Journal of Human Genetics (2009) 54, 62–65

www.nature.com/jhg

ORIGINAL ARTICLE

Two-stage case–control association study of polymorphisms in rheumatoid arthritis susceptibility genes with schizophrenia

Yuichiro Watanabe[1], Ayako Nunokawa[1], Naoshi Kaneko[1], Tatsuyuki Muratake[2], Tadao Arinami[3], Hiroshi Ujike[4], Toshiya Inada[5], Nakao Iwata[6], Hiroshi Kunugi[7], Masanari Itokawa[8], Takeshi Otowa[9], Norio Ozaki[10] and Toshiyuki Someya[1]

There is strong evidence for a negative association between schizophrenia and rheumatoid arthritis (RA). However, the mechanism for this association is unknown. We hypothesize that these two diseases share susceptibility genes. Recently, extensive studies have identified some RA susceptibility genes, including *NFKBIL1*, *SLC22A4*, *RUNX1*, *FCRL3* and *PADI4*, in the Japanese population. To assess whether polymorphisms in these RA susceptibility genes are implicated in vulnerability to schizophrenia, we conducted a two-stage case–control association study in Japanese subjects. In a screening population of 534 patients and 559 control subjects, we examined eight polymorphisms in RA susceptibility genes and found a potential association of padi4_94 in *PADI4* with schizophrenia. However, we could not replicate this association in a confirmatory population of 2126 patients and 2228 control subjects. The results of this study suggest that these polymorphisms in RA susceptibility genes do not contribute to genetic susceptibility to schizophrenia.
Journal of Human Genetics (2009) **54,** 62–65; doi:10.1038/jhg.2008.4; published online 9 January 2009

Keywords: case–control study; Japanese; rheumatoid arthritis; schizophrenia; single-nucleotide polymorphism

INTRODUCTION

Schizophrenia is a chronic mental disorder and rheumatoid arthritis (RA) is a chronic inflammatory disease. Both diseases affect approximately 1% of the world population. There is strong evidence for a negative association between schizophrenia and RA, implying low comorbidity of these disorders.[1–3] However, the mechanism for this association is unknown. Genetic epidemiological studies including family, twin and adoption studies have shown that genetic factors play important roles in the pathogeneses of these two diseases.[4] Interestingly, major histocompatibility complex (MHC), class II, DR β 1 (*HLA-DRB1*) is associated with both schizophrenia[5–7] and RA.[8,9] These findings led us to hypothesize that these two diseases share susceptibility genes.

Recently, extensive studies have identified some RA susceptibility genes in the Japanese population. The study by Okamoto *et al.*[10] identified a second RA susceptibility locus within the MHC region of the short arm of chromosome 6 as the T allele at position −61 in the promoter region of nuclear factor of κ light polypeptide gene enhancer in B-cells inhibitor-like 1 (*NFKBIL1*). The study by Tokuhiro *et al.*[11] reported an association between slc2F2, an intronic single-nucleotide polymorphism (SNP) in a Runt-related transcription factor 1 (RUNX1) binding site of solute carrier family 22, member 4 (*SLC22A4*) on the 5q31 cytokine cluster region and RA. In that study, an SNP (runx1) of *RUNX1* on 21q22.3 was also associated with RA. Kochi *et al.*[12] showed an association of a functional SNP at position −169 (fcrh3_3) in the promoter region of *Fc* receptor-like 3 (*FCRL3*) on 1q21–23 with RA. This SNP altered the binding affinity for nuclear factor-κB and regulated the expression of *FCRL3* mRNA. The study by Suzuki *et al.*[13] found that the haplotype of peptidylarginine deiminase type IV (*PADI4*) on 1p36, which affects the stability of transcripts, was associated with RA. A recent meta-analysis revealed associations of *PADI4*, *FCRL3* and *SLC22A4* with RA in East Asian populations.[14] To assess whether these polymorphisms of RA susceptibility genes are implicated in vulnerability to schizophrenia,

[1]Department of Psychiatry, Niigata University Graduate School of Medical and Dental Sciences, Chuo-ku, Niigata, Japan; [2]Furumachi Mental Clinic, Chuo-ku, Niigata, Japan; [3]Department of Medical Genetics, Doctoral Program in Social and Environmental Medicine, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan; [4]Department of Neuropsychiatry, Okayama University, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan; [5]Seiwa Hospital, Institute of Neuropsychiatry, Shinjuku-ku, Tokyo, Japan; [6]Department of Psychiatry, Fujita Health University School of Medicine, Toyoake, Aichi, Japan; [7]Department of Mental Disorder Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Kodaira, Tokyo, Japan; [8]Schizophrenia Research Project, Tokyo Institute of Psychiatry, Setagaya, Tokyo, Japan; [9]Department of Neuropsychiatry, Graduate School of Medicine, University of Tokyo, Bunkyo, Tokyo, Japan and [10]Department of Psychiatry, School of Medicine, Nagoya University, Showa-ku, Nagoya, Aichi, Japan

Correspondence: Dr Y Watanabe, Department of Psychiatry, Niigata University Graduate School of Medical and Dental Sciences, 757 Asahimachidori-ichibancho, Chuo-ku, Niigata 951-8510, Japan.

E-mail: yuichiro@med.niigata-u.ac.jp

Received 7 October 2008; revised 30 October 2008; accepted 8 November 2008; published online 9 January 2009

we performed a two-stage case–control association study in Japanese subjects.

MATERIALS AND METHODS

Subjects

This study was approved by the Ethics Committee of each participating institute, and written informed consent was obtained from each participant. All the participants were unrelated Japanese subjects. The screening population consisted of 534 patients with schizophrenia (281 men and 253 women; mean age, 40.1 (s.d. 14.2) years) and 559 control subjects (297 men and 262 women; mean age, 37.3 (s.d. 10.1) years). The confirmatory population consisted of 2126 patients with schizophrenia (1137 men and 989 women; mean age, 47.3 (s.d. 14.3) years) and 2228 control subjects (1189 women and 1039 men; mean age, 46.6 (s.d. 13.9) years). The patients were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders Fourth Edition (DSM-IV) criteria consensus of at least two experienced psychiatrists on the basis of all available sources of information, including unstructured interviews, clinical observations and medical records. The control subjects were mentally healthy subjects with no self-reported history of psychiatric disorders who showed good social and occupational skills, although they were not assessed by a structured psychiatric interview.

Genotyping

Genomic DNA was extracted from peripheral blood using a standard phenol/chloroform method. We examined eight polymorphisms in five RA susceptibility genes. We included the SNPs that showed the strongest associations in the original studies, namely T-62A (rs2071592) in *NFKBIL1*,[10] slc2F1 (rs2073838) in *SLC22A4*,[11] runx1 (rs2268277) in *RUNX1*,[11] fcrh3_3 (rs7528684) in *FCRL3*[12] and padi4_94 (rs2240340) in *PADI4*.[13] We also examined slc2F2 (rs3792876), which was found to affect the transcriptional efficiency of *SLC22A4*.[11] For *PADI4*, we genotyped two additional SNPs (padi4_104 (rs1748033) and padi4_102 (rs2240337)) because Ikari *et al*.[15] indicated that padi4_94, padi4_104 and padi4_102 allowed them to describe the four haplotypes detected in the original study.

All the SNPs were genotyped using a TaqMan 5′-exonuclease assay as described earlier.[16]

Statistical analysis

Deviation from the Hardy–Weinberg equilibrium (HWE) was tested using the χ^2 test. Pair-wise linkage disequilibrium (LD) indices (D' and r^2) and haplotype frequencies were determined using Haploview v4.0.[17] Genotypic associations were examined using the χ^2 test or Fisher's exact test. The allele and haplotype frequencies of the patients and control subjects were compared using the χ^2 test. A probability level of $P<0.05$ was considered to indicate statistical significance.

A power calculation was performed using Genetic Power Calculator.[18] The power was estimated with an α of 0.05, assuming that the disease prevalence was 0.01 and the risk allele frequencies were the values observed in control samples.

RESULTS

We genotyped eight SNPs in RA susceptibility genes in a screening population (Table 1). The genotype distributions of these SNPs did not deviate significantly from the HWE in both groups. We found a potential association between padi4_94 and schizophrenia (allelic uncorrected $P=0.047$).

Two SNPs (slc2F2 and slc2F1) in *SLC22A4* were in strong LD ($D'=0.99$; $r^2=0.97$) and three SNPs (padi4_94, padi4_104 and padi4_102) in *PADI4* were in LD ($D'=0.80$–1; $r^2=0.09$–0.82). The degree of LD between the two SNPs in *SLC22A4* was very similar to that of the HapMap data ($D'=1$; $r^2=1$). The degree of LD between the three SNPs in *PADI4* was higher than that of the HapMap data ($D'=0.37$–1; $r^2=0.01$–0.96), but very similar to that found in a previous study ($D'=0.76$–1).[15] Next, we performed haplotype analyses of *SLC22A4* and *PADI4* in the screening population (Table 2). There were no significant differences in the haplotype frequencies of *SLC22A4* and *PADI4* between the two groups.

To confirm the potential association between padi4_94 and schizophrenia, we examined this SNP in a confirmatory population (Table 3). However, we were unable to replicate this association. We were also unable to find this association in a combined population comprising the screening and confirmatory populations.

DISCUSSION

In our two-stage case–control study, we found no associations of eight polymorphisms in RA susceptibility genes with schizophrenia in the

Table 2 Haplotype analyses of *SLC22A4* and *PADI4* in a screening population

Haplotype	*Patients*	*Controls*	P-*value*
slc2F2-slc2F1			0.340[a]
1-1	0.676	0.656	0.334
2-2	0.318	0.337	0.348
padi4_94-padi4_104-padi4_102			0.283[a]
1-1-1	0.574	0.610	0.091
2-2-1	0.297	0.266	0.114
2-2-2	0.073	0.069	0.765
2-1-1	0.050	0.042	0.359

[a]Global *p*.

Table 1 Genotype and allele frequencies of eight polymorphisms in the rheumatoid arthritis susceptibility genes in a screening population

	Patients						*Controls*						P-*value*	
SNP	n	*HWE*	*1/1*[a]	*1/2*[a]	*2/2*[a]	*MAF*	n	*HWE*	*1/1*[a]	*1/2*[a]	*2/2*[a]	*MAF*	*Genotype*	*Allele*
T-62A	531	0.168	178	272	81	0.409	556	0.067	193	251	112	0.427	0.054	0.382
slc2F2	526	0.589	245	224	57	0.321	552	0.210	246	235	71	0.342	0.557	0.320
slc2F1	532	0.792	245	234	53	0.320	556	0.516	247	242	67	0.338	0.536	0.356
runx1	530	0.495	174	266	90	0.421	556	0.148	213	249	94	0.393	0.138	0.188
fcrh3_3	532	0.150	195	267	70	0.383	559	0.997	216	263	80	0.378	0.576	0.841
padi4_94	533	0.898	179	261	93	0.419	556	0.954	215	262	79	0.378	0.139	0.047
padi4_104	533	0.972	212	248	73	0.370	557	0.473	242	256	59	0.336	0.216	0.098
padi4_102	531	0.431	449	80	2	0.079	558	0.658	469	86	3	0.082	0.957[b]	0.775

Abbreviations: HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency; SNP, single-nucleotide polymorphism.
[a]Genotypes, major and minor alleles are denoted by 1 and 2, respectively.
[b]Calculated using Fisher's exact test.

Table 3 Genotype and allele frequencies of padi4_94 in confirmatory and combined populations

	Patients						*Controls*						P-*value*	
Population	n	*HWE*	*1/1*[a]	*1/2*[a]	*2/2*[a]	*MAF*	n	*HWE*	*1/1*[a]	*1/2*[a]	*2/2*[a]	*MAF*	*Genotype*	*Allele*
Confirmatory	2088	0.243	801	964	323	0.386	2209	0.915	787	1061	361	0.404	0.176	0.087
Combined	2621	0.309	980	1225	416	0.392	2765	0.925	1002	1323	440	0.398	0.658	0.527

Abbreviations: HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency.
[a]Genotypes, major and minor alleles are denoted by 1 and 2, respectively.

Japanese population. Although there was a marginally significant association between padi4_94 and schizophrenia in the screening population, this association could not be replicated in the confirmatory population. The positive association observed in the screening population was probably because of a type I error. It is unlikely that the negative results in the confirmatory and combined populations were due to type II errors because the power calculation showed that, when the genotypic relative risk was set at 1.3 for homozygous risk allele carriers under the multiplicative model of inheritance, the power was more than 0.80 in the confirmatory population.

To the best of our knowledge, the RA susceptibility genes examined in this study have not been tested for their associations with schizophrenia, with the exception of *NFKBIL1*. *NFKBIL1* is located on 6p21.3 where a genome scan meta-analysis has suggested a locus of susceptibility to schizophrenia.[19] The study by Shirt *et al*.[20] investigated 26 SNPs covering a 100-kb region centered on D6S2672, including two SNPs in *NFKBIL1*. These two SNPs in *NFKBIL1* were not associated with schizophrenia in their Caucasian samples of 236 patients and 240 control subjects. The study by Morar *et al*.[21] analyzed 36 SNPs in a 165-kb region around tumor necrosis factor, including five SNPs in *NFKBIL1*. There were no significant associations of these five SNPs in *NFKBIL1* with schizophrenia in their sample of 204 families (79 sib-pairs and 125 trios). In this study, we did not find an association between T-62A in *NFKBIL1* and schizophrenia in our Japanese samples of 534 patients and 559 control subjects. Our results are in line with the negative findings reported in the previous studies.[20,21] To draw a definitive conclusion that *NFKBIL1* does not contribute to genetic susceptibility to schizophrenia, however, further studies using larger sample sizes and sufficient markers are required in different ethnic populations.

We recognize several limitations of this study. First, we investigated only eight SNPs in five RA susceptibility genes. Overall, 10, 4, 15, 7 and 14 tagging SNPs for *NFKBIL1*, *SLC22A4*, *RUNX1*, *FCRL3* and *PADI4*, respectively, covering these RA susceptibility gene regions and the 5′ and 3′ flanking regions were selected from the HapMap database (release #23a; population: Japanese in Tokyo; minor allele frequency: more than 0.05). We applied the criterion of an r^2 threshold greater than 0.8 in the 'aggressive tagging: use 2- and 3-marker haplotype' mode using the 'Tagger' program[22] as implemented in Haploview v4.0,[17] and all polymorphisms examined, with the exception of runx 1, were forced to be selected as tagging SNPs. Therefore, our results cannot exclude the possible contributions of other polymorphisms in these RA susceptibility genes to the pathogenesis of schizophrenia. Second, the sample size of the screening population was moderate. The power calculation showed that when the genotypic relative risk was set at 1.69 for homozygous risk allele carriers under the multiplicative model of inheritance, the power was more than 0.80 for seven of the examined SNPs, but only 0.43 for padi4_102. Therefore, we could not exclude the possibility that our negative results in the screening population, especially for padi4_102, were because of type II errors. Third, no standardized structured interview was applied to verify the clinical diagnoses of the enrolled patients, but the diagnosis of schizophrenia was assigned on the basis of all available sources of information. The control samples were not well characterized. We cannot exclude the possibility that our control samples may have contained some younger individuals who will suffer from schizophrenia later in life. To the best of our knowledge, however, there were no control subjects who were likely to develop schizophrenia at their present stage of life. Thus it is unlikely that our failure to find a significant association is attributable to misdiagnosis. Fourth, substantial differences in the susceptibility genes to RA exist between Caucasian and Japanese populations. A missense SNP (R620W) in protein tyrosine phosphatase non-receptor type 22 (*PTPN22*) was associated with RA in a Caucasian population,[23] whereas R620W was not polymorphic in a Japanese population.[24] If R620W in *PTPN22* explains the negative association between schizophrenia and RA, an association of R620W with schizophrenia should only be observed in Caucasian populations, although such association studies have not been conducted. In this study, therefore, we evaluated polymorphisms in RA susceptibility genes identified in the Japanese population. However, there are no solid epidemiological data to support a negative association between schizophrenia and RA in the Japanese population. Thus our strategy based on this negative association may not be effective.

In conclusion, this study suggests that the eight polymorphisms in RA susceptibility genes examined do not contribute to genetic susceptibility to schizophrenia in the Japanese population.

ACKNOWLEDGEMENTS

We thank the patients, their families and the healthy volunteers for their participation. This work was supported by a grant for the Promotion of Niigata University Research Projects (to YW).

1 Mors, O., Mortensen, P. B. & Ewald, H. A population-based register study of the association between schizophrenia and rheumatoid arthritis. *Schizophr. Res.* **40,** 67–74 (1999).
2 Oken, R. J. & Schulzer, M. At issue: schizophrenia and rheumatoid arthritis: the negative association revisited. *Schizophr. Bull* **25,** 625–638 (1999).
3 Leucht, S., Burkard, T., Henderson, J., Maj, M. & Sartorius, N. Physical illness and schizophrenia: a review of the literature. *Acta. Psychiatr. Scand.* **116,** 317–333 (2007).
4 Torrey, E. F. & Yolken, R. H. The schizophrenia-rheumatoid arthritis connection: infectious, immune, or both? *Brain Behav. Immun.* **15,** 401–410 (2001).
5 Arinami, T., Otsuka, Y., Hamaguchi, H., Itokawa, M., Aoki, J., Shibuya, H. *et al.* Evidence supporting an association between the DRB1 gene and schizophrenia in Japanese. *Schizophr. Res.* **32,** 81–86 (1998).
6 Sasaki, T., Matsushita, M., Nanko, S., Fukuda, R., Kennedy, J. L. & Tokunaga, K. Schizophrenia and the HLA-DRB1 gene in the Japanese population. *Am. J. Psychiatry* **156,** 771–773 (1999).
7 Akaho, R., Matsushita, I., Narita, K., Okazaki, Y., Okabe, Y., Matsushita, M. *et al.* Support for an association between HLA-DR1 and schizophrenia in the Japanese population. *Am. J. Med. Genet. (Neuropsychiatr. Genet.)* **96,** 725–727 (2000).

8 Wakitani, S., Murata, N., Toda, Y., Ogawa, R., Kaneshige, T., Nishimura, Y. *et al.* The relationship between HLA-DRB1 alleles and disease subsets of rheumatoid arthritis in Japanese. *Br. J. Rheumatol.* **36,** 630–636 (1997).
9 Kochi, Y., Yamada, R., Kobayashi, K., Takahashi, A., Suzuki, A., Sekine, A. *et al.* Analysis of single-nucleotide polymorphisms in Japanese rheumatoid arthritis patients shows additional susceptibility markers besides the classic shared epitope susceptibility sequences. *Arthritis. Rheum.* **50,** 63–71 (2004).
10 Okamoto, K., Makino, S., Yoshikawa, Y., Takaki, A., Nagatsuka, Y., Ota, M. *et al.* Identification of *IkBL* as the second major histocompatibility complex-linked susceptibility locus for rheumatoid arthritis. *Am. J. Hum. Genet.* **72,** 303–312 (2003).
11 Tokuhiro, 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.* **35,** 341–348 (2003).
12 Kochi, Y., Yamada, R., Suzuki, A., Harley, J. B., Shirasawa, S., Sawada, T. *et al.* A functional variant in *FcRH3*, encoding Fc receptor homolog 3, is associated with rheumatoid arthritis and several autoimmunities. *Nat. Genet.* **37,** 478–485 (2005).
13 Suzuki, A., Yamada, R., Chang, X., Tokuhiro, S., Sawada, T., Suzuki, M. *et al.* Functional haplotypes of *PADI4*, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. *Nat. Genet.* **34,** 395–402 (2003).
14 Takata, Y., Inoue, H., Sato, A., Tsugawa, K., Miyatake, K., Hamada, D. *et al.* Replication of reported genetic associations of *PADI4*, *FCRL3*, *SLC22A4* and *RUNX1* genes with rheumatoid arthritis: results of an independent Japanese population and evidence from meta-analysis of East Asian studies. *J. Hum. Genet.* **53,** 163–173 (2008).
15 Ikari, K., Kuwahara, M., Nakamura, T., Momohara, S., Hara, M., Yamada, H. *et al.* Association between *PADI4* and rheumatoid arthritis: a replication study. *Arthritis. Rheum.* **52,** 3054–3057 (2005).
16 Watanabe, Y., Muratake, T., Kaneko, N., Nunokawa, A. & Someya, T. No association between the *brain-derived neurotrophic factor* gene and schizophrenia in a Japanese population. *Schizophr. Res.* **84,** 29–35 (2006).
17 Barrett, J. C., Fry, B., Maller, J. & Daly, M. J. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* **21,** 263–265 (2005).
18 Purcell, S., Cherny, S. S. & Sham, P. C. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics* **19,** 149–150 (2003).
19 Lewis, C. M., Levinson, D. F., Wise, L. H., DeLisi, L. E., Straub, R. E., Hovatta, I. *et al.* Genome scan meta-analysis of schizophrenia and bipolar disorder, part II: schizophrenia. *Am. J. Hum. Genet.* **73,** 34–48 (2003).
20 Shirts, B. H., Kim, J. J., Reich, S., Dickerson, F. B., Yolken, R. H., Devlin, B. *et al.* Polymorphisms in *MICB* are associated with human herpes virus seropositivity and schizophrenia risk. *Schizophr. Res.* **94,** 342–353 (2007).
21 Morar, B., Schwab, S. G., Albus, M., Maier, W., Lerer, B. & Wildenauer, D. B. Evaluation of association of SNPs in the TNF alpha gene region with schizophrenia. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **144B,** 318–324 (2007).
22 de Bakker, P. I., Yelensky, R., Pe'er, I., Gabriel, S. B., Daly, M. J. & Altshuler, D. Efficiency and power in genetic association studies. *Nat. Genet.* **37,** 1217–1223 (2005).
23 Begovich, A. B., Carlton, V. E., Honigberg, L. A., Schrodi, S. J., Chokkalingam, A. P., Alexander, H. C. *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.* **75,** 330–337 (2004).
24 Mori, M., Yamada, R., Kobayashi, K., Kawaida, R. & Yamamoto, K. Ethnic differences in allele frequency of autoimmune-disease-associated SNPs. *J. Hum. Genet.* **50,** 264–266 (2005).