Rheumatology 2006;45:1345–1348
Advance Access publication 11 May 2006
doi:10.1093/rheumatology/kel169

Concise Report

Haplotype analysis revealed no association between the *PTPN22* gene and RA in a Japanese population

K. Ikari, S. Momohara, E. Inoue, T. Tomatsu, M. Hara, H. Yamanaka and N. Kamatani

***Objective*. The protein tyrosine phosphatase non-receptor type 22 (*PTPN22*) gene is a member of the PTPs that negatively regulate T-cell activation. A missense single nucleotide polymorphism (SNP) in the *PTPN22* gene known as R620W was recently reported to be associated with several autoimmune diseases including rheumatoid arthritis (RA). The association was confirmed repeatedly in the populations of North European ancestry. However, the SNP was reported to be non-polymorphic in the Asian populations. Because the gene confers an impact on autoimmune diseases, we attempt to explore an association between *PTPN22* gene and RA in a Japanese population without restricting to the SNP, R620W.**

***Methods*. We studied 1128 RA patients and 455 controls. In addition to the SNP, R620W, we selected eight testing SNPs spanning 45 kb over the *PTPN22* gene using the International HapMap Project. Genotyping was performed using the TaqMan fluorogenic 5′ nuclease assay. Associations between RA and each of the SNPs were estimated by the Fisher's exact test. Haplotype was constructed using the expectation-maximization algorithm.**

***Results*. R620W was not polymorphic enough in both the patients and the controls, and was therefore excluded from further analysis. Each allele frequency for the eight other SNPs in both groups was compared and no association was detected. Haplotype analysis also revealed that *PTPN22* gene was not associated with RA in a Japanese population.**

***Conclusion*. We found no association between *PTPN22* and RA in a Japanese population. The result suggests that the *PTPN22* gene is associated with RA only in a specific ethnic group.**

KEY WORDS: Rheumatoid arthritis, PTPN22, Association, Haplotype, IORRA.

Introduction

Autoimmune disease (MIM 109100) is one of the common diseases affecting up to 5% of the population. It is characterized by an abnormal immune response to self-antigens. Among all the systemic autoimmune diseases, rheumatoid arthritis (RA, MIM 180300) is the most common, afflicting up to 1% of the adult population worldwide. The disease susceptibility has been estimated to have a genetic component of 60% [1].

Bottini *et al*. [2] first described that a missense single nucleotide polymorphism (SNP) known as R620W (rs2476601) in the protein tyrosine phosphatase non-receptor type 22 (*PTPN22*) gene was associated with susceptibility to type I diabetes. Begovich *et al*. [3] also reported that this gene was associated with RA in a North American population. Since then, the association was confirmed repeatedly in the populations of the European ancestry [4–7]. Furthermore, the SNP was found to be associated with other autoimmune diseases, such as systemic lupus erythematosus, Graves' disease and juvenile idiopathic arthritis [5–7].

T-cells play a central role in the immunopathogenesis of autoimmune diseases and are also the key regulators of the destructive joint lesions [8]. The *PTPN22* gene is a member of the PTPs that are involved in the negative regulation of T-cell signalling through its interaction with C-terminal Src tyrosine kinase (Csk) [9]. The amino change in PTPN22 caused by the functional SNP disrupts the binding to Csk that leads to the overactivity of the immune system [2, 3]. Thus, this susceptibility gene with the functional polymorphism is thought as one of the main players to autoimmune diseases outside the human leucocyte antigen locus [10].

Although the association of the R620W SNP and autoimmune diseases was validated repeatedly in the North European descents including the pathogenetic role of *PTPN22* in the diseases, the functional SNP was non-polymorphic and the association could not be confirmed in the Asian populations [3, 11]. Recently, Carlton *et al*. [12] described that an SNP in the 3′ untranslated region of the gene (rs3789604) is also associated with RA, independent of R620W. This finding suggests that in addition to R620W, the association between the diseases and the *PTPN22* gene in the Asian populations needs to be studied more extensively.

In this study, we attempt to find an association between *PTPN22* gene and RA patients in a large Japanese population using the HapMap-tagged SNPs that covers the *PTPN22* gene.

Materials and methods

Subjects and disease criteria

Approval for this study was granted by Tokyo Women's Medical University Genome Ethics Committee. The study was part of an observational cohort project that included over 4000 Japanese RA patients, established in the year 2000 by the Institute of

Institute of Rheumatology, Tokyo Women's Medical University, Tokyo, Japan.

Received 3 March 2006; revised version accepted 7 April 2006.

Correspondence to: Katsunori Ikari, MD, PhD, Institute of Rheumatology, Tokyo Women's Medical University, 10-22 Kawada, Shinjuku, Tokyo 162-0054, Japan. E-mail: kikari@ior.twmu.ac.jp

© The Author 2006. Published by Oxford University Press on behalf of the British Society for Rheumatology. All rights reserved. For Permissions, please email: journals.permissions@oxfordjournals.org

Downloaded from https://academic.oup.com/rheumatology/article/45/11/1345/2255872 by U.S. Department of Justice user on 16 August 2022

TABLE 1. Distribution of the *PTPN22* polymorphisms in rheumatoid arthritis patients and controls

			Genotypes of patient[a]					Genotypes of control					Allele 1 *vs* 2	
ID	dbSNP ID	Position[b]	1/1	1/2	2/2	Sum	MAF	1/1	1/2	2/2	Sum	MAF	OR (95% CI)	*P*
1	rs17031952	10494939	1058	62	3	1123	0.03	425	27	1	453	0.03	1.06 (0.66–1.67)	0.82
2	rs3789608	10483903	699	359	57	1115	0.21	284	152	19	455	0.21	0.98 (0.81–1.19)	0.85
3	rs3765598	10480578	707	360	54	1121	0.21	280	149	19	448	0.21	1.00 (0.82-1.21)	1.00
4	rs1746853	10469212	489	491	134	1114	0.34	200	199	50	449	0.33	0.97 (0.82–1.14)	0.71
5	rs2476601	10463683	1119	5	0	1124	0.002	451	2	0	453	0.002	NC	NC
6	rs2797415	10463208	385	541	198	1124	0.42	153	208	78	439	0.41	0.99 (0.84–1.16)	0.94
7	rs2476600	10455849	701	348	51	1100	0.20	282	153	17	452	0.21	1.01 (0.83–1.23)	0.88
8	rs3789607	10452549	864	155	4	1023	0.08	385	66	3	454	0.08	0.99 (0.73–1.34)	1.00
9	rs2476599	10449574	835	227	24	1086	0.13	340	99	8	447	0.13	1.02 (0.80–1.29)	0.91

[a]The major allele was always referred to as allele 1 and the minor allele as allele 2. Values indicate number of subjects except for MAF.
[b]Positions are according to genomic contig NT_019273. SNPs are listed in the order from the 5′ end of the gene to the 3′ end.
SNP, single nucleotide polymorphism; MAF, minor allele frequency; OR, odds ratio; 95% CI, 95% confidence intervals; NC, not calculated.

Rheumatology, Tokyo Women's Medical University (IORRA: Institute of Rheumatology RA cohort). The diagnosis of RA was established using the classification criteria of the American College of Rheumatology (formerly, the American Rheumatism Association) 1987 revised criteria [13]. Out of the registered RA patients, DNA samples were obtained from 1284 patients. Informed written consent was obtained from every subject. Of these, 1128 samples were randomly selected for this study. About 88% of the patients were RF positive and they were mostly females (82.6%). A total of 455 population-based control DNA samples were obtained from the Pharma SNP Consortium (http://www.jpma.or.jp/psc/index.html). All control subjects were matched for sex, ethnic origin and geographical area.

Selection of SNPs

In addition to the functional R620W SNP, we selected eight SNPs that allowed us to describe the haplotypes detected in the international HapMap project (release 19 October 2005) (Table 1) [14]. We used SNP 3 (rs3765598) instead of rs3789604, a risk polymorphism for RA independent of R620W, for the study because the supplier (Applied Biosystems, Tokyo, Japan) was unable to manufacture the assay of rs3789604 [12]. SNP3 was almost in absolute linkage disequilibrium (LD) with rs3789604 and minor alleles of these SNPs were carried by a single haplotype according to the HapMap in Japanese samples ($D' = 1.0$, $R^2 = 0.94$) [14]. We finally selected nine SNPs spanning 45 kb over the *PTPN22* gene for genotyping.

Statistical power

Our study was designed to have >90% power at a 5% significance level to detect the odds ratio (OR) of 1.40 conferred by the risk allele of SNP 3 (rs3765598, 15.6% frequency in the controls) [12]. Statistical power was calculated using a web power calculator (http://calculators.stat.ucla.edu/powercalc/).

Genotyping

Genotyping was carried out using the TaqMan fluorogenic 5′ nuclease assay (Applied Biosystems). The final volume of polymerase chain reaction (PCR) was 5 μl, containing 2 ng of genomic DNA and 2.5 μl TaqMan Universal PCR Master Mix, with 0.125 μl of 40× Assay Mix or 0.25 μl of 20× Assay Mix. Thermal cycle conditions were as follows: 50°C for 2 min to activate the uracil *N*-glycosylase and to prevent carry-over contamination, 95°C for 10 min to activate the DNA polymerase, followed by 40 cycles of 92°C for 15 s and 60°C for 1 min. All PCR were performed using 384-well plates by a Dual 384-Well GeneAmp PCR System 9700 (Applied Biosystems) and the endpoint fluorescent readings were performed on an ABI PRISM 7900 HT Sequence Detection System (Applied Biosystems). Duplicate samples and negative controls were included to ensure accuracy of genotyping.

Statistical analysis

Allele frequencies were estimated by the gene counting method. The exact test of Hardy–Weinberg equilibrium was used to compare the observed numbers of each genotype with those expected for a population in the Hardy–Weinberg equilibrium (http://cran.r-project.org/src/contrib/Descriptions/genetics.html). Associations between RA and each of the SNPs or haplotypes were estimated by the Fisher's exact test. These tests were implemented in the R software package version 2.0.1 (http://www.r-project.org/). The expectation-maximization algorithm implemented in the LDSUPPORT program was used for the LD analysis and the haplotype estimation [15].

Results

From the genotyping result, the functional R620W SNP was not polymorphic enough in both the patients and the controls, and therefore it was excluded from further analysis (minor allele frequency was 0.0022 and 0.0022, respectively). This result was consistent with the results reported previously [3, 11]. Allele frequencies for the eight other SNPs that cover the gene were in Hardy–Weinberg equilibrium in both the patients and the controls. Each allelic frequency of the SNP in both the groups was nearly equal and no association was detected when compared independently (OR = 0.97–1.06) (Table 1). Allelic frequency of SNP3 that was substitute for novel risk polymorphism of RA, rs3789604, was completely equal in patients and controls ($P = 1.00$, OR = 1.0) [12]. Stratifying the patients by the presence of RF or sex also revealed no evidence of association with RA (data not shown).

We calculated D′ values for all SNP pairs to assess the LD across the *PTPN22* gene (Fig. 1). The pairwise D′ values in the gene were nearly 1 among almost all SNP pairs, indicating that the SNPs were highly associated with each other and the entire *PTPN22* was contained within a single LD block. Haplotype analysis predicted five common (frequency >1%) haplotypes and revealed that the *PTPN22* gene was not associated with RA in this Japanese cohort (Table 2).

Discussion

In our study, the association between the *PTPN22* gene and RA was investigated using a large Japanese RA patient cohort. Our data revealed no association between RA and the *PTPN22*

Downloaded from https://academic.oup.com/rheumatology/article/45/11/1345/2255872 by U.S. Department of Justice user on 16 August 2022

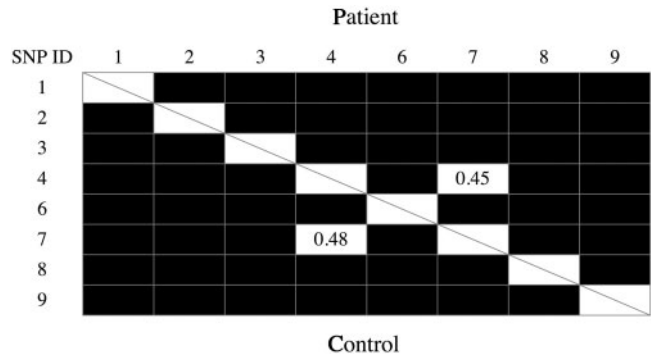

FIG. 1. Pairwise linkage disequilibrium between eight SNPs measured by D' value in the patient and control populations in the *PTPN22* gene: upper triangle, patient population; lower triangle, control population. A black cell means $|D'| > 0.9$, otherwise actual values were shown. SNP 5 was excluded from this analysis because it was not polymorphic enough. SNP, single nucleotide polymorphism.

TABLE 2. *PTPN22* haplotype structure and frequencies[a]

	SNP ID								Haplotype comparison[b]		
Haplotype	1	2	3	4	6	7	8	9	Patients	Controls	*P*
1	T	C	C	T	T	A	T	G	0.55	0.55	0.94
2	T	T	T	G	C	A	T	G	0.21	0.21	1
3	T	C	C	G	C	G	T	A	0.13	0.13	0.95
4	T	C	C	T	C	G	C	G	0.08	0.08	0.77
5	C	C	C	T	T	A	T	G	0.03	0.03	0.82

[a]The program, LDSUPPORT, was used to estimate common (frequency >0.01) haplotypes for eight of the nine SNPs genotyped. The SNP 5, R620W, was not polymorphic enough and was excluded from this analysis.

[b]Each haplotype was compared with the other haplotypes combined. SNP, single nucleotide polymorphism.

gene not only in the functional R620W SNP but also in the eight HapMap tagged SNPs or haplotypes that cover the gene.

Lack of association might be due to a lack of statistical power. However, based on a recent study that detected novel risk allele for RA susceptibility, a statistical power of >90% could be achieved with 2256 chromosomes of patients and 910 chromosomes of controls ($P = 0.05$, OR = 1.40 and a 15.6% frequency of risk allele) [12]. The statistical power is sufficient to detect a risk greater than OR 1.40 and if the OR is lower the negative result might be due to a type II error. It might be noticed that the recent findings on peptidyl arginine deiminase, type IV gene (PADI4) as a risk of RA show a different magnitude of genetic risk according to the ethnic differences [16].

Ethnic differences may play a role in the conflicting results among the genetic association studies [11]. A replication study using a population with a different ethnicity allows us to know whether a reported association is caused by a common variance through different ethnicities. In the case of *PTPN22* gene and RA, the disease responsible polymorphism R620W found in the populations of North European ancestry is extremely rare in the Asian populations, and so the R620W alone cannot explain the disease susceptibility in the Asian populations [3, 11]. Since the causal variant of a disease might differ between populations, the failure to replicate an association may reflect an incomplete analysis of the variation within the gene of interest. For this reason, we conducted an expanded analysis using the HapMap to clarify the association in a Japanese population. We still did not find evidence of the association between RA and each of the eight HapMap-tagged SNPs that covered the gene nor the haplotypes.

In conclusion, our study suggests that *PTPN22* gene may be associated with RA only in a specific ethnic group.

Rheumatology	Key messages
	• *PTPN22* is not associated with RA in a Japanese population. • *PTPN22* may be associated with RA only in a specific ethnic group.

Acknowledgements

We thank all the DNA donors for making this study possible. We are grateful to A. C. Tang for her assistance in preparing the manuscript and K. Arai for her technical efforts. We appreciate A. Taniguchi and other members of our Institute for their efforts on the cohort project. This work was supported by a grant provided by the Japan Rheumatism Foundation (to K.I.) and a Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (to S.M.).

The authors have declared no conflicts of interest.

References

1. MacGregor AJ, Snieder H, Rigby AS *et al*. Characterizing the quantitative genetic contribution to rheumatoid arthritis using data from twins. Arthritis Rheum 2000;43:30–7.
2. Bottini N, Musumeci L, Alonso A *et al*. A functional variant of lymphoid tyrosine phosphatase is associated with type I diabetes. Nat Genet 2004;36:337–8.
3. Begovich AB, Carlton VE, Honigberg LA *et al*. A missense single-nucleotide polymorphism in a gene encoding a protein tyrosine phosphatase (PTPN22) is associated with rheumatoid arthritis. Am J Hum Genet 2004;75:330–7.
4. Ladner MB, Bottini N, Valdes AM, Noble JA. Association of the single nucleotide polymorphism C1858T of the PTPN22 gene with type 1 diabetes. Hum Immunol 2005;66:60–4.
5. Hinks A, Barton A, John S *et al*. Association between the PTPN22 gene and rheumatoid arthritis and juvenile idiopathic arthritis in a UK population: further support that PTPN22 is an autoimmunity gene. Arthritis Rheum 2005;52:1694–9.
6. Orozco G, Sanchez E, Gonzalez-Gay MA *et al*. Association of a functional single-nucleotide polymorphism of PTPN22, encoding lymphoid protein phosphatase, with rheumatoid arthritis and systemic lupus erythematosus. Arthritis Rheum 2005;52:219–24.
7. Smyth D, Cooper JD, Collins JE *et al*. Replication of an association between the lymphoid tyrosine phosphatase locus (LYP/PTPN22) with type 1 diabetes, and evidence for its role as a general autoimmunity locus. Diabetes 2004;53:3020–3.
8. Klimiuk PA, Yang H, Goronzy JJ, Weyand CM. Production of cytokines and metalloproteinases in rheumatoid synovitis is T cell dependent. Clin Immunol 1999;90:65–78.
9. Cloutier JF, Veillette A. Association of inhibitory tyrosine protein kinase p50csk with protein tyrosine phosphatase PEP in T cells and other hemopoietic cells. EMBO J 1996;15:4909–18.
10. Deighton CM, Walker DJ, Griffiths ID, Roberts DF. The contribution of HLA to rheumatoid arthritis. Clin Genet 1989;36:178–82.

Downloaded from https://academic.oup.com/rheumatology/article/45/11/1345/2255872 by U.S. Department of Justice user on 16 August 2022

11. Mori M, Yamada R, Kobayashi K, Kawaida R, Yamamoto K. Ethnic differences in allele frequency of autoimmune-disease-associated SNPs. J Hum Genet 2005;50:264–6.
12. Carlton VE, Hu X, Chokkalingam AP *et al.* PTPN22 genetic variation: evidence for multiple variants associated with rheumatoid arthritis. Am J Hum Genet 2005;77:567–81.
13. Arnett FC, Edworthy SM, Bloch DA *et al.* The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
14. Altshuler D, Brooks LD, Chakravarti A, Collins FS, Daly MJ, Donnelly P. A haplotype map of the human genome. Nature 2005;437:1299–320.
15. Kitamura Y, Moriguchi M, Kaneko H *et al.* Determination of probability distribution of diplotype configuration (diplotype distribution) for each subject from genotypic data using the EM algorithm. Ann Hum Genet 2002;66:183–93.
16. Iwamoto T, Ikari K, Nakamura T *et al.* Association between PADI4 and rheumatoid arthritis: a meta-analysis. Rheumatology 2006; [Epub ahead of print].

Downloaded from https://academic.oup.com/rheumatology/article/45/11/1345/2255872 by U.S. Department of Justice user on 16 August 2022