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Association of *STAT4* With Susceptibility to Rheumatoid Arthritis and Systemic Lupus Erythematosus in the Japanese Population

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Objective. ***STAT4*** **encodes a transcriptional factor that transmits signals induced by several key cytokines, and it might be a key molecule in the development of autoimmune diseases. Recently, a** ***STAT4*** **haplotype was reported to be associated with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) in Caucasian populations. This was replicated in a Korean RA population. Interestingly, the degree of risk of RA susceptibility with the** ***STAT4*** **haplotype was similar in the Caucasian and Korean populations. The present study was undertaken to investigate the effect of** ***STAT4*** **on susceptibility to RA and SLE in the Japanese.**

Methods. **We performed an association study using 3 independent Japanese RA case–control populations (total 3,567 cases and 2,199 controls) and 3 independent Japanese SLE populations (total 591 cases). All samples were genotyped using the TaqMan fluorogenic 5′ nuclease assay for single-nucleotide polymorphism (SNP) rs7574865, which tags the susceptibility haplotype. The association of the SNP with disease susceptibility in each case–control study was calculated using Fisher's exact test, and the results were combined, using the Mantel-Haenszel method, to obtain combined odds ratios (ORs).**

Results. **We observed a significant association of the** ***STAT4*** **polymorphism with susceptibility to both RA and SLE. The combined ORs for RA and SLE, respectively, were 1.27 ($P = 8.4 \times 10^{-9}$) and 1.61 ($P = 2.1 \times 10^{-11}$) for allele frequency distribution; these ORs were quite similar to those previously observed in the Caucasian population.**

Conclusion. **We conclude that** ***STAT4*** **is associated with RA and SLE in the Japanese. Our results indicate that** ***STAT4*** **is a common genetic risk factor for autoimmune diseases, with similar strength across major racial groups.**

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Rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) are chronic inflammatory autoimmune diseases characterized by pathologic infiltration of lymphocytes in target organs. Although the pathogenesis of these diseases remains unclear, dysregulated lymphocyte activation via the breakdown of self tolerance is believed to be implicated in their pathogenesis, and multiple genetic and environmental factors are important in the development of these diseases.

Recently, Amos et al conducted a genome-wide linkage scan using >5,700 single-nucleotide polymorphisms (SNPs) in 642 Caucasian families with affected sibling pairs; they found the best evidence of linkage at chromosomes 2q33 (1). Following the linkage analysis, Remmers et al performed a large case–control study of 13 selected candidate genes within the linkage region and found an association between a common haplotype located in the third intron of *STAT4* and susceptibility to RA and SLE (2). The association was replicated in several independent Caucasian RA and SLE populations, and also in a Korean RA population (2,3).

STAT4 encodes signal transducer and activator of transcription 4, the STAT protein family member that is uniquely activated by interleukin-12 (IL-12) through its receptor, which has an essential downstream role in Th1 cell differentiation and proliferation (4). In addition, it has been reported that STAT-4 is necessary for the development of Th17 cells (IL-17–producing CD4+ T cells) (5). Since Th1 cells and Th17 cells play an important role in chronic inflammatory disorders and since STAT-4 is considered to be a key molecule in both the Th1 and Th17 lineages, STAT-4 may play a crucial role in the development of autoimmune diseases such as RA and SLE.

Genetic association between HLA–DRB1 and RA susceptibility has been well established, and several other risk genes for RA outside the HLA region have been identified. However, while DRB1 has been repeatedly shown to be an RA risk locus in Caucasian and Asian populations, the other reported RA risk genes, such as *PTPN22*, *PADI4*, and *FCRL3*, have been difficult to replicate in other ethnic populations aside from the original populations first reported (6). These conflicting results suggest that the genetic background of the disease may vary among ethnic groups.

Interestingly, the degree of risk of RA susceptibility observed with the *STAT4* haplotype was found to be similar in the Caucasian and Korean populations (2,3). This finding indicates that the risk haplotype for RA susceptibility might be common across major racial groups. In the present study, we investigated the association of *STAT4* with RA susceptibility using large series of Japanese RA cohorts. We also tried to evaluate whether the gene is associated with RA outcome measures in a Japanese RA cohort. In addition, we tested the association between the gene and susceptibility to SLE in the Japanese population. This study is the first to investigate the association of *STAT4* with SLE in an Asian population.

PATIENTS AND METHODS

Subjects and disease criteria. All patients with RA fulfilled the American College of Rheumatology (ACR; formerly, the American Rheumatism Association) 1987 revised criteria for the disease (7). All patients with SLE met the ACR 1982 revised criteria for the disease (8).

DNA samples were obtained from subjects in 3 RA case–control series (Table 1). DNA from the case subjects in the Tokyo Women's Medical University (TWMU) Institute of Rheumatology RA cohort (IORRA) case–control series was obtained from the IORRA DNA collection. The IORRA is an observational RA cohort with an enrollment of nearly 5,000 Japanese RA patients, and DNA samples were collected from 1,504 of these patients (mean age 59.3 years, 84% female, 88% rheumatoid factor [RF] positive) (9). This DNA collection was also used to analyze the effect of the single-nucleotide polymorphism (SNP) (see below) on RA outcome measures. Demographic, clinical, and treatment information on IORRA patients as of the spring of 2003 was obtained from the IORRA database, which includes the Disease Activity Score in 28 joints (DAS28) (10) and the Japanese version of the Health Assessment Questionnaire (J-HAQ) (11). Radiographs of the hands and feet of the IORRA patients, obtained when the duration of disease was 5 years, were reviewed retrospectively, and radiographic joint damage was assessed by a single skilled reader, using the modified Sharp/van der Heijde score (SHS) (12). The SHS includes a count of erosions and joint space narrowing in the hands and feet and has a range of 0 (no damage) to 448 (highest damage). DNA samples from popu-

Table 1. Case–control series for the studies of rheumatoid arthritis and systemic lupus erythematosus

Series	No. of patients	No. of controls
Rheumatoid arthritis		
IORRA	1,504	752
RIKEN	1,113	940
Tokushima	950	507
Systemic lupus erythematosus		
TWMU	238	752*
RIKEN	188	940†
Tokushima/Fukuoka	165	212

* Genotype information was obtained from the controls in the Tokyo Women's Medical University (TWMU) Institute of Rheumatology Rheumatoid Arthritis cohort (IORRA) rheumatoid arthritis series.
† Genotype information was obtained from the controls in the Institute of Physical and Chemical Research (RIKEN) series.

lation controls were obtained from the Pharma SNP consortium (http://www.jpma.or.jp/psc/index.html).

DNA from the case subjects in the Institute of Physical and Chemical Research (RIKEN) RA case–control series (mean age 60.4 years, 82% female, 70% RF positive) was obtained from the BioBank Japan Project DNA collection. As part of the BioBank Japan Project, DNA and serum samples along with clinical data have been collected from 300,000 patients with 47 diseases, including RA (13). Sixty-six hospitals affiliated with 12 institutions are participating in the project. Population-based control subjects were recruited through the Rotary Club of Osaka-Midosuji District 2660 Rotary International in Japan.

Patients and controls in the Tokushima RA case–control series were recruited through the orthopedics clinic at University of Tokushima Hospital, its community affiliates, and the rheumatology clinic at Tokushima Kensei Hospital (Tokushima, Japan) (14). The mean age of the patients was 61.8 years, and 79% were female.

Cases with SLE were also obtained from 3 sources (Table 1). TWMU patients were recruited from Institute of Rheumatology and Aoyama Hospital, TWMU. RIKEN patients were recruited through the Specified Disease Treatment Research Program of the Japanese Ministry of Health, Labor, and Welfare. Several medical institutions nationwide are participating in the program. These 2 series included only cases; control genotype information was obtained from the RA case–control series in the IORRA and RIKEN, respectively. Patients and control subjects in the Tokushima/Fukuoka lupus case–control series were recruited from Kyushu University Hospital (Fukuoka, Japan) (15).

The ethics committee of each institution (TWMU, RIKEN, and University of Tokushima) granted approval for the study, and each individual subject signed an informed consent form after receiving a verbal explanation of the study.

SNP genotyping. A polymorphism located within intron 3 of *STAT4*, rs7574865, which tags the susceptibility haplotype, was selected for this study because it exhibited the best evidence for association in the primary study (2) and was one of the SNPs most significantly associated with RA susceptibility in the Korean replication study (3). This SNP has been considered to be in strong linkage equilibrium with a putative functional variant. Genotyping was performed using the TaqMan fluorogenic 5′ nuclease assay, according to the instructions of the manufacturer (Applied Biosystems, Tokyo, Japan). All polymerase chain reactions were performed using GeneAmp PCR System 9700 (Applied Biosystems), and end point fluorescence readings were performed with an ABI Prism 7900 HT Sequence Detection System (Applied Biosystems).

Statistical analysis. Allele frequencies of SNP rs7574865 in each case–control series were estimated by the allele counting method. Chi-square testing was used to identify significant departure from Hardy-Weinberg equilibrium.

Association of the SNP with susceptibility to RA or SLE in each study was estimated by Fisher's exact test; we compared the allelic effect of T (suspected risk allele) with G (common allele), and the genotypic effect of the homozygous genotypes with other genotypes. After assessing heterogeneity among the studies as determined based on Woolf's method, the Mantel-Haenszel test was used to evaluate combined odds ratios (ORs) and 95% confidence intervals (95% CIs), demonstrating the population-wide impact of the polymorphism on disease susceptibility.

Differences in patient characteristics among IORRA subjects with different rs7574865 genotypes were assessed by Kruskal-Wallis test or Fisher's exact test. The allelic effect of rs7574865 on the SHS was analyzed by linear regression analysis.

All statistical tests were implemented using the R software package, version 2.6.0 (http://www.r-project.org/).

RESULTS

On average, we achieved a genotyping success rate of 98.9%, with call rates of >98.2% for each case–control series. The genotype concordance rate was 100% as assessed by random retyping across different plates. Genotype distributions for SNP rs7574865 were in Hardy-Weinberg equilibrium in each case–control series.

Association of *STAT4* polymorphism with RA. The data summarized in Table 2 show the allele fre-

Table 2. Association of single-nucleotide polymorphism rs7574865 with RA in Japanese subjects*

Series, subjects	Genotype					Allele		GG vs. others		TT vs. others	
	GG	GT	TT	Total	MAF	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
IORRA											
RA	588	694	199	1,481	0.37	1.29 (1.13–1.48)	1.7×10^{-4}	1.38 (1.15–1.66)	3.9×10^{-4}	1.41 (1.05–1.89)	0.020
Controls	355	316	74	745	0.31						
RIKEN											
RA	447	502	160	1,109	0.37	1.31 (1.15–1.50)	4.8×10^{-5}	1.38 (1.16–1.66)	3.0×10^{-4}	1.48 (1.12–1.96)	0.0048
Controls	453	389	96	938	0.31						
Tokushima											
RA	365	448	128	941	0.37	1.17 (0.99–1.38)	0.056	1.20 (0.96–1.50)	0.11	1.30 (0.92–1.86)	0.13
Controls	216	230	54	500	0.34						
Combined						1.27 (1.17–1.37)	8.4×10^{-9}	1.34 (1.20–1.49)	1.9×10^{-7}	1.41 (1.19–1.67)	8.5×10^{-5}

* RA = rheumatoid arthritis; MAF = minor allele frequency; OR = odds ratio; 95% CI = 95% confidence interval (see Table 1 for other definitions).

Table 3. Association of single-nucleotide polymorphism rs7574865 with SLE in Japanese subjects*

Series, subjects	Genotype					Allele		GG vs. others		TT vs. others	
	GG	GT	TT	Total	MAF	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
TWMU											
SLE	76	103	48	227	0.44	1.73 (1.38–2.15)	8.3×10^{-7}	1.81 (1.31–2.50)	1.8×10^{-4}	2.43 (1.59–3.68)	2.9×10^{-5}
Controls	355	316	74	745	0.31						
RIKEN											
SLE	70	85	28	183	0.39	1.40 (1.10–1.77)	0.0059	1.51 (1.08–2.12)	0.015	1.58 (0.97–2.53)	0.053
Controls	453	389	96	938	0.31						
Tokushima/Fukuoka											
SLE	51	83	31	165	0.44	1.79 (1.31–2.45)	1.4×10^{-4}	2.07 (1.33–3.25)	0.0010	2.34 (1.22–4.59)	0.0059
Controls	102	91	19	212	0.30						
Combined						1.61 (1.40–1.85)	2.1×10^{-11}	1.74 (1.43–2.12)	4.9×10^{-8}	2.08 (1.59–2.72)	8.5×10^{-8}

* SLE = systemic lupus erythematosus; MAF = minor allele frequency; OR = odds ratio; 95% CI = 95% confidence interval (see Table 1 for other definitions).

quency and genotype distribution in RA patients and controls in each case–control series. We observed a significant difference in allele frequency and genotype distribution of the *STAT4* polymorphism between RA patients and controls in the IORRA and the RIKEN cohorts, while no significant difference was found in the Tokushima series. When study-specific ORs were combined using the Mantel-Haenszel method, the differences in allele frequency and genotype distributions of the SNP between patients and controls were significant (combined OR 1.27 [95% CI 1.17–1.37, $P = 8.4 \times 10^{-9}$]–1.41 [95% CI 1.19–1.67, $P = 8.5 \times 10^{-5}$]). There was no significant heterogeneity among the studies, as assessed by Woolf's method ($P > 0.05$).

Association of *STAT4* polymorphism with SLE. Table 3 shows the genotype distribution and minor allele frequency in the 3 SLE series. As with RA, we found differences in the allele frequency and genotype distributions of SNP rs7574865 between SLE patients and controls; these were significant in all 3 case–control series. No significant evidence of heterogeneity among the studies was identified by Woolf's method ($P > 0.05$), and the combined OR for the polymorphism as calculated by Mantel-Haenszel testing was 1.61 (95% CI 1.40–1.85, $P = 2.1 \times 10^{-11}$). Combined ORs for the recessive trait and the dominant trait were 2.08 (95% CI 1.59–2.72) and 1.74 (95% CI 1.43–2.12), respectively.

Stratified analyses of clinical and laboratory variables in RA patients. Among 1,504 patients with available DNA samples, 1,335 participated in the IORRA clinical data collection in the spring of 2003, and information on their demographic, clinical, and treatment details as of that time could be obtained from the IORRA database (Table 4). Consistent with previous findings by Lee et al in a Korean population (3), there was no significant genotypic association with age at disease onset or sex. We also found no significant differences among the genotypes in age, disease duration, family history of RA, RF status, DAS28 score, or J-HAQ score. There was a trend toward an association of risk allele with elevated levels of inflammation markers and patient's assessment of global health, but these were not significant. Only glucocorticoid usage and glucocorticoid dosage were found to differ significantly among the genotypes, with the difference increasing in a stepwise manner according to the number of risk alleles (median dosage 0, 1, and 2.5 mg equivalent prednisolone, respectively, among patients with the GG, GT, and TT genotypes).

The SHS after 5 years of disease could be measured in 163 patients, of whom 160 were genotyped. Although a trend toward an effect of the risk allele on the SHS was observed, it was not significant ($P = 0.22$) (median score 40, 45, and 46, respectively, among patients with the GG, GT, and TT genotypes [n = 67, 79, and 14, respectively]).

We did not perform a stratified analysis on anti–cyclic citrullinated peptide antibody (anti-CCP) positivity, since anti-CCP data were not available on most of the patients from the IORRA DNA collection. However, Lee and colleagues suggested that, at least among Asians, the risk of RA susceptibility associated with the *STAT4* variant may not be restricted to the anti-CCP positive disease subset (3).

DISCUSSION

This study is the first to investigate the association of a *STAT4* polymorphism with genetic susceptibility to lupus in any Asian population, and susceptibility to

Table 4. Genotypic differences in clinical or laboratory variables in RA patients*

	Total	Genotype			*P*†
		GG	GT	TT	
No. (%) of patients	1,335	521 (40)	610 (46)	183 (14)	
Age, years	60 (53–68)	61 (53–68)	60 (53–68)	60 (52–66)	0.14
Female, no. (%)	1,125 (84)	441 (85)	507 (83)	157 (86)	0.63
Disease duration, years	10 (5–16)	10 (5–17)	10 (5–16)	10 (5–17)	0.71
Age at RA onset, years	48 (39–57)	49 (40–57)	49 (40–57)	48 (38–55)	0.18
Family history of RA, no. (%)	415 (32)	157 (31)	187 (31)	63 (35)	0.54
RF positive, no. (%)‡	1,195 (90)	468 (90)	543 (89)	164 (90)	0.92
RF titer, IU/ml‡	116 (48–282)	115.5 (49–296)	116 (49–278)	122 (46–283)	0.97
Treatment§					
NSAID, no. (%)	980 (73)	378 (73)	446 (73)	143 (78)	0.32
DMARD, no. (%)	1,228 (92)	478 (92)	560 (92)	171 (93)	0.78
Glucocorticoid, no. (%)	719 (54)	266 (51)	325 (53)	117 (64)	0.01
Prednisolone, mg	1 (0–4.9)	0 (0–4)	1 (0–5)	2.5 (0–5)	0.01
DAS28	3.6 (2.7–4.5)	3.5 (2.7–4.4)	3.6 (2.8–4.5)	3.8 (2.8–4.6)	0.39
TJC	1 (0–3)	1 (0–3)	1 (0–3)	1 (0–3)	1.00
SJC	1 (0–3)	1 (0–3)	1 (0–3.75)	1 (0–4)	0.25
Patient's global assessment by VAS, mm	27 (10–54)	24 (9–54)	27 (11–55)	33 (14–53)	0.12
ESR, mm/hour	28 (16–48)	27 (16–46)	29 (16–48)	31 (16–54)	0.51
CRP, mg/dl	0.7 (0.2–1.6)	0.6 (0.2–1.6)	0.7 (0.2–1.6)	0.95 (0.3–1.9)	0.08
J-HAQ	0.625 (0.125–1.375)	0.625 (0.125–1.375)	0.625 (0.125–1.25)	0.625 (0.125–1.375)	0.96

* Data on some variables were missing for a small number of patients (maximum 3.1%). Data on genotype were missing for 21 patients (1.6%); therefore, values in the individual columns under Genotype are for 1,314 patients (521 for GG, 610 for GT, 183 for TT). Except where indicated otherwise, values are the median (interquartile range). RA = rheumatoid arthritis; NSAID = nonsteroidal antiinflammatory drug; DMARD = disease-modifying antirheumatic drug; DAS28 = Disease Activity Score in 28 joints; TJC = tender joint count (28 joints); SJC = swollen joint count (28 joints); VAS = visual analog scale; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; J-HAQ = Japanese version of the Health Assessment Questionnaire.

† By Kruskal-Wallis test or Fisher's exact test.

‡ The highest rheumatoid factor (RF) value measured in the cohort project during 2000–2006 for each individual was used. Cutoff for positivity = 15.0 IU/ml.

§ Biologic agents were not available in Japan at this time (spring 2003). Glucocorticoid dosage was calculated as the prednisolone equivalent dosage in milligrams.

RA in a Japanese population. Although replication studies using other ethnic populations are essential for establishing any genetic association, results are often reported as negative in the other populations. One of the reasons for this is that the degree of genetic risk differs among ethnic groups.

Concerning RA genetics, many study groups worldwide have made great efforts to newly identify susceptibility genes and to replicate findings of other groups, particularly using Caucasian or Asian populations. However, findings for most susceptibility genes identified outside the HLA region have not been replicated in the populations different from the population used in the primary study. A typical example of this is an association between *PTPN22* and susceptibility to RA. A missense SNP in *PTPN22* known as R620W was discovered as a common genetic risk factor for several autoimmune diseases including RA in a Caucasian population, and the finding has been replicated in many Caucasian RA cohorts (16). However, the risk allele is extremely rare in Asians, and attempts to validate the association in Asian populations have been unsuccessful (17). In contrast, the association between *PADI4* and RA susceptibility is thought to be strong among Asian populations, and indeed, most replication studies in Asian populations have succeeded in validating this association. However, a meta-analysis of studies using Caucasian populations revealed the combined OR for the association to be as low as 1.1 (18), and as a result, replication studies in Caucasian populations have seldom validated the association.

Failure to replicate a genetic association in a different ethnic population from the population used in the primary study is often due to low statistical power. To avoid this problem, it is important to make the sample size as large as possible, as we did in the present multicenter Japanese case–control study. We collected 3,567 RA cases and 591 SLE cases to validate the association between *STAT4* and susceptibility to RA and lupus in the Japanese. Consistent with previous reports (2,3), we observed a significant association of the *STAT4* polymorphism with both RA and SLE susceptibility in

the Japanese. Although the risk allele frequency in control populations is slightly different between Caucasians (22%) and Japanese (30–34%), the OR shown by investigation of the allele frequency distribution of rs7574865, 1.27, is exactly the same as in the Caucasian populations. Also, the impact of the risk allele on susceptibility to SLE in the Japanese population was found to be similar to that obtained in the previous meta-analysis of studies of Caucasian populations (1.61) (2). These results suggest that the responsible functional variant, which remains unknown, is ancient in origin. Further independent studies using populations of other ethnicities would help to prove the hypothesis.

Autoimmune diseases are initiated by breakdown of self tolerance, and thus, they may share a common pathogenesis. Indeed, some RA susceptibility genes have been identified as common risk factors for clinically different autoimmune phenotypes. One of them is *PTPN22*, which has been reported as a disease susceptibility gene for type 1 diabetes, autoimmune thyroid disease, lupus, Addison's disease, and juvenile idiopathic arthritis, in addition to RA (16). *CTLA4*, one of the genes associated with lupus and RA, especially in Asians, has also been suggested to be a disease-associated gene in a variety of other autoimmune diseases (19). Both *PTPN22* and *CTLA4* negatively regulate T cell activation and maintain peripheral tolerance, and T cells play a central role in the immunopathogenesis of autoimmune diseases. STAT-4 is suggested to be a key molecule in both the Th1 and Th17 lineages, and therefore may be involved in a common pathway of pathogenesis in autoimmune diseases.

It is reasonable to speculate that a variant on *STAT4* could also affect disease activity in autoimmune diseases through dysregulation of the Th1 and Th17 pathways. Although we did not find evidence of association between *STAT4* and disease activity in RA, we did observe a trend toward an effect of the risk allele on elevated levels of inflammation markers and patient's global assessment. Both the fact that glucocorticoid usage and dosage increased significantly in a stepwise manner in parallel with the number of risk alleles and the knowledge that glucocorticoid treatment significantly reduces levels of inflammation markers suggest that the polymorphism on *STAT4* might be associated with disease activity in RA. Although a trend toward an effect of risk allele on radiographic damage in the first 5 years was observed, it was not significant, similar to findings in the Korean study (3). However, while differences in other clinical variables among the genotypes were tested using DNA from 1,335 patients, the effect on radiographic severity was tested only in 163 patients, due to the unavailability of suitable radiographs in the others (20). As a result, the statistical power of the study of association with radiographic severity was rather limited. There were also other potential sources of artifacts that should be considered in interpretation of these preliminary data. A large prospective study, accounting for the genotypes of *STAT4*, is needed to definitively answer the question of its associations with clinical and laboratory features.

The functional variant in *STAT4* that is responsible for increased disease susceptibility remains unknown. Since the susceptibility haplotype is located within intron 3 of *STAT4*, it is considered to be responsible for splice variation or regulatory effects of STAT-4. However, it might be also possible that the putative functional variant could be responsible for a biologic effect on intragenic RNA or other factors. Studies to investigate the functional variant on the susceptibility haplotype remain to be performed.

In conclusion, using Japanese RA and SLE case–control series with large samples, we confirmed *STAT4* polymorphism as a common genetic risk factor for these autoimmune diseases. The strength of the association was found to be similar across major racial groups.

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AUTHOR CONTRIBUTIONS

Dr. Ikari had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study design. Ikari, Kochi, Inoue.

Acquisition of data. Ikari, Kochi, Yamamoto, Shimane, Nakamura, Toyama, Kawaguchi, Terai, Hara, Tomatsu, Yamanaka, Horiuchi, Tao, Yasumoto, Hamada, Yasui, Inoue, Itakura, Okamoto, Kamatani, Momohara.

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