SLC22A4 Polymorphism and Rheumatoid Arthritis Susceptibility: A Replication Study in a Japanese Population and a Metaanalysis

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ABSTRACT. ***Objective.*** The *SLC22A4* polymorphisms slc2F1 (rs2073838) and slc2F2 (rs3792876) are reported to be associated with rheumatoid arthritis (RA) in Japanese, but the associations have not been replicated. We assessed the RA susceptibility of slc2F1/F2 polymorphisms.

Methods. We conducted a metaanalysis for slc2F1/F2 polymorphisms to RA susceptibility, which included the replication study of an independent Japanese population consisting of 924 cases and 940 controls. A total of 9 studies (4 Japanese studies, 5 Caucasian studies) consisting of 8076 cases and 6837 controls were included in the metaanalysis.

Results. The replication study demonstrated significant associations in a Japanese population (OR 1.20, 95% CI 1.04–1.37, p = 0.0099, in the allelic mode; OR 1.29, 95% CI 1.08–1.55, p = 0.006, in the dominant mode; p = 0.011 in the trend mode). Significant ethnic diversities of allele frequencies of slc2F1/F2 polymorphisms were found ($p = 8.6*10^{-8}$) between Caucasian and Japanese populations (0.07–0.08 and 0.30–0.32, respectively). The metaanalysis demonstrated significant associations for all studies (fixed-effect OR 1.11, 95% CI 1.05–1.18, p = 0.00084; random-effect OR 1.10, 95% CI 1.02–1.19, p = 0.017 in the allelic mode). Although subgroup analysis did not detect a significant association within Caucasian studies, significant associations were found within Japanese studies (fixed-effect and random-effect OR 1.16, 95% CI 1.07–1.25, p = 0.00012 in the allelic mode).

Conclusion. The associations in Caucasian studies were not significant. Since the significantly low frequency of the risk allele made statistical power lower in Caucasians than in Japanese, whether significant relative risks existed in Caucasian populations was inconclusive. The significant relative risks in Japanese populations were confirmed. (First Release Aug 15 2008; J Rheumatol 2008;35:1723–8)

Key Indexing Terms:
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Rheumatoid arthritis (RA) is a common chronic autoimmune disease characterized by inflammation and destruction of synovial joints, affecting up to 1% of the population worldwide[1]. Although the precise etiology of RA is unknown, both genetic and environmental components to RA susceptibility have been suggested[2]. Familial aggregation and linkage demonstrated significant causative genetic factors in the pathogenesis of RA. The crucial role of the histocompatibility locus antigen (HLA)-DRB1 gene has been confirmed, which accounts for at least 30% of the overall genetic susceptibility[3]. Although identifying a non-HLA susceptibility gene has been challenging, recent case-

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control association studies have reported novel RA susceptible genes, such as *PADI4*[4], *PTPN22*[5], *FCRL3*[6], and *TRAF1-C5*[7].

SLC22A4 (solute carrier family 22, member 4) gene, which is located on chromosome 5q31 and encodes the organic cation transporter OCTN1, was reported to be strongly associated with RA in a Japanese population[8]. The most significant association was observed with a G/A single-nucleotide polymorphism (SNP) in intron 2, called slc2F1 (rs2073838). A C/T polymorphism in intron 1, called slc2F2 (rs3792876), was in strong linkage disequilibrium (LD) with slc2F1 and located within runt-related transcription factor 1 (*RUNX1*) binding site of *SLC22A4*; this polymorphism was suspected to regulate the transcription of *SLC22A4*. Interestingly, inflammatory bowel disease (IBD) 5 region on 5q31, which contains *SLC22A4*, was also reported to have the potential causal variants for Crohn's disease, a chronic inflammatory disease of the gastrointestinal tract, in Caucasian populations[9-11]. Although the association between the SNP and Crohn's disease is controversial[11] these findings suggest that the region containing *SLC22A4* possibly increases the risk of multiple inflammatory diseases in multiple ethnic groups. Attempts to replicate associations for *SLC22A4* polymorphisms and RA have been made in both Caucasian and Japanese populations[12-18]. However, significant associations have not been found. The study conducted by Kuwahara, *et al*[14] in a Japanese population (882 cases, 948 controls) failed to reveal an association, although it had more than 99% statistical power to detect the relative risk reported in the initial study[8]. Studies by Barton, *et al*[13] in a Caucasian population (880 cases, 594 controls in the United Kingdom), Plenge, *et al*[15] (2345 cases, 1721 controls in Swedish and North American populations), and Orozco, *et al*[17] (886 cases, 987 controls in a Spanish population) also had more than 80% statistical power. These power estimations were based on the initial report[8], following the methodology of epidemiology. Conversely, the study conducted by Martinez, *et al*[16] (416 cases, 501 controls in a Spanish population) pointed out the insufficient powers in Caucasian populations due to lower allele frequencies than in Japanese populations. A recent study by Takata, *et al*[18] (940 cases, 500 controls in a Japanese population) failed to replicate the association. However, it suggested the evidence of significant association through a metaanalysis of Japanese studies.

We conducted a replication study in an independent Japanese sample set and a metaanalysis in which we synthesized previous studies and our replication study to evaluate the effects of *SLC22A4* polymorphisms on RA susceptibility[19-21].

MATERIALS AND METHODS

Subjects. A total of 924 patients with RA were recruited through several medical institutes in Japan under the support of the BioBank Japan Project[22]. All RA patients fulfilled the American College of Rheumatology revised criteria for RA[23]. Among the RA patients, 81.5% were women; the age at disease onset was 47.8 ± 14.4 years (mean ± SD), age at enrollment was 60.4 ± 11.6 years, 69.8% were rheumatoid factor (RF)-positive, and 83.6% were positive for antibodies of cyclic citrullinated peptide (CCP). A total of 940 controls were recruited from the general population through several medical institutes in Japan. Among the controls, 25.0% were women, age at enrollment was 52.8 ± 14.7 years. All subjects were Japanese, and had not been enrolled in any previous studies of *SLC22A4* polymorphisms and RA susceptibility. All subjects provided written informed consent to participate in the study, or their parents gave consent if they were younger than age 20 years, according to the procedure approved by the Ethical Committee of BioBank Japan Project and the SNP Research Center, The Institute of Physical and Chemical Research (RIKEN), Yokohama.

Genotyping methods. In the replication study, we selected slc2F1 (rs2073838) for genotyping. We genotyped SNP using TaqMan assays in accord with the manufacturer's instructions (assay ID C_26479222_10; Applied Biosystems, Foster City, CA, USA) with the ABI-Prism 7900HT sequence detection system (Applied Biosystems).

Statistical analysis. For each of the individual studies, Hardy-Weinberg equilibrium (HWE) of the controls was assessed using the Exact test[24]. The significance of differences in genotype and allele frequencies in the case and control groups was assessed by chi-square test and Armitage's trend test. Chi-square tests were performed for the allelic, recessive (11 vs [12+22]), and dominant ([11+12] vs 22) mode, in which A/G alleles of slc2F1 (rs2073838) and T/C alleles of slc2F2 (rs3792876) registered in NCBI dbSNP build 128 were referred to as alleles 1/2. The odds ratio (OR) and the 95% confidence interval (95% CI) were estimated using Woolf's method. When the generated contingency table had the expected cell values < 5, Fisher's exact test was performed. The ethnic diversity of allele frequencies of slc2F1/F2 polymorphisms in the controls between Japanese and Caucasian populations was tested by the unpaired t-test.

In the metaanalysis, we performed a literature search of the NCBI PubMed database with key words "*SLC22A4*," "rheumatoid arthritis," and "susceptibility" from the publication of the first report[8] up to February 29, 2008. Among the search results, we filtered the studies if they were case-control studies for RA, if they reported original data, if they were published in English, if slc2F1/F2 polymorphisms were genotyped, and if numbers of alleles or genotypes were available. The following information was extracted from each of the identified studies: first author, year of publication, number of cases and controls, and available genotype data of slc2F1/F2 polymorphisms. When both SNP were genotyped in the same study, we used slc2F2, which was suspected to be functionally responsible for RA susceptibility in the initial study by Tokuhiro, *et al*[8]. The metaanalysis was performed using the Mantel-Haenszel approach[25] as a fixed-effect model and the DerSimonian-Laird method as a random-effect model[26]. The metaanalysis was conducted for 5 groups — all studies (A), replication studies (B), Caucasian studies (C), Japanese studies (D), and Japanese replication studies (E). We quantified OR heterogeneity among studies by Cochran's Q-statistic and its metric of I^2 [27,28]. Publication bias in the metaanalysis was evaluated with Egger's regression test[29]. Statistical calculations were done using StatsDirect statistical software (V. 2.6.2; StatsDirect Ltd., Sale, UK) and the R software package (V. 2.5.0; available at http://www.r-project.org/).

RESULTS

Replication study. The success rate of genotyping of the SNP was > 99.7% of samples. The genotypes of slc2F1 were in HWE in controls (p = 0.09). The frequencies of allele 1 were 0.306 in controls and 0.346 in cases. Statistically significant associations of the polymorphism and RA were found (OR 1.20, 95% CI 1.04–1.37, p = 0.0099, in the allelic mode; OR 1.29, 95% CI 1.08–1.55, p = 0.006, in the dom-

inant mode; and p = 0.011 in the trend mode). Stratification analysis according to sex did not show significant differences of OR among men and women, although associations were not significant due to reduced sample size (OR 1.10, 95% CI 0.86–1.42, p = 0.44, in men; OR 1.21, 95% CI 0.97–1.51, p = 0.098, in women in the allelic mode). The summary of our replication study appears in Table 1 and Figure 1.

Eligible studies for metaanalysis. A total of 8 studies on the association between RA and SNP in *SLC22A4* gene met the inclusion criteria for the metaanalysis. We included these 8 studies and our replication study in the metaanalysis, which consisted of 8076 cases and 6837 controls. Five studies were performed in Caucasian populations (4527 cases, 3803 controls from the UK[13], Swedish[15], North American[15] and Spanish populations[16,17]) and 4 studies were performed in Japanese populations[8,14,18] (3549 cases, 3034 controls). The study by Orozco, *et al*[17] was included in only the allelic mode metaanalysis, and the study conducted by Newman, *et al*[12] was not included due to restrictions in available genotype data. Slc2F2 was used in the study conducted by Barton, *et al*[13]. The genotypes of slc2F1/F2 were identical in the study conducted by Orozco, *et al*[17]. The characteristics of the 9 case-control studies are summarized in Table 1 and Figure 1. The genotypes of the polymorphism in controls of each study held the HWE. The frequencies of allele 1 in controls were < 0.09 in Caucasian populations (0.066–0.083), whereas they were > 0.29 in Japanese populations (0.298–0.323). Significant interethnic diversity of the allele frequencies was found ($p = 8.6*10^{-8}$).

Association between slc2F1/F2 polymorphisms and RA. Among the individual studies, the initial study conducted by Tokuhiro, *et al*[8] and our replication study showed significant associations in Japanese populations (p < 0.01). Significant associations were not indicated in the replication studies performed in Caucasian populations[13,15-17]. The summary of the metaanalysis appears in Table 2 and Figure 1. OR heterogeneity among studies was suggested for all studies (Group A) and Japanese studies (Group D) in the recessive mode ($I^2 > 50\%$ or Cochran's Q-statistic p < 0.1), and publication bias was suggested for all studies (Group A) in the allelic and dominant mode (Egger's p value < 0.05). However, the OR heterogeneity and the publication bias diminished in other modes of genotype contrasts or corresponding replication-specific groups. The pooled OR showed significant associations for the metaanalysis of all studies in both fixed-effect and random-effect models (Group A) (fixed-effect OR 1.11, 95% CI 1.05–1.18, p = 0.00084; random-effect OR 1.10, 95% CI 1.02–1.19, p = 0.017 in the allelic mode). In the replication-specific subgroup analysis (Group B), the significant associations remained in a fixed-effect model (OR 1.09, 95% CI 1.02–1.17, p = 0.016, in the allelic mode), but diminished in a random-effect model. No association was detected in subgroup analysis of Caucasian studies (Group C). Significant associations were found in both the Japanese studies (Group D) (fixed-effect and random-effect OR 1.16, 95% CI 1.07–1.25, p = 0.00012, in the allelic mode) and Japanese replication studies (Group E) (fixed-effect OR 1.13, 95% CI 1.04–1.23, p = 0.0045; random-effect OR 1.13, 95% CI 1.04–1.23, p = 0.0042, in the allelic mode).

Table 1. Characteristics of individual studies included in the metaanalysis.

Study	Population	SNP	No. of Subjects	Genotypes* 11	12	22	Allele Freq.**	HWE*** p	OR (95% CI), p Allelic	11 vs (12+22)	(11+12) vs 22	Trend†
Tokuhiro[8]	Japanese	slc2-F1	Case 819	132	321	366	0.357		1.25 (1.07–1.46),	1.98 (1.43–2.75),	1.11 (0.90–1.36),	—
		(rs2073838)	Control 656	58	288	310	0.308	0.47	0.0049	0.000034	0.33	0.0066
Barton[13]	UK	slc2-F2	Case 880	2	112	766	0.066		0.78 (0.59–1.03),	0.22 (0.02–1.26),	0.80 (0.60–1.08),	—
		(rs3792876)	Control 594	6	87	501	0.083	0.28	0.074	0.067	0.14	0.075
Kuwahara[14]	Japanese	slc2-F1	Case 867	104	383	380	0.341		1.08 (0.94–1.24),	1.24 (0.92–1.67),	1.06 (0.88–1.27),	—
		(rs2073838)	Control 940	93	422	425	0.323	0.46	0.27	0.15	0.55	0.27
Plenge[15]	Swedish	slc2-F1	Case 1516	13	210	1293	0.078		1.13 (0.90–1.42),	1.07 (0.40–3.18),	1.15 (0.90–1.47),	—
		(rs2073838)	Control 874	7	107	760	0.069	0.18	0.28	0.88	0.26	0.28
Plenge[15]	North American	slc2-F1	Case 829	5	131	693	0.085		1.03 (0.81–1.32),	0.85 (0.26–2.80),	1.04 (0.80–1.35),	—
		(rs2073838)	Control 847	6	128	713	0.083	0.82	0.80	0.78	0.74	0.80
Martinez[16]	Spanish	slc2-F2	Case 416	3	51	362	0.069		0.91 (0.64–1.30),	1.81 (0.21–21.8),	0.87 (0.60–1.28),	—
		(rs3792876)	Control 501	2	71	428	0.075	1.00	0.60	0.66	0.49	0.60
Orozco[17]	Spanish	slc2-F2	Case 886	129	1643††		0.073		1.11 (0.87–1.43),	—	—	—
		(rs3792876)	Control 987	130	1844††		0.066	—	0.40	—	—	—
Takata[18]	Japanese	slc2-F2	Case 940	93	415	432	0.320		1.11 (0.94–1.31),	1.11 (0.76–1.61),	1.15 (0.92–1.43),	—
		(sr3792876)	Control 500	45	208	247	0.298	0.91	0.23	0.58	0.21	0.23
Okada, present study	Japanese	slc2-F1	Case 923	112	414	397	0.346		1.20 (1.04–1.37),	1.17 (0.88–1.56),	1.29 (1.08–1.55),	—
		(rs2073838)	Control 938	99	376	463	0.306	0.09	0.0099	0.28	0.006	0.011

*A/G alleles of slc2F1 (rs2073838) and T/C alleles of slc2F2 (rs3792876) referred to as alleles 1/2. **Frequency of allele 1. *** Hardy-Weinberg equilibrium p value of genotypes of controls. †Armitage's trend test for 3 genotypes. ††Allelic-mode data only. SNP: single-nucleotide polymorphism.

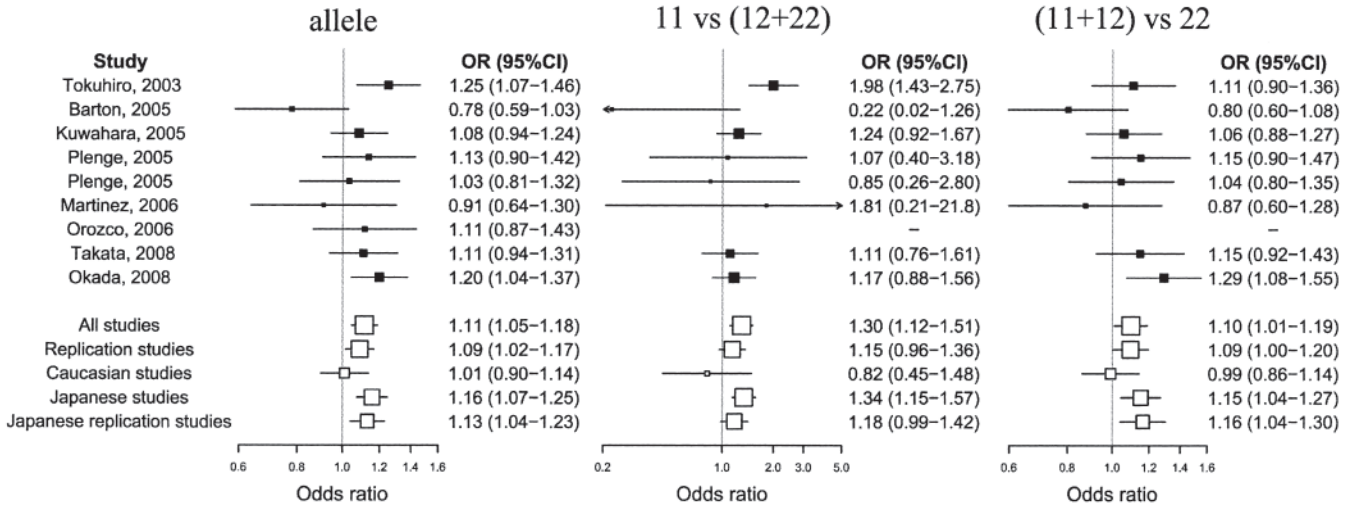

Figure 1. OR and 95% CI of individual studies and pooled results of the metaanalysis (fixed-effect model) for the associations of slc2F1/F2 polymorphisms and RA.

Table 2. Meta-analysis of *SLC22A4* polymorphisms and RA association.

Group (no. of studies)	Mode of Genotypes*	Test of Heterogeneity: Cochran Q	p	I^2, % (95% CI)	Test of Association** (fixed-effect model): Pooled OR (95% CI)	p	Test of Association*** (random-effect model): Pooled OR (95% CI)	p
A: All studies (9)	Allelic	11.44	0.18	30.1 (0–83.9)	**1.11 (1.05–1.18)**	**0.00084**	**1.10 (1.02–1.19)**	**0.017**
	11 vs (12+22)	13.07	0.071	46.4 (2.9–89.9)	**1.30 (1.12–1.51)**	**0.00084**	1.25 (0.98–1.61)	0.076
	(11+12) vs 22	9.36	0.23	25.2 (0–84.3)	**1.10 (1.01–1.19)**	**0.026**	1.09 (0.99–1.20)	0.084
B: Replication studies (8)	Allelic	8.89	0.26	21.3 (0–81.6)	**1.09 (1.02–1.17)**	**0.016**	1.08 (1.00–1.17)	0.065
	11 vs (12+22)	4.84	0.56	0 (0–100)	1.15 (0.96–1.36)	0.13	1.15 (0.97–1.37)	0.11
	(11+12) vs 22	9.35	0.16	35.8 (0–91.2)	**1.09 (1.00–1.20)**	**0.045**	1.08 (0.96–1.21)	0.19
C: Caucasian studies (5)	Allelic	5.37	0.25	25.6 (0–72.5)	1.01 (0.90–1.14)	0.89	1.00 (0.87–1.15)	0.97
	11 vs (12+22)	3.61	0.31	16.9 (0–73.0)	0.82 (0.45–1.48)	0.61	0.83 (0.41–1.67)	0.60
	(11+12) vs 22	3.98	0.26	24.6 (0–75.1)	0.99 (0.86–1.14)	0.94	0.98 (0.83–1.16)	0.84
D: Japanese studies (4)	Allelic	2.35	0.50	0 (0–100)	**1.16 (1.07–1.25)**	**0.00012**	**1.16 (1.07–1.25)**	**0.00012**
	11 vs (12+22)	7.55	0.056	60.3 (16.7–100)	**1.34 (1.15–1.57)**	**0.00030**	**1.34 (1.04–1.72)**	**0.024**
	(11+12) vs 22	2.45	0.48	0 (0–100)	**1.15 (1.04–1.27)**	**0.0052**	**1.15 (1.04–1.27)**	**0.0048**
E: Japanese replication studies (3)	Allelic	1.14	0.57	0 (0–100)	**1.13 (1.04–1.23)**	**0.0045**	**1.13 (1.04–1.23)**	**0.0042**
	11 vs (12+22)	0.22	0.90	0 (0–100)	1.18 (0.99–1.42)	0.078	1.18 (0.99–1.42)	0.070
	(11+12) vs 22	2.28	0.32	12.3 (0–100)	**1.16 (1.04–1.30)**	**0.0081**	**1.16 (1.03–1.31)**	**0.013**

*A/G alleles of slc2F1(rs2073838) and T/C alleles of slc2F2 (rs3792876) referred to as alleles 1/2. **Pooled OR and p value estimated by Mantel-Haenszel. ***Pooled OR and p value estimated by DerSimonian-Laird approach. Statistically significant values printed in bold type.

DISCUSSION

We performed a metaanalysis for the susceptibility of slc2F1/F2 polymorphisms to RA. As reported[21,30], we found significant interethnic diversity of allele frequencies of risk alleles between Caucasian and Japanese populations. To account for the interethnic OR heterogeneity, we adopted both fixed-effect and random-effect models. Although the fixed-effect model can lead to inaccurate results when significant OR heterogeneity exists[31], no apparent heterogeneity was observed. Significant associations were found in the metaanalysis of all studies and Japanese studies in both fixed-effect and random-effect models, but not in Caucasian studies.

Among the Japanese studies, the OR in the recessive mode of the initial study by Tokuhiro, *et al*[8] deviated from others' OR. This seemed due to the bias that the initial positive study tends to overestimate the relative risk[32] and also due to the sampling bias of the excess of the risk allele homozygotes. The genotypic associations were not consistent among the studies. Which genotypic modes optimally accounted for RA susceptibility was not concluded, and requires the accumulations of independent studies.

When *SLC22A4* plays common roles responsible for RA susceptibility in both populations, the relative risks of the polymorphisms might be equivalent irrespective of their different allele frequencies. One explanation for the inconsistent results among the ethnic groups was the different statistical powers of the studies. We evaluated the powers of individual studies with a pooled OR, the allele frequencies in controls of the individual studies, and the significance level (α) of 0.05 (Table 3). Caucasian replication studies had a severe lack of power (4.5%–15.0%) due to the lower allele frequencies compared with Japanese studies (13.0%–44.3%). When we estimate the power of the metaanalysis simply from the pooled OR and pooled subjects, Japanese studies achieved 81.6% power for the allelic mode, whereas Caucasian studies were powered as low as 45.4% ($\alpha = 0.05$). We found that the powers reported originally in individual replication studies[12-15,17] (> 99% or 80%) were higher than our estimation (< 50%). These reported powers were based on the OR of the initial study[8], whereas we adopted the pooled OR. The initial positive study tends to overestimate the relative risk[32], and power estimation based on it may increase the false-negative error of the replication studies. The powers reported by Barton, *et al*[13] and Orozco, *et al*[17] were based on the allele frequencies of the initial study[8], and the heterogeneity of allele frequencies and relative risk between ethnic groups might produce the deviation of estimated powers. Powers are generally calculated prior to the study based on the available published reports, and their power calculations were correct. Recently, the development of the HapMap database[33] enabled the estimation of allele frequencies in different populations. We also propose that accounting for the ethnic differences of allele frequencies would give useful insights into power estimation.

Another potential explanation may be the difference of relative risk among ethnic groups. It is known that relative risks of some of the disease-associated alleles that have a genetic risk to phenotypes vary among ethnic groups[34]. When different ethnic susceptibilities of slc2F1/F2 polymorphisms are anticipated, the random-effect model is more appropriate in a metaanalysis synthesizing both populations. Significant association diminished in the replication-specific metaanalysis under the random-effect model. In this case, the magnitudes of underlying relative risks in Caucasian populations were weaker compared with Japanese populations or did not exist. Consequently, whether the relative risks of slc2F1/F2 polymorphisms differ among the populations remains to be determined because of the significantly lower allele frequencies in Caucasian populations, and more independent studies into metaanalysis are required.

We also implemented a metaanalysis in which slc2F1 was used in the studies genotyping both slc2F1/F2. Since slc2F1/F2 were in strong LD in both Japanese and Caucasian populations ($r^2 = 1$ and 0.91, respectively, according to the HapMap database[33]), the results did not change significantly (fixed-effect: OR 1.11, 95% CI 1.04–1.18, p = 0.0011, in the allelic mode; OR 1.30, 95% CI 1.12–1.51, p = 0.00078, in the recessive mode; and OR 1.09, 95% CI 1.02–1.17, p = 0.033, in the dominant mode, for all studies). The minimum p values were given in different genotypic modes. However, the modes that optimally accounted for RA susceptibility were not concluded, and therefore the variant that played a more essential role in RA susceptibility remains to be determined. It is possible that the exact causal variants responsible for RA susceptibility are in LD with slc2F1/F2 in Japanese populations, but not in Caucasian populations, yielding the inconsistent relative risks among ethnic groups. To further elucidate the causal variants of *SLC22A4* polymorphisms to RA susceptibility, fine mapping of the region containing *SLC22A4* and assessment of different LD structures between populations would be instructive. Although the estimated OR of slc2F1/F2 (1.10–1.11) seemed too small to directly improve the effects of clinical rheumatology, exploring their roles in RA susceptibility will contribute to rheumatology overall.

In conclusion, the associations in Caucasian studies were not significant. Since the significantly low frequency of the risk allele made statistical power lower in Caucasian studies than Japanese studies, whether significant relative risks existed in Caucasian populations remained inconclusive. The significant relative risks in Japanese populations were confirmed. Additional independent studies are required to clarify the role of *SLC22A4* polymorphisms in the etiology of RA.

Table 3. Power comparisons of individual studies.

Study	Population	Power Estimated by Pooled OR*, %		
		Allelic	11 vs (12+22)	(11+12) vs 22
Tokuhiro[8]	Japanese	27.0	31.9	14.0
Barton[13]	UK	12.4	7.5	9.4
Kuwahara[14]	Japanese	32.9	41.3	16.2
Plenge[15]	Swedish	15.0	8.4	11.2
Plenge[15]	North American	13.8	6.9	10.4
Martinez[16]	Spanish	8.9	4.5	7.2
Orozco[17]	Spanish	12.9	—	—
Takata[18]	Japanese	24.3	29.5	13.0
Okada, present study	Japanese	33.0	44.3	16.8

* Power was calculated with estimated pooled OR of all studies for each mode by a fixed-effect model. Significance level was set at 0.05.

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