PRKCH polymorphism is associated with rheumatoid arthritis in a Chinese population

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Summary
Genetic factors have been widely considered to have a substantial effect on the susceptibility to rheumatoid arthritis (RA). The purpose of this study was to determine whether the four newly discovered polymorphisms in a genome-wide association study (GWAS) meta-analysis confer susceptibility to RA in a Chinese Han population. We conducted a case-control study involving 359 RA cases and 873 age-and gender-matched controls and performed genotyping of four single nucleotide polymorphisms (SNPs), rs227163, rs726288, rs3783782 and rs2469434, using the dye terminator-based SNaPshot method. Consequently, we detected significant differences of genotype distribution of rs3783782 in PRKCH between RA and controls. The minor allele frequencies (MAFs) of rs3783782 were significantly higher in RA patients compared to control subjects. Moreover, the rs227163 in TNFRSF9 had higher MAFs in male RA compared with male controls. In addition, the polymorphism of rs3783782 in PRKCH was significantly associated with RA susceptibility (OR = 1.67, 95% CI = 1.32-2.11, \( p = 1.32 \times 10^{-5} \)). After stratification by gender, the minor (A) allele was strongly associated with increased risk for RA in males (OR = 1.87, 95% CI = 1.34-2.60; \( p = 1.62 \times 10^{-4} \)) and in females (OR = 1.51, 95% CI = 1.08-2.10; \( p = 0.014 \)). For rs227163, the minor (C) allele was found to be associated with RA risk only in males (OR = 1.34, 95% CI = 1.02-1.75; \( p = 0.036 \)). These findings for the first time confirmed that rs3783782 in PRKCH was associated with RA susceptibility in a Chinese population, and rs227163 in TNFRSF9 was associated with RA risk in Chinese males; these SNPs may serve as genetic markers for RA.

Keywords: Rheumatoid arthritis, single nucleotide polymorphisms, PRKCH

1. Introduction
Rheumatoid arthritis (RA) is one of the most common autoimmune diseases, characterized by progressive joint destruction, autoantibody formation, and synovitis, eventually leading to functional disability. Epidemiological data has estimated that RA affects approximately 0.5-1.0% of the world's population and 0.2-0.37% of the Chinese population (1,2). Particularly, the prevalence of RA is estimated to be 2-4% in siblings, 5-10% in same-sex dizygotic twins, and even 12-30% in monozygotic twins, supporting the critical role of genetic factors in RA susceptibility (3,4). Human leukocyte antigen (HLA) class II molecules are well-studied genetic factors closely associated with RA development (5). However, the contribution of the HLA is considered to only account for 30% of the total genetic factors of susceptibility (6). Thus, it is critical to identify novel biomarkers responsible for RA susceptibility.

A genome-wide association study (GWAS) meta-analysis discovered 42 novel non-HLA RA risk loci at a genome-wide level of significance. Interestingly, four risk single nucleotide polymorphisms (SNPs) were found to be significantly different between the European and Asian populations, including rs3783782...
in PRKCH, rs227163 in TNFRSF9, rs726288 in SFTPD, and rs2469434 in CD226 (7). The PRKCH gene encodes protein kinase C η, a member of protein kinase C (PKC). Although PRKCH was identified in 1990, its specific functions in the pathogenesis of RA have not been elucidated. It has been reported that PRKCH may be a susceptibility gene for RA in the Japanese population, but not in the French Caucasian population, suggesting the genetic diversity of patients with RA (8,9).

Tumor Necrosis Factor α (TNF-α) is a prototypical pro-inflammatory cytokine, which is highly expressed in the synovitis of RA patients and targeting TNF-α by monoclonal antibodies proves to be an effective therapeutic approach for this disease. TNF receptor superfamily member 9 (TNFRSF9) is a key factor for communication signals between many cell types during development of multiple organs (10). Surfactant protein D gene (SFTPD) is mainly synthesized in alveolar type II cells of the lung. Studies have shown that SFTPD plays a diverse role in the innate immune system, and suppression of T-lymphocyte proliferation and cytokine production (11). The Cluster of Differentiation-226 (CD226) is expressed on immune cells such as T lymphocytes, monocytes and natural killer (NK) cells. There is some evidence regarding the potential role of CD226 gene polymorphisms in autoimmune diseases (12).

Currently, few studies have explored the potential association between these SNPs (rs3783782 in PRKCH, rs227163 in TNFRSF9, rs726288 in SFTPD, and rs2469434 in CD226) and RA susceptibility in the Chinese population. In this study, we selected these four SNPs for RA association in a Chinese Han population.

2. Materials and Methods

2.1. Ethical approval

This study was approved by the Ethics Committee of Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital. Informed consent was obtained from all individual participants included in the study. All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

2.2. Subjects

A total of 1,232 Chinese subjects, including 359 patients with RA (201 men and 158 women; mean age 51.3 ± 13.07 years) were recruited from the Sichuan Provincial People's Hospital. 873 age-and-gender-matched healthy controls (481 men and 392 women; mean age 51.2 ± 15.81 years) were recruited from the physical examination center. All patients were diagnosed with RA according to the American College of Rheumatology criteria (2). Demographic data and laboratory testing were obtained by reviewing hospital records of the hospital.

2.3. Genomic DNA extraction

Genomic DNA from all participants was isolated from peripheral blood leukocytes using a Gentra Puregene Blood DNA kit (Minneapolis, MN, USA) according to the manufacturer's protocol. The concentration of DNA samples was measured by NanoDrop 2000 (Thermo Scientific, USA).

2.4. Genotyping

The DNA sequences containing the target SNPs were amplified by polymerase chain reaction (PCR) with designed primers (Table 1). PCR amplification was performed on each sample in a 20-μL reaction volume containing 50 ng of genomic DNA and 2×Taq Master Mix. After PCR amplification, the concentration of each primer was defined. Then, the products of the four SNPs were added together in the same concentration for multiplex reaction. SNP genotyping was performed using the dye terminator-based SNAPSHOT method according to the protocol (Applied Biosystems, USA). The SNAPSHOT primers included the 20-60bp upstream sequence or reverse complement of the downstream sequence of each SNP position (Table 1). All probe Primers were synthesized by Sangon Biotech (Shanghai, China). The SNAPSHOT analysis was performed on the ABI 3130XL genetic analyzer (Applied Biosystems, USA). The genotypes of the SNPs were determined using Genemapper software 5.0 (Applied Biosystems, USA).

2.5. Statistical analysis

All of the statistical analyses were performed using the software Statistical Product and Service Solutions (SPSS) version 17.0 (Prentice Hall International, Chicago, USA). The p values of the SNPs were calculated using an additive model. Hardy-Weinberg equilibrium was tested for each allele using the χ² test. The unadjusted odds ratios of the alleles and genotypes were estimated by the χ² test. Odds ratios (ORs) and 95 % confidence intervals (95 % CIs) were calculated. P < 0.05 was considered statistically significant.

3. Results and Discussion

3.1. Clinical characteristics in RA patients and controls

Between 10 February 2017 and 30 May 2018, 359 patients with RA and 873 sex-and-age-matched controls were enrolled. All subjects were Han Chinese. In RA
The protein kinase C (PKC) family plays an important role in T lymphocyte activation and autoimmune disorders (16). Therefore, PRKCH, as a member of the PKC family, is speculated to be associated with the pathophysiologic mechanism of RA (17). Indeed, Takata et al. reported PRKCH as a susceptibility gene for RA in a Japanese population. They found that multiple SNPs of PRKCH may influence susceptibility to this disease (8). Nevertheless, another study by Teixeira et al. confirmed that these susceptibility alleles of PRKCH were not associated with RA in a French Caucasian population (9).

These contrary findings suggest the variation of PRKCH across different ethnic populations and identification of these variants in other populations is necessary. In our study, we observed a significant difference of genotype distribution of PRKCH rs3783782 between RA patients and control subjects ($p = 3.09 \times 10^{-5}$). The minor allele frequencies (MAFs) of rs3783782 were significantly higher in RA patients compared to control subjects both in males (18.1% in RA vs. 10.6% in control, $p = 1.62 \times 10^{-7}$) and females (22.1% in RA vs. 15.8% in control, $p = 0.014$).

Controlling the excessive production of pro-inflammatory cytokines such as tumor necrosis factor (TNF) represents a remarkable therapeutic approach for RA treatment (18). TNFRSF9 is a 30 kDa membrane glycoprotein which belongs to the tumor necrosis factor receptor (TNFR) family. TNFRSF9 is widely expressed in the synovial membrane of patients with RA, and its expression is increased in the synovial tissue of RA patients. TNFRSF9 is involved in the pathogenesis of RA through its interaction with TNF-alpha and other cytokines (19-21).

### Table 1. Primers used in this study

| Primer Name          | Sequence                              | Tm (°C) |
|----------------------|---------------------------------------|---------|
| rs726288-F           | AGTCCCTGAGGATCTCAGG               | 54      |
| rs726288-R           | AGGAGCAGCAGGAAATCCAAAA             | 50      |
| rs726288-SNapSHOT-primer | AGGCAAAATGTGCACCACACTCCGCAGGC      | 52      |
| rs3783782-F          | TGTTTTTCTAGGGATCTTGGCAGTTTTTTGTT  | 56      |
| rs3783782-R          | TTTAAAGCAGGCTCAGTCGCTG             | 52      |
| rs3783782-SNapSHOT-primer | AGGAAATGTGCACCACACTCCGCAGGC      | 52      |
| rs2469434-F          | CCTGGAAAGATCTCAGGTC                | 54      |
| rs2469434-R          | CCAACAGCAGTCAGTCGCTG              | 52      |
| rs2469434-SNapSHOT-primer | AGAAGATGTGCACCACACTCCGCAGGC      | 52      |
| rs227163-F           | CCACTCCCTACCACACAC                 | 54      |
| rs227163-R           | CCTCCTTTTACCACTACAC               | 54      |
| rs227163-SNapSHOT-primer | AGAAGATGTGCACCACACTCCGCAGGC      | 52      |

Tm, DNA melting temperature.

### Table 2. Characteristics of the RA cases and controls

| Characteristics               | Total RA cohort ($n = 359$) | Healthy Controls ($n = 873$) | $p$ value |
|-------------------------------|-------------------------------|-------------------------------|----------|
| Male, n (%)                   | 201 (56)                      | 481 (55)                      | 0.08     |
| Age (years), mean (SD)        | 51.3 (13.07)                  | 51.2 (15.81)                  | 0.97     |
| BMI (kg/m$^2$), mean (SD)     | 21.6 (3.16)                   | 20.4 (4.32)                   | 0.53     |
| Age of onset (years), mean (SD)| 45.2 (13.84)                   | -                             | -        |
| Disease duration (years), median (p25-p75) | 3.0 (1.0-9.0)               | -                             | -        |

RA, rheumatoid arthritis; BMI, Body Mass Index; p25-p75, 25th to 75th percentile.
in a variety of immune cells, including activated T/B, NK, and NKT cells (10). Previous studies have found that SNPs of TNFRSF9 are associated with autoimmune disorders, such as psoriatic arthritis in Europeans by GWAS, and RA in African Americans (19, 20). In our study, we did not find a significant difference in genotype distribution of TNFRSF9 rs227163 between RA patients and controls ($p = 0.21$), but we found that rs227163 appeared to have higher MAFs in male RA compared male controls (27.1% in male RA vs. 21.8% in male controls, $p = 0.036$). However, we failed to detect any differences between RA and control subjects with respect to rs726288 and rs2469434 genotype distribution and allele frequencies.

### Table 3. Genotype frequencies of the four SNPs in RA patients and controls

| SNPs   | Gene    | Gender | RA, $n$ (%) | Control, $n$ (%) | $p$ value |
|--------|---------|--------|-------------|------------------|-----------|
| rs726288 | SFTP D | Male   | 200         | 475              | 0.056     |
|        |         | TT     | 7 (3.50)    | 19 (4.0)         |           |
|        |         | CT     | 29 (14.50)  | 155 (32.63)      |           |
|        |         | CC     | 164 (82.00) | 301 (63.37)      |           |
|        |         | Female | 157         | 387              | 0.54      |
|        |         | TT     | 10 (6.37)   | 20 (5.17)        |           |
|        |         | CT     | 64 (40.76)  | 143 (36.95)      |           |
|        |         | CC     | 83 (52.87)  | 224 (57.88)      |           |
|        |         | All    |             | 481 (56.27)      | 0.44      |
| rs3783782 | PRK CH | Male   | 196         | 478              | 3.99 x 10^{-4} |
|        |         | AA     | 5 (2.55)    | 8 (1.67)         |           |
|        |         | AG     | 61 (31.12)  | 85 (17.78)       |           |
|        |         | GG     | 130 (66.33) | 385 (80.54)      |           |
|        |         | Female | 154         | 386              | 0.035     |
|        |         | AA     | 4 (2.60)    | 6 (1.55)         |           |
|        |         | AG     | 60 (38.96)  | 110 (28.50)      |           |
|        |         | GG     | 90 (58.44)  | 270 (69.95)      |           |
|        |         | All    |             | 322 (56.18)      | 0.041     |
| rs2469434 | CD226  | Male   | 199         | 455              | 3.40 x 10^{-5} |
|        |         | CC     | 28 (14.07)  | 46 (10.11)       |           |
|        |         | CT     | 93 (46.73)  | 222 (48.79)      |           |
|        |         | TT     | 78 (39.20)  | 187 (41.10)      |           |
|        |         | Female | 157         | 384              | 0.85      |
|        |         | CC     | 17 (10.83)  | 48 (12.50)       |           |
|        |         | CT     | 75 (47.77)  | 177 (46.09)      |           |
|        |         | TT     | 65 (41.40)  | 159 (41.41)      |           |
|        |         | All    |             | 297 (40.01)      | 0.77      |
| rs227163 | TNFRS F9 | Male  | 199         | 454              | 7.7 x 10^{-3} |
|        |         | CC     | 14 (7.04)   | 25 (5.50)        |           |
|        |         | CT     | 80 (40.20)  | 148 (32.60)      |           |
|        |         | TT     | 105 (52.76) | 281 (61.89)      |           |
|        |         | Female | 150         | 366              | 0.98      |
|        |         | CC     | 9 (6.0)     | 20 (5.46)        |           |
|        |         | CT     | 55 (36.67)  | 128 (34.97)      |           |
|        |         | TT     | 91 (60.67)  | 218 (59.56)      |           |
|        |         | All    |             | 294 (41.07)      | 0.21      |

RA, Rheumatoid Arthritis; MAF, minor allele frequency; SNPs, single nucleotide polymorphisms; OR, odds ratio.

### Table 4. MAFs of the four SNPs in RA patients and controls

| SNPs (MAF) | Gene    | Gender | RA, n (%) | Control, n (%) | OR (95% CI) | $p$ value |
|------------|---------|--------|-----------|----------------|-------------|-----------|
| rs726288 (T) | SFTP D | Male   | 43 (10.8) | 193 (20.3) | 1.129 (0.85-1.50) | 0.399     |
|            |        | Female | 84 (26.8) | 183 (23.6) | 1.179 (0.87-1.59) | 0.28      |
|            |        | All    | 127 (17.9) | 376 (21.8) | 1.15 (0.94-1.42) | 0.181     |
| rs3783782 (A) | PRK CH | Male   | 71 (18.1) | 101 (10.6) | 1.87 (1.34-2.60) | 1.62 x 10^{-4} |
|            |        | Female | 68 (22.1) | 122 (15.8) | 1.51 (1.08-2.10) | 0.014     |
|            |        | All    | 139 (19.9) | 223 (29.2) | 1.67 (1.32-2.11) | 1.32 x 10^{-3} |
| rs2469434 (C) | CD226  | Male   | 149 (37.4) | 314 (34.5) | 1.135 (0.89-1.45) | 0.308     |
|            |        | Female | 109 (26.7) | 273 (35.5) | 0.964 (0.73-1.27) | 0.79      |
|            |        | All    | 258 (36.2) | 587 (35.0) | 1.056 (0.88-1.27) | 0.558     |
| rs227163 (C) | TNFRSF9 | Male  | 108 (27.1) | 198 (21.8) | 1.34 (1.02-1.75) | 0.036     |
|            |        | Female | 73 (19.3) | 168 (20.0) | 1.03 (0.76-1.42) | 0.83      |
|            |        | All    | 181 (25.9) | 366 (22.3) | 1.20 (0.97-1.47) | 0.087     |

SNPs, single nucleotide polymorphisms; MAF, minor allele frequency; RA, rheumatoid arthritis; OR, odds ratio.

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3.4. Polymorphisms of the four SNPs and risk of RA

Furthermore, we evaluated the effects of rs3783782 in PRKCH and rs227163 in TNFRSF9 for RA risk, as shown in Table 4. As a result, we found rs3783782 polymorphism was significantly associated with RA susceptibility (OR = 1.67, 95% CI = 1.32-2.11, p = 1.32 × 10^-5). After stratification by gender, the minor (A) allele was strongly associated with increased risk for RA in males (OR = 1.87, 95% CI = 1.34-2.60; p = 1.62 × 10^-3) and in females (OR = 1.51, 95% CI = 1.08-2.10; p = 0.014). For rs227163, the minor (C) allele was found to be associated with RA risk only in males (OR = 1.34, 95% CI = 1.02-1.75; p = 0.036). Unfortunately, we failed to find any obvious association of rs227163 polymorphism with female RA (OR = 1.03, 95% CI = 0.76-1.42; p = 0.83).

Previous studies have shown that SFTPD and CD226 is critically involved in the innate immune system by suppressing T cell proliferation and cytokine production (11,12). Accumulating evidence also supports the role of SFTPD polymorphisms in a diversity of human diseases, such as chronic obstructive pulmonary disease, obesity, and RA (21-23). However, we found no association of SFTPD (rs726288) and CD226 (rs2469434) with RA in a Chinese Han population, probably due to the SNP loci and genetic differences in different populations.

There are some limitations in this study. First, the study sample size is relatively small, and more participants need to be involved to confirm our findings. Second, the physiological and pathophysiological functions of SNPs of PRKCH and TNFRSF9 in RA have not yet been investigated.

In conclusion, these findings for the first time confirmed that rs3783782 in PRKCH was associated with RA susceptibility in a Chinese population, and rs227163 in TNFRSF9 was associated with RA risk in Chinese males. These SNPs may serve as genetic markers for RA. Further functional investigations are needed to elucidate the precise role of PRKCH and TNFRSF9 in RA pathogenesis.

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References

1. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. N Engl J Med. 2011; 365:2205-2219.
2. Gibofsky A. Overview of epidemiology, pathophysiology, and diagnosis of rheumatoid arthritis. Am J Manag Care. 2012; 18:S295-S302.
3. Karami J, Aslani S, Jamshidi A, Garshabsi M, Mahmoudi M. Genetic implications in the pathogenesis of rheumatoid arthritis; An updated review. Gene. 2019; 702:8-16.
4. Deane KD, Demoruelle MK, Kelmenson LB, Kuhn KA, Norris JM, Holers VM. Genetic and environmental risk factors for rheumatoid arthritis. Best Pract Res Clin Rheumatol. 2017; 31:3-18.
5. van Drongelen V, Holoshitz J. Human leukocyte antigen-disease associations in rheumatoid arthritis. Rheum Dis Clin North Am. 2017; 43:363-376.
6. Pratt AG, Isaacs JD. Genotyping in rheumatoid arthritis: A game changer in clinical management? Expert Rev Clin Immunol. 2015; 11:303-305.
7. Okada Y, Wu D, Trynka G, et al. Genetics of rheumatoid arthritis contributes to biology and drug discovery. Nature. 2014; 506:376-381.
8. Takata Y, Hamada D, Miyatake K, Nakano S, Shinomiya F, Scafe CR, Reeve VM, Osabe D, Moritani M, Kumita K, Kamatani N, Inoue H, Yasui N, Itakura M. Genetic association between the PRKCH gene encoding protein kinase ceta isoforms and rheumatoid arthritis in the Japanese population. Arthritis Rheum. 2007; 56:30-42.
9. Teixeira VH, Jacq L, Moore J, Lasbleiz S, Hilliquin P, Resende Oliveira C, Cornelis F, Petit-Teixeira E. Association and expression study of PRKCH gene in a French Caucasian population with rheumatoid arthritis. J Clin Immunol. 2008; 28:115-121.
10. El Krakami N, Elsherbiny NM, El-Gayar AM, Ebrahim MA, Al-Gayar MMH. Clinical significance of the TNF-α receptors, TNFRSF2 and TNFRSF9, on cell migration molecules Fas and Varensin in acute leukemia. Cytokine. 2018; 111:523-529.
11. Sarashina-Kida H, Negishi H, Nishio J, Suda W, Nakajima Y, Yasui-Kato M, Iwasaki K, Kang S, Endo N, Yanai H, Asagiri M, Kida H, Hattori M, Kumanogoh A, Taniguchi T. Gallbladder-derived surfactant protein d regulates gut commensal bacteria for maintaining intestinal homeostasis. Proc Natl Acad Sci U S A. 2017; 114:10178-10183.
12. Gaud G, Roncagalli R, Chauoi K, Bernard I, Familiades J, Colacios C, Kassem S, Monsarrat B, Burlet-Schiltz O, de Peredo AG, Malissen B, Saoudi A. The costimulatory molecule CD226 signals through VAV1 to amplify TCR signals and promote IL-17 production by CD4+ T cells. Sci Signal. 2018; 11. pii: eaar3083.
13. Yang X, Chang Y, Wei W. Endothelial dysfunction and inflammation: immunity in rheumatoid arthritis. Mediators Inflam. 2016; 2016:6813016.
14. Lv M, Miao J, Zhao P, Luo X, Han Q, Wu Z, Zhang K, Zhu P. CD147-mediated chemotaxis of CD4+CD161+ T cells may contribute to local inflammation in rheumatoid arthritis. Clin Rheumatol. 2018; 37:59-66.
15. Byng-Maddick R, Ehrenstein MR. The impact of biological therapy on regulatory T cells in rheumatoid arthritis. Rheumatology (Oxford). 2015; 54:768-775.
16. Bai G. The PKC gene module: molecular biosystematics to resolve its T cell functions. Immunol Rev. 2003; 192:64-79.
17. Wang L, Wu LF, Lu X, Mo XB, Tang ZX, Lei SF, Deng FY. Integrated analyses of gene expression profiles digs out common markers for rheumatic diseases. Plos One.
18. McInnes IB, Buckley CD, Isaacs JD. Cytokines in rheumatoid arthritis - shaping the immunological landscape. Nat Rev Rheumatol. 2016; 12:63-68.

19. Stuart PE, Nair RP, Tsoi LC, et al. Genome-wide association analysis of psoriatic arthritis and cutaneous psoriasis reveals differences in their genetic architecture. Am J Hum Genet. 2015; 97:816-836.

20. Danila MI, Laufer VA, Reynolds RJ, Yan Q, Liu N, Gregersen PK, Lee A, Kern M, Langefeld CD, Arnett DK, Bridges SL Jr. Dense genotyping of immune-related regions identifies loci for rheumatoid arthritis risk and damage in African Americans. Mol Med. 2017; 23:177-187.

21. Issac MS, Ashur W, Mousa H. Genetic polymorphisms of surfactant protein D rs2243639, Interleukin (IL)-1β rs16944 and IL-1RN rs2234663 in chronic obstructive pulmonary disease, healthy smokers, and non-smokers. Mol Diagn Ther. 2014; 18:343-354.

22. Lin Z, Thorenoor N, Wu R, DiAngelo SL, Ye M, Thomas NJ, Liao X, Lin TR, Warren S, Floros J. Genetic association of pulmonary surfactant protein genes, SFTPA1, SFTPA2, SFTPB, SFTPC, and SFTPD with cystic fibrosis. Front Immunol. 2018; 9:2256.

23. Mosaad YM, El-Toraby EE, Tawhid ZM, Abdelsalam AI, Enin AF, Hasson AM, Shafeek GM. Association between CD226 polymorphism and soluble levels in rheumatoid arthritis: relationship with clinical activity. Immunol Invest. 2018; 47:264-278.

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