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

Lack of association of *IL-2RA* and *IL-2RB* polymorphisms with rheumatoid arthritis in a Han Chinese population

J. Zhu[1]*, F. He[2]*, D.D. Zhang[2]*, J.Y. Yang[2], J. Cheng[1], R. Wu[1], B. Gong[2], X.Q. Liu[2], S. Ma[2] and B. Zhou[1]

[1]Department of Rheumatology and Immunology,
Sichuan Academy of Medical Sciences,
Sichuan Provincial People's Hospital, Chengdu, Sichuan, China
[2]Sichuan Key Laboratory for Disease Gene Study,
Sichuan Academy of Medical Sciences,
Sichuan Provincial People's Hospital, Sichuan, China

*These authors contributed equally to this study.
Corresponding author: B. Zhou
E-mail: bingb2917@126.com

Genet. Mol. Res. 12 (1): 581-586 (2013)
Received August 23, 2012
Accepted December 14, 2012
Published February 27, 2013
DOI http://dx.doi.org/10.4238/2013.February.27.7

ABSTRACT. Polymorphisms in *IL-2RA* and *IL-2RB* genes have been reported to confer susceptibility to rheumatoid arthritis (RA) in European populations. We investigated a possilbe association between SNPs in *IL-2RA* and *IL-2RB* genes and RA in a Han Chinese population. rs2104286 in *IL-2RA* and rs743777 in *IL-2RB* genes were genotyped in a Han Chinese cohort composed of 500 patients with RA and 600 controls. The levels of anti-cyclic citrullinated peptide antibodies (CCP) and rheumatoid factor were determined in all patients and controls. The genotype and allele frequencies of the two SNPs were compared in patients and controls. Additionally, serum concentrations of anti-CCP and rheumatoid factor were analyzed in the three genotype groups of *IL-2RA* and *IL-2RB* genes. There was no overall difference in the genotype and allele frequencies of the two SNPs, rs2104286 in

IL-2RA and rs743777 in *IL-2RB*, between the patients with RA and controls. In addition, none of the subgroups showed any significant association with RA risk after stratification by CCP and rheumatoid factor levels. We conclude that the two genetic variants within *IL-2RA* and *IL-2RB* are not associated with genetic susceptibility to RA in Han Chinese. Also, the rs2104286 and rs743777 genotypes were not significantly associated with the concentrations of anti-CCP antibodies or rheumatoid factor.

Key words: Rheumatoid arthritis; *IL-2RA*; *IL-2RB*; Association study

INTRODUCTION

Rheumatoid arthritis (RA) is a common, complex autoimmune inflammatory joint disease targeting the synovial membrane, cartilage and bone. As a systemic disease, RA can affect organs and areas of the body other than the joints and result in significant disability and early mortality (Silman and Pearson, 2002). Both multiple genes in combination with environmental and hormonal factors contribute to disease etiology (Firestein, 2003). Genetic mutation or polymorphisms are inherited prior to disease onset. Risk alleles could help subset patients into clinical categories that predict outcome (Isaacs, 2010). Genetic susceptibility of RA has been reported to be ethnic-specific (Foster and Freeman, 1998). Some polymorphisms could be unique to a specific ethnic group, and some are common across multiple ethnic groups (Mori et al., 2005). Investigations of ethnic heterogeneity of genetic factors will provide basic information for disease heterogeneity and facilitate individualized medicine. Genome-wide association studies (GWAS) and candidate gene approaches have shown the association of a number of genetic susceptibility loci with RA (Hardy and Singleton, 2009). So far, at least 31 susceptibility loci/genes responsible for RA have been identified (Plenge, 2009).

Recently, GWAS has identified nine new putative RA susceptibility loci, with none of them mapping to the loci previously associated with RA. Of particular interest is the association of SNPs mapping close to both the alpha and beta chains of IL2-receptor (IL-2R; rs2104286 in *IL-2RA* and rs743777 in *IL-2RB*).

In the present study, the two genes *IL-2RA* and *IL-2RB* were selected as candidate genes for case-control association study to explore the association between the polymorphisms in these genes and the susceptibility to RA in a Chinese population.

MATERIAL AND METHODS

Study population

This study was approved by the Institutional Review Boards of the Sichuan Academy of Medical Sciences, Provincial People's Hospital. All subjects provided informed consent before participating in the study. The diagnosis of RA was established using the classification criteria of the American College of Rheumatology 1987 revised criteria (Arnett et al., 1988). In total, 500 patients with RA and 600 normal controls were recruited from the Rheumatology and Immunology Clinics of Sichuan Provincial People's Hospital. The

patients had a mean disease duration of five years. In the normal controls, no sign of joint or other autoimmune disease was detected. Clinical information about the cases and controls is listed in Table 1.

Cyclic citrullinated peptide (CCP) and rheumatoid factor (RF) measurements

Serum samples were obtained and assayed for RF and anti-CCP in all patients and controls. In brief, the concentrations of anti-CCP antibodies were determined with an anti-CCP-ELISA kit (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany) on a Bio-Rad Bench Mark apparatus (Bio-Rad, Hercules, CA, USA). RF was determined with an endpoint nephelometry kit (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) on a DADE BEHRING BNII apparatus (GMI, MN, USA) according to manufacturer instructions. Results were corroborated by validation in the Clinical Laboratory, Sichuan Provincial People's Hospital, Sichuan, China. Cases with anti-CCP levels higher than 5 U/mL were considered to be positive for anti-CCP antibodies; cases with RF levels higher than 20 U/mL were considered to be positive for RF.

Genotyping

Genomic DNA was extracted from blood by serial phenol/chloroform extraction and ethanol precipitation. rs2104286 and rs743777 were genotyped by the SNaPshot method (Applied Biosystems, CA, USA). The following primers were used to genotype rs2104286 - PCR forward primer: 5'-CAGCCAGCATGACCCACTGCTT-3', PCR reverse primer: 5'-AACAGC AGAGGACCCCGCCC-3', SNaPshot primer: 5'-GGCATAGATATAGTCATGGTAACACAA GTC-3', and rs743777 - PCR forward primer: 5'-TGTCAGGGCTTTGTGCATGCTGAG-3', PCR reverse primer: 5'-CCCAGGTCTGGGTAGGCCCG-3', SnaPshot primer: 5'-CCACAT TCTGGTAGGATGACTCATGAGGCC-3'. SNP analysis was performed on an ABI 3130 genetic analyzer (Applied Biosystems).

Statistical analysis

Hardy-Weinberg equilibrium (HWE) for the SNP was tested by the χ^2 test. All analyses were adjusted for age and gender. Allele and genotype frequencies between cases and controls were compared by χ^2 analysis. Statistical significance was defined as $P < 0.05$. All statistical analyses were performed using the SPSS version 10.0 software.

RESULTS

The demographic and clinical features of this cohort are listed in Table 1. There was no significant difference of gender and age between RA cases and the control group (Table 1). Genotype distributions in controls and cases were consistent with HWE. Allele and genotype frequencies of rs2104286 and rs743777 were comparable in RA cases and controls, and thus, the two SNPs showed no association with RA (Table 2). The associations of the two SNPs with RA were also analyzed after stratification by CCP and RF. However, none of the subgroups showed any significant association with RA risk (Table 3).

Table 1. Characteristics of rheumatoid arthritis cases and controls matched for ages and ethnicity.

Subject	Cases	Controls
Male (N)	221	352
Female (N)	279	248
Average age [(years) ± SD]	64 ± 6.6	65 ± 7.8
Anti-CCP positive	346	0
RF positive	427	0

CCP = cyclic citrullinated peptide; RF = rheumatoid factor.

Table 2. Association between rs2104286 and rs743777 and rheumatoid arthritis in a Han Chinese cohort.

SNP	Genotype	Case (frequency)	Control (frequency)	Allelic P	OR (95%CI)
rs2104286	AA	380 (0.760)	451 (0.752)	0.776	1.04 (0.81-1.33)
	AG	110 (0.220)	137 (0.228)		
	GG	10 (0.020)	12 (0.020)		
	A	0.870	0.866		
	G	0.130	0.134		
rs743777	AA	386 (0.772)	483 (0.805)	0.214	0.85 (0.65-1.10)
	AG	108 (0.216)	110 (0.183)		
	GG	6 (0.012)	7 (0.012)		
	A	0.880	0.897		
	G	0.120	0.103		

SNP = single nucleotide polymorphism; OR = odds ratio; 95%CI = 95% confidence interval.

Table 3. Association of rs2104286 in *IL-2RA* and rs743777 in *IL-2RB* and subgroup rheumatoid arthritis in a Han Chinese cohort.

SNP	Group	HWE	Genotype count and frequency		Allele frequency		Allelic P	OR (95%CI)
			Case (frequency)	Control (frequency)	Case	Control		
rs2104286	Anti-CCP-positive	Case: 0.379 Control: 0.673	AA: 262 (0.757) AG: 76 (0.22) GG: 8 (0.023)	AA: 451 (0.752) AG: 137 (0.228) GG: 12 (0.020)	A: 0.867 G: 0.133	A: 0.866 G: 0.134	0.941	1.01 (0.76-1.33)
	Anti-CCP-negative	Case: 0.798 Control: 0.673	AA: 118 (0.766) AG: 34 (0.221) GG: 2 (0.013)	AA: 451 (0.752) AG: 137 (0.228) GG: 12 (0.020)	A: 0.877 G: 0.123	A: 0.866 G: 0.134	0.619	1.10 (0.75-1.6)
	RF-positive	Case: 0.97 Control: 0. 673	AA: 324 (0.759) AG: 96 (0.225) GG: 7 (0.016)	AA: 451 (0.752) AG: 137 (0.228) GG: 12 (0.020)	A: 0.871 G: 0.129	A: 0.866 G: 0.134	0.725	1.05 (0.81-1.36)
	RF-negative	Case: 0.107 Control: 0.673	AA: 56 (0.767) AG: 14 (0.192) GG: 3 (0.041)	AA: 451 (0.752) AG: 137 (0.228) GG: 12 (0.020)	A: 0.863 G: 0.137	A: 0.866 G: 0.134	0.926	0.98 (0.67-1.22)
rs743777	Anti-CCP-positive	Case: 0.831 Control: 0.794	AA: 272 (0.786) AG: 70 (0.202) GG: 4 (0.012)	AA: 483 (0.805) AG: 110 (0.183) GG: 7 (0.012)	A: 0.887 G: 0.113	A: 0.897 G: 0.103	0.525	0.91 (0.67-1.22)
	Anti-CCP-negative	Case: 0.554 Control: 0.794	AA: 114 (0.74) AG: 38 (0.247) GG: 2 (0.013)	AA: 483 (0.805) AG: 110 (0.183) GG: 7 (0.012)	A: 0.864 G: 0.136	A: 0.897 G: 0.103	0.098	0.73 (0.5-1. 06)
	RF-positive	Case: 0.766 Control: 0.794	AA: 334 (0.782) AG: 88 (0.206) GG: 5 (0.012)	AA: 483 (0.805) AG: 110 (0.183) GG: 7 (0.012)	A: 0.885 G: 0.115	A: 0.897 G: 0.103	0.411	0.89 (0.67-1.17)
	RF-negative	Case: 0.548 Control: 0.794	AA: 52 (0.712) AG: 20 (0.247) GG: 1 (0.014)	AA: 483 (0.805) AG: 110 (0.183) GG: 7 (0.012)	A: 0.856 G: 0.144	A: 0.849 G: 0.103	0.083	0.65 (0.40-1.06)

SNP = single nucleotide polymorphism; HWE = Hardy-Weinberg equilibrium; OR = odds ratio; 95%CI = 95% confidence interval; CCP = cyclic citrullinated peptide; RF = rheumatoid factor.

DISCUSSION

Although the well-known susceptibility gene is the *HLA-DRB1* gene, it accounts for only approximately one-third of the total genetic effects. Non-HLA susceptibility genes contribute to a major portion of genetic susceptibility to RA. However, it has been much more difficult to identify risk genes outside the HLA region. Recently, GWAS has identified nine putative RA susceptibility loci. In that study, SNPs close to both the alpha and beta chains of the IL-2R (rs2104286 within the *IL-2RA* gene and rs743777 within the *IL-2RB* gene) were identified to be associated with RA. The results were replicated in a Dutch population (Kurreeman et al., 2009) and North American juvenile idiopathic arthritis population (Hinks et al., 2009). IL-2 is a key cytokine involved in promoting peripheral tolerance through the generation and maintenance of regulatory T cell subsets (Malek, 2008). The IL-2 receptor mediates IL-2 stimulation of T lymphocytes and is thereby thought to have an important role in preventing autoimmunity. The high-affinity IL-2R is comprised of three distinct subunits: IL-2RA, IL-2RB and IL-2RG. Because of the central role of the IL-2/IL-2R system in the mediation of the immune system, IL-2 receptor is a potential target of immunologic therapy in autoimmune disease (Morris and Waldmann, 2000). *IL-2RA* was initially identified as a candidate gene for type 1 diabetes mellitus and autoimmune thyroid diseases (Vella et al., 2005).

However, there is still some controversy regarding the association of *IL-2R* and RA. Danoy et al. (2011) investigated the polymorphisms of 19 distinct RA genomic regions including *IL-2RA* and *IL-2RB* (571 cases, 880 controls), and revealed that *IL-2RA* and *IL-2RB* were not associated with RA in the Han Chinese population.

In the present study, we investigated the association between rs2104286 in *IL-2RA* and rs743777 in *IL-2RB* and RA in a Han Chinese group composed of 500 RA patients (346 of them were anti-CCP-positive and 427 of them were RF-positive) and 600 controls. Neither of the two SNPs showed significant association with RA ($P > 0.05$). Genetic heterogeneity between populations may be explained by the following factors: first, dozens of genetic variations are now thought to be involved in disease pathogenesis; second, the allele frequency of each susceptibility variant differs between populations; third, gene-environment interactions and gene-gene interactions may affect the contribution of each genetic factor to disease susceptibility, and account for the genetic heterogeneity between populations (Kochi et al., 2010).

The associations of the two SNPs with RA were also analyzed after stratification by CCP and RF. However, none of the subgroups showed any significant association with RA risk. The lack of a significant association in our patients with RA could reflect a relatively small contribution of *IL-2RA* and *IL-2RB* polymorphisms to disease susceptibility in Chinese RA patients compared with Europeans.

In conclusion, the two genetic variants within *IL-2RA* and *IL-2RB* are not associated with genetic susceptibility to RA in the Han Chinese population. In addition, stratification by CCP and RF does not reveal significant association with RA risk, and genotypes of rs2104286 and rs743777 are not significantly associated with concentrations of anti-CCP antibodies or RF. Our data suggest that there is genetic heterogeneity between different ethnicities. Investigations of the ethnic heterogeneity of genetic factors will provide information about disease heterogeneity and facilitate individualized medicine.

ACKNOWLEDGMENTS

Research supported by the Department of Public Health of Sichuan Province (#100447 to J. Zhu and #110140 to F. He) and the Department of Science and Technology of Sichuan Province (#06SG1964 to D.D. Zhang). We thank all patients and family members for their participation.

REFERENCES

Arnett FC, Edworthy SM, Bloch DA, McShane DJ, et al. (1988). The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum.* 31: 315-324.

Danoy P, Wei M, Johanna H, Jiang L, et al. (2011). Association of variants in MMEL1 and CTLA4 with rheumatoid arthritis in the Han Chinese population. *Ann. Rheum. Dis.* 70: 1793-1797.

Firestein GS (2003). Evolving concepts of rheumatoid arthritis. *Nature* 423: 356-361.

Foster MW and Freeman WL (1998). Naming names in human genetic variation research. *Genome Res.* 8: 755-757.

Hardy J and Singleton A (2009). Genomewide association studies and human disease. *N. Engl. J. Med.* 360: 1759-1768.

Hinks A, Ke X, Barton A, Eyre S, et al. (2009). Association of the IL2RA/CD25 gene with juvenile idiopathic arthritis. *Arthritis Rheum.* 60: 251-257.

Isaacs JD (2010). The changing face of rheumatoid arthritis: sustained remission for all? *Nat. Rev. Immunol.* 10: 605-611.

Kochi Y, Suzuki A, Yamada R and Yamamoto K (2010). Ethnogenetic heterogeneity of rheumatoid arthritis-implications for pathogenesis. *Nat. Rev. Rheumatol.* 6: 290-295.

Kurreeman FA, Daha NA, Chang M, Catanese JJ, et al. (2009). Association of IL2RA and IL2RB with rheumatoid arthritis: a replication study in a Dutch population. *Ann. Rheum. Dis.* 68: 1789-1790.

Malek TR (2008). The biology of interleukin-2. *Annu. Rev. Immunol.* 26: 453-479.

Mori M, Yamada R, Kobayashi K, Kawaida R, et al. (2005). Ethnic differences in allele frequency of autoimmune-disease-associated SNPs. *J. Hum. Genet.* 50: 264-266.

Morris JC and Waldmann TA (2000). Advances in interleukin 2 receptor targeted treatment. *Ann. Rheum. Dis.* 59 (Suppl 1): i109-i114.

Plenge RM (2009). Recent progress in rheumatoid arthritis genetics: one step towards improved patient care. *Curr. Opin. Rheumatol.* 21: 262-271.

Silman AJ and Pearson JE (2002). Epidemiology and genetics of rheumatoid arthritis. *Arthritis Res.* 4 (Suppl 3): S265-S272.

Vella A, Cooper JD, Lowe CE, Walker N, et al. (2005). Localization of a type 1 diabetes locus in the IL2RA/CD25 region by use of tag single-nucleotide polymorphisms. *Am. J. Hum. Genet.* 76: 773-779.