and Crete [12], but the same as the results reported in Japanese [29], Indian [30], Iranian [31], Turkey [32], Egyptian [33], Colombian [13], Spanish [34] and Russian [35]. This discrepancy may relate to different genetic background in sample selection.

Significant association was observed between the rs2476601 and rheumatoid factor(RF) in RF-positive patients and healthy control with an OR equal to 1.55 ($p=0.03$) in Dutch population[36].And, the susceptible TT and TC genotypes of *PTPN22* rs2476601 were strongly associated with rheumatoid factor–positive (RF) disease in Caucasian[9]. Similar results were reported by Lee et al[37]. and Helen et al[38]. In contrast with the studies above, no association was found between the *PTPN22* rs2476601 and the presence of rheumatoid factor–positive disease in Spanish RA patients[14]. Interesting, the rs2476601 was reported to be lack of polymorphism in Asian population including Chinese Han, Japanese, Indian and Iranian et al. In our study, the frequency of rs2476601 was $\leq 0.1\%$ in RA indicated that the rs2476601T carriers were rare in this population. Genetic and environmental heterogeneity between the Caucasian and Chinese Han populations from which the RA cohorts might explain the inconsistency.

Given the *PTPN22* R620W was not polymorphic in the Chinese Han population, it is a matter of debate whether the rs2476601 polymorphism is the only *PTPN22* disease-

associated variant in Asian populations. It is unlikely that a single locus in the *PTPN22* gene is solely responsible for the T cell dysfunction in autoimmune disorders. As reported in our previous study [11], a significant association was detected between an intron polymorphism (rs1217414) and ankylosing spondylitis (AS) in the Chinese Han population. Before the course of this work, similar studies have suggested statistically significant associations between the two SNPs (rs1217414 and rs3811021) and autoimmune disorders including SLE and RA. The results of this study supported the previous findings in UK and European-American populations. Carton et al. firstly revealed a significant association between the SNP1 (rs1217414) and SNP36 (rs3811021) and RA in two unrelated white North Americans populations [39], which was confirmed by a meta-analysis study and a case-control study later [40, 41]. In addition, a significant association was found between rs1217414 and Type I psoriasis in UK [42], as well as SLE in European-Americans [25]. Moreover, protective effect was also observed between rs3811021 and RA in Caucasian populations [36, 43]. These results suggested a potential role for rs1217414 and rs3811021 in the development of immune-mediated diseases. There are an increasing number of new pathogenic variants located in non-coding region [44] and studies have suggested that many disease-related non-coding region SNPs are responsible for aberrant splice processes [45-47]. These observations indicated that the intronic and 3'-UTR SNPs in the *PTPN22* gene might affect the splice processes of *PTPN22*, leading to aberrant expression of *PTPN22* and thereby influencing the susceptibility to autoimmune diseases. Moreover, although the rs1217414 and rs3811021 may not have any functional significance itself, it may be linked to some functional polymorphisms in *PTPN22* gene. This was just a hypothesis, and further study should be carried out to search for more significant evidence.

Interesting, rs3765598 was found to be associated with SLE, but not with RA. And significant association was detected between this locus and ANA1 in SLE as well. Rs3765598 is absolute LD with rs3789604, which was identified to be associated with autoimmune diseases. This discovery support that other SNP in 3'UTR outside R620W is associated with human autoimmune diseases. Thus, the function of this SNP is still hardly known.

To evaluate the combined influence of multiple SNPs of the *PTPN22* gene, association between haplotypes and SLE and RA was also analyzed in the current study. Statistical association was detected between the haplotype ACGTC, GCTTT and ACTTC and SLE and RA after using Bonferroni's correction. Logistic regression analysis indicated that Haplotype ACTTC may significantly decrease the risk of RA and SLE with odds ratio equal to 0.02 and

0.11 separately. Similarly, Logistic regression analysis indicated that haplotypes ACGTC and GCTTT significantly increase the risk of RA and SLE separately. This is the first report on the association between haplotypes containing rs1217418, rs3765598, rs1746853, rs1970559 and rs3811021 of *PTPN22* with RA and SLE, which supported our previous finding that conducted on Ankylosing spondylitis in Chinese Han population [11]. Never the less, further investigation with a larger sample size and haplotype analysis with more SNPs should be required to confirm the present result.

Conclusions

Our results show that two non-coding polymorphisms (rs1217414 and rs3811021) might decrease the genetic susceptibility of both SLE and RA, and rs3765598 might be a risk factor for SLE but not RA in Chinese Han population, which highlighted the need to further explore the primary association between *PTPN22* and autoimmune diseases.

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Figure 1. PTPN22 SNPs and SLE and interaction effects with control. The numbers in the seques refer to the pariwise linkage disequilibrium measured as r^2 . Haplotype blocks were defined using a setting of average pariwise r^2 within block of ≥ 70 .

Figure 2. PTPN22 SNPs and RA and interaction effects with control. The number in the squares refer to pairwise linkage disequilibrium measured as r^2 . Haplotype blocks were defined using a setting of average pairwise r^2 within block of ≥ 70 .

Table 1. Clinical Characteristics of RA and SLE patients

RA patients			SLE patients		
Clinical Characteristics	Mean ± SD	Healthy Control	Clinical Characteristics	Mean ± SD	Healthy Control
Male/female	94/264	126/438		102/611	97/575
Age(years)	41.7 ± 11.6 (18 ~ 74 years)	39.6±13.2(22~77 years)		36.7 ± 13.4 (16 ~ 80 years)	31.4±16.9(19~ 82 years)
ESR (mm/h)	33.2 ± 12.5	18.2±11.5	SLEDAI	18.44±8.12	
CRP (mg/l)	25 ± 12.2	4.9±13.3	Sm ⁺ , %	252/683(36.9%)	0/672(0%)
PLT (×10 ³ /μL)	294 ± 11.3	216±18.4	dsDNA ⁺ , %	436/674 (64.7%)	0/672(0%)
HLA-B27 ⁺ , %	1/358 (0.27%)	0/564(0%)	Pro ⁺ , %	406/547 (74.2%)	0/672(0%)
IgA mg/dl	87.31±21.5	71±15.4	ANA ⁺ , %	294/698 (42.1%)	0/672(0%)
IgG mg/dl	104±19.7	55.7±13.3	RNP ⁺ , %	292/677 (43.1%)	0/672(0%)
IgM mg/dl	168±24.1	112±14.6	SSB ⁺ , %	183/655 (27.9%)	0/672(0%)
ANA ⁺ , %	12/358 (3.35%)	0/564(0%)	But ⁺ , %	506/679 (74.5%)	0/672(0%)
RF ⁺ , %	237/308 (76.9%)	0/564(0%)	Pho ⁺ , %	139/647 (21.5%)	0/672(0%)

Abbreviation: SLEDAI, Systemic Lupus Erythematosus Disease Activity Index SD: Standard Deviation; Sm: Anti-Sm antibody; But: butterfly erythema; Pho: photosthesia; ANA: antinuclear antibodies;

SSB: single strand DNA-binding protein; dsDNA: Anti-double-stranded DNA; PLT: Platelets; Pro: urine protein; RNP: Ribonucleoprotein; ESR, erythrocyte sedimentation rate; CRP,C-reactive protein; RF: rheumatoid factor

Table 1s The information of restriction enzymes and sequencing primers for SNPs

SNPs	region	restriction enzymes	annealing temperature(°C)	Sequencing primers(5'-3')
rs2470601	Exon-14	Rsa1	59	CATCTGCTATCCGGTACTCGA
rs1217414	Intron-1	Xho I	55	GAAATTACACGGGGTGACTGC
rs1217418	Intron-3	PstI	57	GCTATTTCAAGTCCACAAGTCA
rs1746853	Intron-11	Rsa1	57	AATTGCATTGCACTTGTAGATC
rs1970559	Intron-14	Taq1	58	ATTTTGGACATTTTGGATAGCA
rs3765598	Intron-10	Hinf 1	58	ATAAGGATTTGGGAACATTTGG
rs3811021	3'UTR	Taq1	60	TCAGGTGGATTCTTTGTAAAATC

Table 2. Allele distributions of *PTPN22* gene polymorphisms in RA and SLE cases and healthy controls

SNP ID	positi on	Mi no r Alle	RA					po wer ^c	SLE					po wer ^c
			Case(freq.)	control (freq.)	<i>p</i>	<i>P</i> _{adj} ^a	OR(95 % CI) ^b		case(freq.)	control (freq.)	<i>p</i>	<i>P</i> _{adj} ^a	OR(95 % CI) ^b	
rs1217414	1:114124709	T	0.031	0.11	2.86e-009	2.01e-008	0.26[0.17-0.42]	84	0.062	0.10	8.67e-005	6.07e-004	0.57[0.43-0.76]	69
rs1217418	1:11413273	G	0.12	0.14	0.22	-	1.12[0.90-1.58]	85	0.11	0.13	0.12	-	1.20[0.95-1.51]	48.2
rs3765598	1:114106505	T	0.11	0.09	0.48	-	1.12[0.81-1.52]	85	0.17	0.095	9.38e-009	6.57e-008	1.93[1.54-2.43]	93.6
rs1746853	1:114095139	G	0.27	0.28	0.57	-	1.06[0.86-1.31]	74	0.25	0.27	0.24	-	1.11[0.93-1.31]	53
rs2476601	1:114089610	T	0.001	0.0008	0.74	-	1.57[0.09-5.24]	61	0	0	-	-	-	-
rs1970559	1:114089190	C	0.04	0.038	0.68	-	1.10[0.68-1.77]	50	0.04	0.038	0.78	-	1.05[0.71-1.54]	41
rs3811021	1:114068705	C	0.17	0.23	0.04	0.028	0.70[0.55-0.89]	48.9	0.17	0.24	6.68e-006	4.68e-005	0.65[0.54-0.78]	86.2

^a The Bonferroni's correction was carried out to adjust the *p* value.

^b OR and 95% CI are calculated for the minor allele of each SNP.

^c Power calculations assume $\alpha = 0.05$ and small effect size (0.1) or OR = 1.5.

Abbreviation: SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence intervals; -, not calculated; RA, Rheumatoid arthritis; SLE, Systemic lupus erythematosus; Freq., frequency; *P*_{adj}, *P*_{adjusted}

Table 3. Distribution of the genotypes of *PTPN22* gene polymorphisms in RA and SLE cases and controls.

SNPs, rs, (minor allele)	RA		OR, P^a (Add model)	P^b (adj)	OR, P^a (Dom model)	P^b (adj)	OR, P^a (Rec model)	P^b (adj)	SLE		OR, P^a (Add model)	P^b (adj)	OR, P^a (Dom model)	P^b (adj)	OR, P^a (Rec model)	P^b (adj)
	Case(n)	Control(n)							Case(n)	Control(n)						
rs12174 14,(T)	1/20/3 37	4/112/ 448	-	-	0.2 4, <.0 001	<.0 001	-	-	0/89/6 24	6/127/ 539	-	-	0.5 8, 0.0 002	0.0 014	-	-
rs12174 18,(G)	6/77/2 75	5/158/ 401	-	-	0.7 4, 0.0 6	-	-	-	10/14 5/558	6/174/ 482	1.4 4, 0.4 8	-	0.7 4, 0.0 2	0.1 4	0.9 4, 0.9 2	-
rs37655 98,(T)	4/67/2 87	4/99/4 61	1.58, 0.52	-	1.1 1, 0.5 5	-	-	-	25/19 3/495	4/121/ 547	6.9 1, <.0 001	<.0 001	1.9 3, <.0 001	<.0 001	5.1 6, 0.0 008	0.0 07
rs17468 53,(G)	31/13 0/197	24/31 6/224	1.47, 0.18	-	0.5 3, <.0 001	<.0 001	2.1 3, 0.0 06	0.0 42	48/26 4/401	33/29 7/342	1.2 4, 0.3 6	-	0.8 1, 0.0 5	-	1.3 9, 0.1 5	-
rs24766 01,(T)	0/1/35 7	0/1/56 3	-	-	-	-	-	-	0/0/71 3	0/0/67 2	-	-	-	-	-	-
rs19705 59,(C)	1/28/3 29	0/43/5 12	-	-	1.0 4, 0.8 4	-	-	-	0/58/6 55	0/52/6 20	-	-	1.0 6, 0.7 9	-	-	-
rs38110 21,(C)	12/10 1/245	39/18 3/342	0.43, 0.01	0. 07	0.7 1, 0.0 2	0.1 4	0.4 7, 0.0 2	0.1 4	35/17 7/501	50/22 6/396	0.5 5, 0.0 09	0.0 63	0.6 1, <.0 001	<.0 001	0.5 4, 0.0 06	0.0 42

^a P value were calculated using Fisher's exact test.

^b The Bonferroni's correction was carried out to adjust the P value.

Abbreviation: SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence intervals; -, not calculated; RA, Rheumatoid arthritis; SLE, Systemic lupus erythematosus, Add model, Additive model; Dom model, Dominant model;

Rec model, Recessive model; P_{adj} , $P_{adjusted}$.

Table 4. Haplotype analysis of RA and SLE cases and the healthy controls in the *PTPN22* genes

Haplotype ^a	RA					Haplotype ^a	SLE				
	Case(freq.)	control(freq.)	<i>P</i> ^b	<i>P</i> _{adj} ^c	OR[95%CI]		case(freq.)	control(freq.)	<i>P</i> ^b	<i>P</i> _{adj} ^c	OR[95%CI]
A C G T	0.098	0.056	0.006	0.004	1.85 [1.29-2.63]	A C G T	0.07	0.02	7.74e-011	6.81e-010	2.21 [1.12-3.34]
C	0.04	0.01				C	0.01	0.01			
A C G T			5.72e-005	3.90e-004	3.53 [1.83-6.77]	A C G T			-	-	-
T						T					
A C T T	0.002	0.09	7.73e-016	5.51e-015	0.02 [0.002-0.10]	A C T T	0.01	0.09	8.29e-018	5.80e-017	0.11[0.06-0.21]
C						C					
A C T T	0.63	0.67	0.05	-	0.82 [0.66-1.00]	A C T T	0.64	0.66	0.26	-	0.90[0.75-1.08]
T						T					
A T G T	0.07	0.08	0.77	-	0.95 [0.66-1.35]	A T G T	0.12	0.08	0.004	0.003	1.64[1.24-2.15]
C						C					
G C T T	0.08	0.04	2.62e-005	1.85e-004	2.40 [1.57-3.65]	G C T T	0.08	0.03	3.23e-007	2.26e-006	2.64 [1.79-3.87]
T						T					
G C G C	0.03	0.01	0.008	0.006	2.35 [1.22-4.49]	G C G C	0.03	0.014	-	-	-
T						T					

^a Haplotype structure of *PTPN22* for RA and SLE were rs1217414, rs1217418, rs3765598, rs1746853, rs1970559, rs3811021

^b *P* value were calculated using Fisher's exact test.

^c The Bonferroni's correction was carried out to adjust the *p* value.

Abbreviation: SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence intervals; -, not calculated; RA, Rheumatoid arthritis; SLE, Systemic lupus erythematosus; Freq., frequency; *P*_{adj}, *P*_{adjusted}

Table 5. The association between clinical characteristics in SLE and RA and *PTPN22* polymorphisms

SNPs	RA	SLE					
	RF(n=308) (<i>p</i> ³ , OR, 95%CI)	But(n=679) (<i>p</i> ³ , OR 95%CI)	ANA1(n=698) (<i>p</i> ³ , OR 95%CI)	dsDNA(n=674) (<i>p</i> ³ , OR 95%CI)	Anti-Sm(n=683) (<i>p</i> ³ , OR 95%CI)	RNP(n=677) (<i>p</i> ³ , OR 95%CI)	Anti-SSB(n=655) (<i>p</i> ³ , OR 95%CI)
rs1217414	0.56,0.44(0.12-1.03)	0.71,0.90(0.52-1.56)	0.15,0.69(0.41-1.15)	0.11,0.65(0.38-1.11)	0.79,0.93(0.53-1.62)	0.49,1.21(0.70-2.10)	0.92,1.03(0.56-1.91)
rs1217418	0.11,1.33(0.45-2.41)	0.20,1.16(0.50-2.15)	0.69,1.22(0.61-2.98)	0.23,1.29(0.84-1.97)	0.65,1.10(0.72-1.68)	0.96,0.99(0.65-1.52)	0.38,1.17(0.48-1.72)
rs1746853	0.32,1.14(0.75-2.33)	0.41,0.87(0.63-1.20)	0.25,1.19(0.88-1.61)	0.74,0.95(0.70-1.28)	0.72,1.06(0.77-1.44)	0.64,0.93(0.68-1.27)	0.10,1.33(0.94-1.86)
rs1970559	0.66,0.57(0.21-1.67)	0.12,0.45(0.23-0.87)	0.36,0.74(0.39-1.42)	0.81,0.92(0.47-1.78)	0.18,0.59(0.27-1.28)	0.43,0.75(0.35-1.56)	0.83,0.91(0.40-2.06)
rs3765598	0.79,1.37(0.32-2.55)	0.93,0.98(0.68-1.41)	0.006,1.63(1.15-2.31)	0.86,1.03(0.73-1.45)	0.39,1.16(0.82-1.64)	0.69,1.07(0.75-1.52)	0.18,1.29(0.88-1.90)
rs3811021	0.93,0.78(0.24-1.88)	0.62,1.10(0.75-1.59)	0.20,1.25(0.89-1.76)	0.65,1.08(0.76-1.53)	0.29,1.21(0.85-1.72)	0.05,1.42(0.99-2.01)	0.26,1.25(0.85-1.85)

^a The alleles were compared between the clinical features -positive group and clinical features -negative group. *P* value were calculated using Fisher's exact test.

Abbreviations: SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence intervals; -, not calculated; RA, Rheumatoid arthritis; SLE, Systemic lupus erythematosus

RF: rheumatoid factor; But: butterfly erythema; Sm: Anti-Sm antibody; ANA1: antinuclear antibodies 1; SSB: single strand DNA-binding protein; dsDNA: Anti-double-stranded DNA; RNP: Ribonucleoprotein;