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Rheumatol Int (2012) 32:3323–3324
DOI 10.1007/s00296-011-2101-x

LETTER TO THE EDITOR

The increasing disease duration of patients at the time of orthopaedic surgery for rheumatoid arthritis

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Received: 24 March 2011 / Accepted: 21 August 2011 / Published online: 7 September 2011

Dear Sir,

In recent years, disease-modifying antirheumatic drugs (DMARDs), including biological agents, have been used to inhibit or halt the underlying immune process and prevent long-term damage in rheumatoid arthritis (RA). The introduction of biological DMARDs has increased treatment options for RA, and clinical remission is now considered to be the primary goal of RA treatment. Despite the availability of such aggressive therapies, ongoing progressive destruction of joints occurs in a subgroup of RA patients, who eventually require joint surgery [1]. Orthopaedic procedures can substantially improve the overall function and quality of life in such patients. However, some reports have suggested that improvements in medical treatment might partially explain the reduction in the incidence of orthopaedic joint surgery, resulting in a worldwide trend towards better long-term outcomes [2–5].

In this study, we investigated the disease duration at the time of orthopaedic surgery among RA patients who participated in a large observational cohort study established by our institute (IORRA) [4]. Outpatients who fulfilled the American College of Rheumatology criteria for RA and/or 2010 rheumatoid arthritis classification criteria were registered in this cohort study [6, 7]. An average of 4,990 outpatients with RA were seen each month from 2003 to 2009. Patient information was collected biannually (April/May and October/November) when the patients visited the outpatient unit of the Institute of Rheumatology, Tokyo Women's Medical University [8].

Figure 1a shows the average RA disease activity from 2003 to 2009. As previously reported, disease activity decreased during this period [9]. Figure 1b shows the disease duration trend at the time of surgery. Surprisingly, the disease duration of patients who underwent total knee arthroplasty (TKA) gradually increased during this period. In addition, the disease duration of patients who underwent any type of surgery, including TKA, total hip arthroplasty, wrist and finger joint surgeries, and foot surgeries, was also increased.

The reason for the observed increase in disease duration at the time of RA-associated surgery may be related to the decreased number of synovectomies and suppression of disease activity that has resulted from the use of methotrexate (MTX) and biological agents [9]. MTX was approved for the treatment for RA by the Japanese Ministry of Health, Labor and Welfare in 1999, while infliximab was approved in 2003, etanercept was approved in 2005, adalimumab and tocilizumab were approved in 2008, and abatacept was approved in 2009. In addition, the use and dosage of MTX and the number of use of biological agents have been increasing every year [9]. Conversely, disease activity score (CDAI: clinical disease activity index, SDAI: simplified disease activity index) has been decreasing. Therefore, the recent implementation of medical therapy in RA patients may have suppressed and delayed the progression of destruction in arthritic joints, thereby resulting in postponement of surgery. We believe that these clinical results also provide evidence that these recently introduced medical therapies result in better long-term outcomes for RA patients. However, further studies, including analyses of RA databases that collect long-term data on a variety of surgical interventions, are required to confirm this hypothesis.

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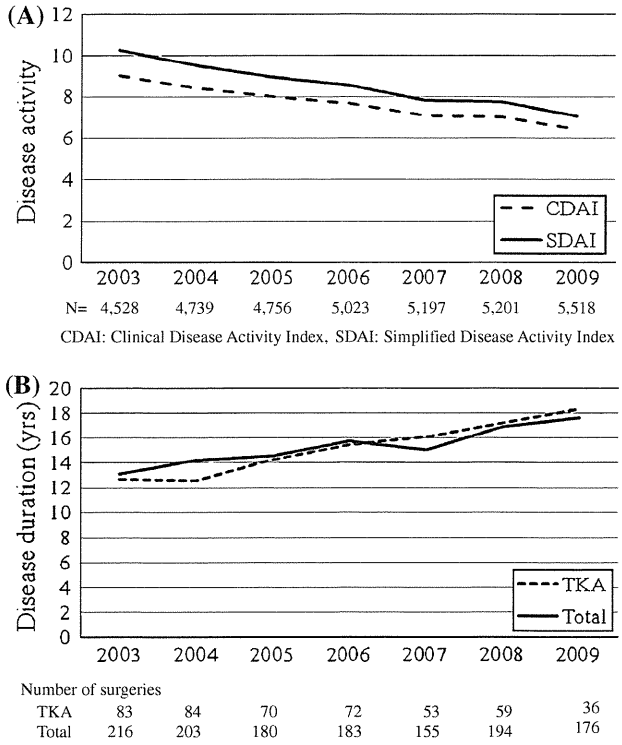

Fig. 1 **a** Clinical disease activity index (CDAI), simplified disease activity index (SDAI), and number of RA outpatients by year. CDAI and SDAI data were collected in October/November each year from 2003 to 2009 in a single institute-based large observational cohort (IORRA). **b** Number of surgeries and disease duration of patients at the time of surgery by year

Acknowledgments This study was supported by a research grant from 38 pharmaceutical companies for the large observational cohort study of RA at the Institute of Rheumatology, Tokyo Women's Medical University.

Conflict of interest None.

Ethics approval Ethics approval was obtained. These patients have participated in a large-scale Japanese RA cohort project, IORRA. Study details were explained to each patient by rheumatologists during clinic visits, and informed consent was received from each patient.

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DOI 10.1007/s10165-011-0495-7

ORIGINAL ARTICLE

Comparison of characteristics and therapeutic efficacy in rheumatoid arthritis patients treated by rheumatologists and those treated by orthopedic surgeons under a team medicine approach at the same institute

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Received: 27 April 2011 / Accepted: 14 June 2011 / Published online: 15 July 2011

Abstract The treatment of rheumatoid arthritis (RA) has improved dramatically with the advent of the latest generation of disease-modifying antirheumatic drugs. Despite these advances, in some patients inflammation is not diminished sufficiently to prevent irreversible musculoskeletal damage, thereby necessitating surgical intervention to reduce pain and improve function. For RA treatment, Japanese orthopedic surgeons also prescribe medication. In this study, we examined whether this Japanese system is effective for RA treatment. We analyzed the clinical condition of RA patients treated by rheumatologists and those treated by orthopedists in a linked registry study using information from a large observational cohort of RA patients followed every half year from 2000 to 2010 (the IORRA cohort). Two groups of patients were compared: patients treated by rheumatologists (rheumatologic group) and patients treated by orthopedists (orthopedic group). The results revealed that patients in the orthopedic group were older, more likely to be female, and had a longer disease duration than patients in the rheumatologic group. The proportion of patients with a history of joint surgery was also much higher in the orthopedic group than in the rheumatologic group. The average scores on the Japanese version of the Health Assessment Questionnaire, and the remission ratio determined using a Boolean-based definition gradually increased from 2000 until 2010, and these findings were consistently better in the rheumatologic group than in the orthopedic group. These data suggest that patients treated primarily by orthopedists are more likely to have long-standing RA compared to patients treated by rheumatologists. Therefore, it is critical for rheumatologists and orthopedists to complement each other medically in the treatment of RA patients.

Keywords Health Assessment Questionnaire (HAQ) · Orthopedist · Rheumatoid arthritis · Rheumatologist · Remission

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Introduction

The course of rheumatoid arthritis (RA) varies greatly from a mild disease to a severe destructive variant that progresses rapidly over a few years. In recent years, disease-modifying antirheumatic drugs (DMARDs), including biological agents, have been used to inhibit or halt the underlying immune process and prevent long-term damage in RA. Biological DMARDs have increased treatment options for RA patients, and clinical remission is considered to be the primary goal of RA treatment. However, despite aggressive treatment with biological DMARDs, progressive destruction of joints continues to occur in a subgroup of RA patients, who eventually require joint surgery [1, 2]. Orthopedic procedures can substantially improve the overall function and quality of life of RA patients. For example, total joint arthroplasty (TJA), such as total knee arthroplasty (TKA) and total hip arthroplasty (THA), has proven to be one of the most successful and most frequently performed orthopedic interventions for reducing pain and enhancing physical function in RA patients [3, 4]. It is generally agreed that TJA is the treatment of choice for relieving pain and improving

function. Therefore, we consider that cooperation between rheumatologists and surgeons is imperative for the proper treatment of RA patients. In western countries, rheumatologists are specially trained to do the investigative work necessary to discover the causes of swollen and painful joints, including osteoarthritis (OA), gout, lupus, back pain, osteoporosis, fibromyalgia, and/or tendonitis, whereas orthopedic surgeons focus on performing surgical procedures. While orthopedic surgeons are familiar with all aspects of the musculoskeletal system, many orthopedists specialize in certain areas, such as the foot and ankle, hand and wrist, spine, shoulder, hip, or knee. Therefore, specific expertise is required among orthopedic surgeons.

In contrast with systems in other countries, the medical service system in Japan differs in that, in Japan, no system exists in which general and primary care physicians consult with hospital-based musculoskeletal services, represented in part by the departments of orthopedics and rheumatology in western countries [5]. Moreover, in addition to performing surgery, orthopedic surgeons in Japan also see patients who are suffering from OA, back pain, shoulder pain, and tendonitis, and prescribe medications. For RA treatment, Japanese orthopedic surgeons also prescribe both non-biological and biological DMARDs. In this study, we examined whether this Japanese system is effective for RA treatment. We compared the therapeutic efficacy in patients with RA treated by rheumatologists versus the efficacy in those treated by orthopedists, using information from a large prospective observational cohort.

Subjects and methods

Patients and study design

The clinical condition of RA patients treated by rheumatologists and orthopedists in a linked registry study was analyzed using information from a large observational cohort of RA patients followed at the Institute of Rheumatology, Tokyo Women's Medical University (IORRA) [6, 7]. The IORRA is an observational study of RA patients that has been conducted at the Institute of Rheumatology, Tokyo Women's Medical University, since October 2000. The medical staff of this institute includes both rheumatologists and orthopedists (36 rheumatologists and 12 orthopedists in 2010). All patients in the cohort fulfilled the 1987 revised American College of Rheumatology criteria for RA and/or the 2010 RA classification criteria [8–10], and more than 98% of the RA patients at our institute have been registered in this study. An average of 5,000 outpatients with RA was seen each month from 2000 to 2010. Patient information was collected biannually (April/May and October/November) when the patients visited the outpatient unit of the Institute of Rheumatology, Tokyo Women's Medical University. This information was used to construct a database that consists of three sections: evaluation data generated by trained rheumatologists and orthopedists, information contributed by patients, and results of laboratory evaluation. RA disease activity was measured using the Disease Activity Score 28 (DAS28), Simplified Disease Activity Index (SDAI), and the ratio of remission determined using a Boolean-based definition derived from RA clinical trials [11, 12]; health-related quality of life was evaluated using a Japanese version of the Health Assessment Questionnaire (J-HAQ) [13].

As mentioned above, these patients had participated in IORRA, and the Tokyo Women's Medical University ethics committee approved the present study. Study details were explained to each patient by rheumatologists and orthopedists during clinic visits, and informed consent was obtained from each patient.

Statistical analysis

Patient characteristics in October 2000 and April 2010 are expressed using the mean, standard deviation, and median. Characteristics in 2000 and 2010 were compared using the Mann–Whitney test and Fisher's exact test. A P value of <0.05 was considered to be statistically significant. All analyses were performed using the statistical package, R, version 2.9.1.

Results

Tables 1 and 2 show the characteristics of the total study population in October 2000 (Table 1) and April 2010 (Table 2). The characteristics of the registered RA patients were actually investigated every half year from October 2000 until April 2010. However, as it would have been very complicated to check the data from each phase, we elected to compare the data from the first and final phases. Table 1 shows the baseline characteristics of the total study population in 2000; at that time, biological agents could not be prescribed for RA patients in Japan. We investigated two groups: patients treated by rheumatologists (rheumatologic group) and patients treated by orthopedists (orthopedic group). Significant differences in background characteristics, including the percentage of females, age, and RA disease duration, were observed between the groups. The orthopedic group included older patients, more women, and patients with a longer disease duration compared to patients in the rheumatologic group. Due to the condition of the patients in the orthopedic group, disease activity as measured by DAS28 and SDAI was also significantly different. Otherwise, the incidence of a history of

Table 1 Baseline characteristics of the total study population and of each patient group in 2000

Variable	Rheumatologic group (n = 2,694)						Orthopedic group (n = 1,142)						P value
	NA	Mean	SD	Median	25%	75%	NA	Mean	SD	Median	25%	75%	
Gender (female/all)	0	0.81	–	–	–	–	0	0.85	–	–	–	–	0.0049
Age (years)	0	57.08	12.84	58.34	49.9	66.2	0	59.23	11.7	60.02	52.3	67.6	5.65E−06
Duration of disease (years)	1	10.07	8.46	8	4	14	0	11.74	8.36	10	6	16	3.48E−14
BMI (kg/m^2)	39	21.34	3	21.09	19.3	23.1	12	21.46	3.02	21.32	19.3	23.2	0.22
DAS28	535	4.03	1.13	3.98	3.19	4.77	223	4.47	1.25	4.4	3.6	5.22	2.87E−18
SDAI	541	13.6	8.7	11.8	7.3	17.7	237	17.35	11.7	14.8	9.2	21.9	1.76E−17
J-HAQ	6	0.74	0.72	0.56	0.13	1.25	2	0.96	0.82	0.75	0.25	1.63	5.45E−14
RF (IU/ml)	132	129.8	217.4	57	20	132	103	148	297	60	23	142	0.096
Remission rate (Boolean-clinical trial-based definition) (%)	528	5.63	–	–	–	–	223	3.59	–	–	–	–	0.27
DMARD use (%)	0	75.84	–	–	–	–	0	74.52	–	–	–	–	0.39
PSL use (%)	0	45.51	–	–	–	–	0	55.78	–	–	–	–	6.62E−09
MTX use (%)	0	35.67	–	–	–	–	0	30.65	–	–	–	–	0.0029
MTX dose (mg/week)	1,777	5.89	2.56	5	4	7.5	810	4.75	2.49	4	4	6	1.63E−06
PSL dose (mg/day)	1,472	5.03	3.5	5	3	6	505	4.4	2.8	4	2.5	5	6.17E−05
Joint surgery (%)	0	10.8	–	–	–	–	0	33.36	–	–	–	–	1.18E−58
TKA (%)	0	2.45	–	–	–	–	0	11.47	–	–	–	–	1.00E−27
THA (%)	0	0.74	–	–	–	–	0	3.33	–	–	–	–	1.65E−08

Mean and median values are shown for continuous variables. Frequency (%) is used for dichotomous variables. Ranges for variables are as follows: J-HAQ [0–3; 3, worst score]

NA number of subjects unavailable for analysis, *BMI* body-mass index, *DAS28* Disease Activity Score in 28 joints, *SDAI* Simplified Disease Activity Index, *J-HAQ* Japanese version of the Health Assessment Questionnaire, *RF* rheumatoid factor, *DMARD* disease-modifying anti-rheumatic drug, *MTX* methotrexate, *PSL* prednisolone, *TKA* total knee arthroplasty, *THA* total hip arthroplasty

joint surgery, TKA, and THA was much higher in RA patients in the orthopedic group than in those in the rheumatologic group. No significant differences in rheumatoid factor positivity or DMARD use were observed between the groups. In this phase, the Boolean-defined ratio of remission was not significantly different between the groups.

Table 2 shows the characteristics of the total study population in the year 2010, at which time biological agents (infliximab, etanercept, adalimumab, tocilizumab, and abatacept) could be prescribed for RA patients in Japan. Similar to the 2000 data, significant differences in background characteristics were observed between the two groups. In 2010, the patients in the orthopedic group were also older, more likely to be female, and had longer disease durations compared to patients in the rheumatologic group. Moreover, the incidence of a history of joint surgery, TKA, and THA was also higher in the orthopedic group than in the rheumatologic group. It follows that disease activity as measured by DAS28 and SDAI was higher in patients in the orthopedic group than in those in the rheumatologic group.

Figure 1 illustrates the increasing trends of the female-to-all patient ratio, mean age, and mean disease duration from October 2000 to April 2010. Similar trends were observed in both the rheumatic and orthopedic groups. However, the female-to-all patient ratio, age, and disease duration of patients in the orthopedic group were always higher than those in the rheumatologic group.

As shown in Fig. 2, methotrexate (MTX) use and dose, glucocorticoid dose, and DMARD use were similar in the rheumatologic and orthopedic groups. Over the 10-year study period, there was a trend toward a decreased average glucocorticoid [prednisolone (PSL)] dose and an increased MTX dose. Compared to rheumatologists, orthopedists prescribed lower PSL and MTX doses. However, no significant difference in DMARD or biological agent use was observed between the rheumatologic and orthopedic groups (this point is also illustrated in Table 2).

Figure 3 shows disease activity as presented by the DAS28 and SDAI scores. In both groups, DAS28 and SDAI scores gradually decreased during the 10-year period; however, the scores in the rheumatologic group were slightly but consistently lower than those in the orthopedic group.

Table 2 Characteristics of the total study population and of each patient group in 2010

Variable	Rheumatologic group (n = 4,490)						Orthopedic group (n = 1,071)						P value
	NA	Mean	SD	Median	25%	75%	NA	Mean	SD	Median	25%	75%	
Gender (female/all)	0	0.84	–	–	–	–	0	0.86	–	–	–	–	0.04
Age (years)	0	59.05	13.37	60.92	51	68.6	0	63.3	11.82	64.46	56.4	71.2	1.94E−20
Duration of disease (years)	189	12.65	9.59	11	5	17	30	16.74	10	16	9	22	4.88E−41
BMI (kg/m^2)	98	21.4	3.17	21	19.2	23.2	19	21.47	3.02	21.23	19.5	23.3	0.14
DAS28	83	3.11	1.11	3.001	2.31	3.81	33	3.3	1.1	3.23	2.54	3.96	9.93E−08
SDAI	80	7.49	6.81	5.87	2.52	10.4	32	8	6.43	6.69	3.5	10.8	3.06E−05
J-HAQ	4	0.65	0.72	0.38	0	1	4	0.88	0.84	0.625	0.13	1.5	3.86E−15
RF (IU/ml)	53	121.8	283.8	48	14	110	27	120.8	216	53	17	112	0.1
Remission rate (Boolean-clinical trial-based definition) (%)	80	22.13	–	–	–	–	32	17.13	–	–	–	–	0.0037
DMARD use (%)	0	91.14	–	–	–	–	0	89.82	–	–	–	–	0.19
PSL use (%)	0	41.65	–	–	–	–	0	48.46	–	–	–	–	5.79E−05
MTX use (%)	0	70.38	–	–	–	–	0	68.81	–	–	–	–	0.32
Biological agent use (%)	0	11.87	–	–	–	–	0	10.46	–	–	–	–	0.2
MTX dose (mg/week)	1,355	8.47	3.44	8	6	10	339	7.05	3.18	6	5	8	3.11E−13
PSL dose (mg/day)	2,620	4.12	3.18	3.95	2	5	552	3.54	2.2	3	2	5	0.0041
Joint surgery (%)	0	16.57	–	–	–	–	0	39.21	–	–	–	–	6.26E−54
TKA (%)	0	6.08	–	–	–	–	0	16.99	–	–	–	–	5.92E−27
THA (%)	0	1.4	–	–	–	–	0	4.11	–	–	–	–	1.51E−07

Mean and median values are shown for continuous variables. Frequency (%) is used for dichotomous variables. Ranges for variables are as follows: J-HAQ [0–3; 3, worst score]

NA number of subjects unavailable for analysis, *BMI* body-mass index, *DAS28* Disease Activity Score in 28 joints, *SDAI* Simplified Disease Activity Index, *J-HAQ* Japanese version of the Health Assessment Questionnaire, *RF* rheumatoid factor, *DMARD* disease-modifying anti-rheumatic drug, *MTX* methotrexate, *PSL* prednisolone, *TKA* total knee arthroplasty, *THA* total hip arthroplasty

Finally, Fig. 4 illustrates the changes in functional ability as measured by J-HAQ and the ratio of remission determined using a Boolean-based definition derived from RA clinical trials. In contrast to the DAS28 and SDAI results, the average J-HAQ score and Boolean-defined remission ratio gradually increased from 2000 until 2010. However, the J-HAQ scores and Boolean-defined remission ratios were consistently better in the rheumatic group than in the orthopedic group.

Discussion

The course of RA varies greatly from mild and sometimes self-limiting disease to a severe destructive variant that progresses rapidly [14]. In general, the immunopathologic characteristics of patients with early RA have few differences compared to those of patients with established RA, suggesting that any unique changes in the synovial microenvironment of early RA patients may occur at very early disease stages. Therefore, from an immunomodulatory standpoint, the "therapeutic window of opportunity" may be more critical than previously thought.

However, recent findings regarding cytokine-independent pathways of joint inflammation might explain the basic disease activity that remains despite the use of currently available pharmaceutical therapies [15], and the progression of radiological damage in patients in clinical remission [16]. In particular, patients who fail to respond to pharmaceutical therapies require surgical intervention to prevent further loss of function [1, 2, 17]. Orthopedic procedures can substantially improve the overall function and quality of life in such patients. Therefore, it is believed that medication and surgical intervention are required for proper RA treatment, and that medical care and treatment for RA should be conducted by a rheumatologist and/or an orthopedist.

The system for RA treatment in Japan is unique in that orthopedists perform surgery and also prescribe RA medications, including biological agents. In the present study, we validated the current status of RA treatment by orthopedists in Japan.

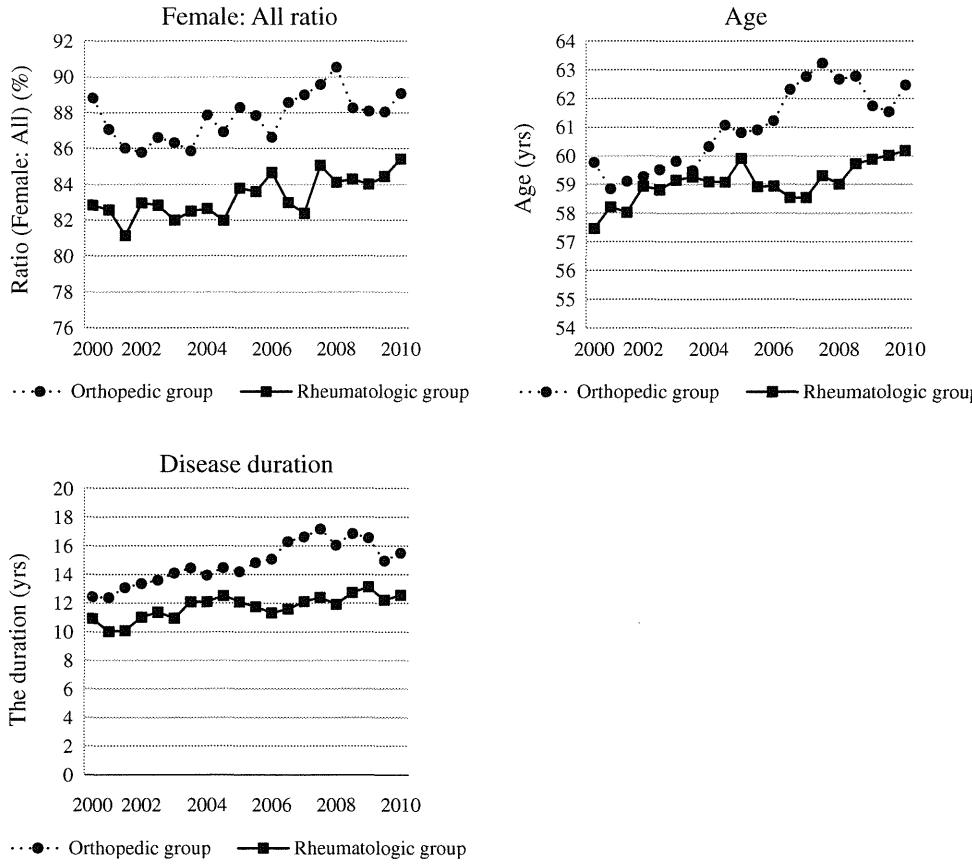

Fig. 1 Trends in the female-to-all patient (*female: all*) ratio, mean age, and mean disease duration from 2000 to 2010. *yrs* years

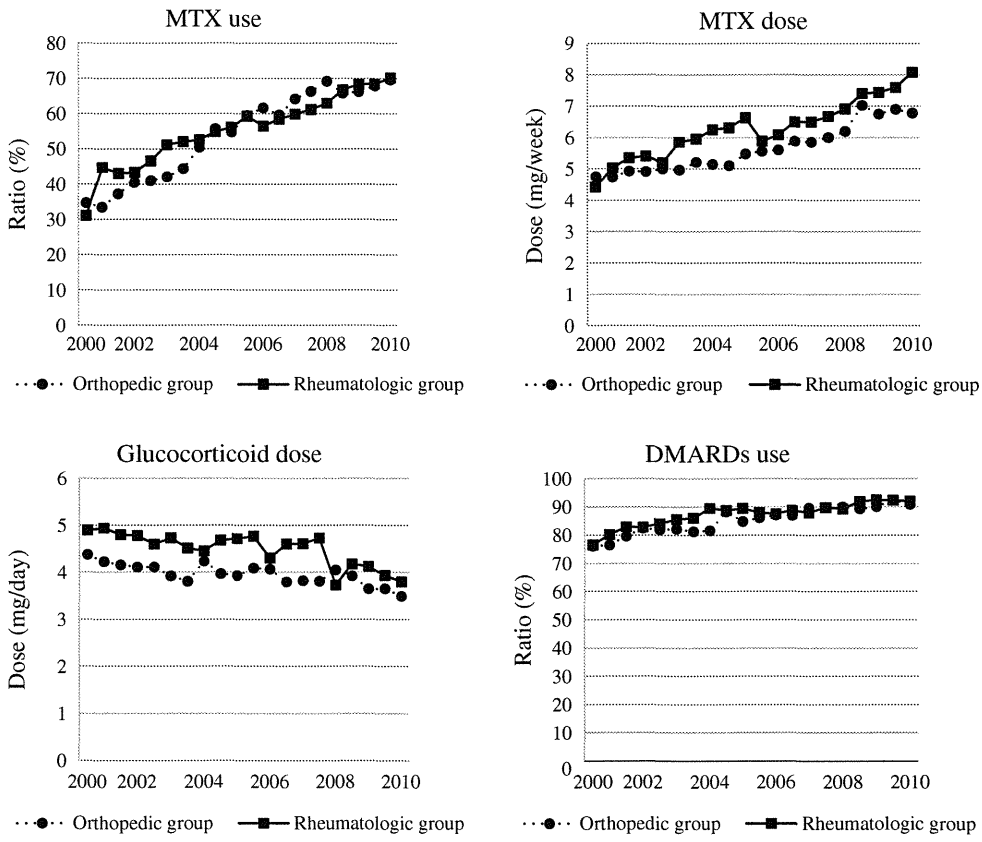

Fig. 2 Use and dose of MTX and use of glucocorticoids and DMARDs from 2000 to 2010. *DMARD* disease-modifying antirheumatic drug, *MTX* methotrexate

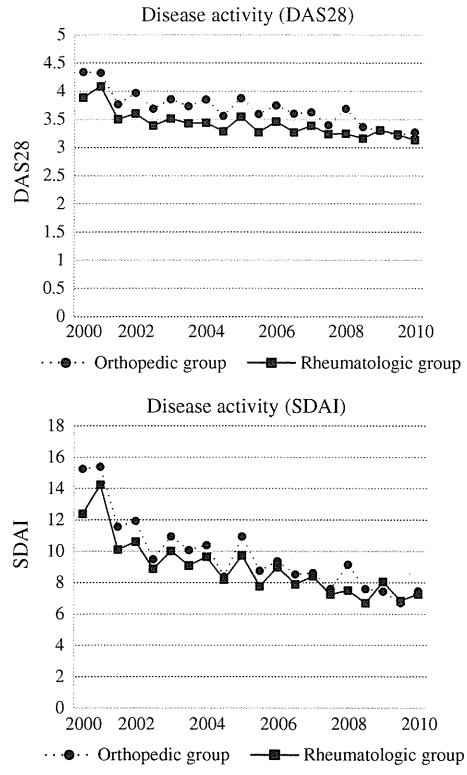

Fig. 3 Disease activity as measured by DAS28 and SDAI scores from 2000 to 2010. *DAS28* Disease Activity Score (DAS) using 28 joint counts, *SDAI* Simplified Disease Activity Index

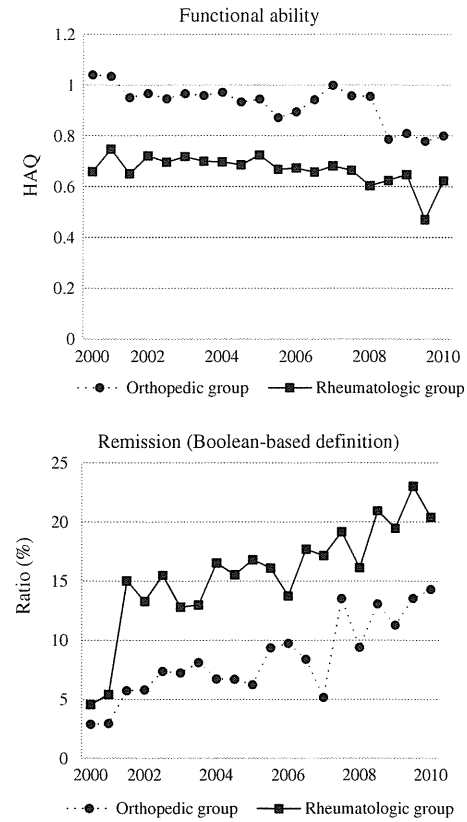

Fig. 4 J-HAQ scores and the ratio of remission determined using a Boolean-based definition from 2000 to 2010. *J-HAQ* Japanese version of the Health Assessment Questionnaire

We analyzed data acquired over a period of 10 years from the IORRA database, which was established as a large observational cohort of RA patients followed at the Institute of Rheumatology, Tokyo Women's Medical University, in 2000. The medical staff of this institute includes both rheumatologists and orthopedists. While these physicians have their own distinctive styles for treating RA patients, they work in an atmosphere of cooperation. For example, if the rheumatologist thinks there is an indication for surgery in a patient with RA, it is easy to consult with the orthopedist.

In the IORRA database, the female-to-all patient ratio, age, and disease duration of patients treated by orthopedists were higher than those of patients treated by rheumatologists. Many studies have reported that female patients have higher RA disease activity and are prone to greater and faster progression of disability over time [18, 19].

Moreover, joint surgery, including TKA and THA, showed a significantly higher rate in patients treated by orthopedists than in patients treated by rheumatologists. However, the patients in the two groups did not receive significantly different medications, with the exception of the doses of MTX and PSL. These data suggested that patients treated primarily by orthopedists were more likely to have established RA compared to patients treated by rheumatologists. The increasingly long life-spans in RA patients are believed to be due to the use of more aggressive pharmacological treatment than that used previously, including biological DMARDs. Thus, these characteristics might explain the greater age and longer disease duration of patients in the orthopedic group.

Disease activity as measured by DAS28 and SDAI was very similar in both groups, albeit that the scores were slightly lower in patients treated by rheumatologists. However, the mean J-HAQ score and the rate of the remission determined using a Boolean-based definition derived from RA clinical trials were consistently better in the rheumatologic group, likely because patient background directly influences both functional ability and remission. Comparison between the backgrounds of patients in the two groups revealed that the rheumatologic group included younger patients and patients with earlier RA compared to patients in the orthopedic group. These results suggest that disease activity in patients seen by rheumatologists could be relatively well controlled with medication, whereas patients seen by orthopedists were more likely to have relatively long-standing active RA. Therefore, the surgery rate was higher and functional ability and remission rates were worse in patients treated by the orthopedists. These observations suggest that patients with long-standing RA were more likely to be treated by orthopedists.

We have previously reported that the declining use of orthopedic surgery at our institute appeared to be primarily influenced by increased treatment with MTX and biologicals [20, 21]. However, a recent multicenter study that included our institute has revealed that the total number of RA-associated surgeries has actually not decreased, and that the numbers have been relatively stable from 1998 to 2008 [22]. Although the number of synovectomies has definitely declined, the numbers of upper limb surgeries and foot arthroplasties have increased. Moreover, when disease activity is increased in joints undergoing damage and destruction in established RA patients, surgical intervention should be considered as a therapeutic option [3]. Together, these studies suggest that RA treatment consisting of both medical treatment and orthopedic joint surgery may lead to a greater improvement in patient quality of life.

We conclude that these clinical data suggest that patients treated primarily by orthopedists are more likely to have long-standing RA compared to patients treated by rheumatologists. Therefore, it is critical for rheumatologists and orthopedists to complement each other medically in the treatment of RA patients.

Acknowledgments The authors would like to thank all participating rheumatologists and orthopedic surgeons, as well as the staff of the IORRA cohort study group. This study was funded through a research grant from 37 pharmaceutical companies given for the support of the large observational cohort study of RA at the Institute of Rheumatology, Tokyo Women's Medical University.

Conflict of interest None.

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nature genetics

Meta-analysis identifies nine new loci associated with rheumatoid arthritis in the Japanese population

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Rheumatoid arthritis is a common autoimmune disease characterized by chronic inflammation. We report a meta-analysis of genome-wide association studies (GWAS) in a Japanese population including 4,074 individuals with rheumatoid arthritis (cases) and 16,891 controls, followed by a replication in 5,277 rheumatoid arthritis cases and 21,684 controls. Our study identified nine loci newly associated with rheumatoid arthritis at a threshold of $P < 5.0 \times 10^{-8}$, including *B3GNT2, ANXA3, CSF2, CD83, NFKBIE, ARID5B, PDE2A-ARAP1, PLD4* and *PTPN2*. *ANXA3* was also associated with susceptibility to systemic lupus erythematosus ($P = 0.0040$), and *B3GNT2* and *ARID5B* were associated with Graves' disease ($P = 3.5 \times 10^{-4}$ and 2.9×10^{-4}, respectively). We conducted a multi-ancestry comparative analysis with a previous meta-analysis in individuals of European descent (5,539 rheumatoid arthritis cases and 20,169 controls). This provided evidence of shared genetic risks of rheumatoid arthritis between the populations.

Rheumatoid arthritis is a complex autoimmune disease characterized by inflammation and the destruction of synovial joints and affects up to 1% of the population worldwide. To date, more than 35 rheumatoid arthritis susceptibility loci, including *HLA-DRB1*, *PTPN22*, *PADI4*, *STAT4*, *TNFAIP3* and *CCR6*, among others, have been identified by GWAS in multiple populations[1–12] and by several meta-analyses of the original GWAS[13–16]. In particular, each meta-analysis of these GWAS uncovered a number of loci that were not identified in the single GWAS, leading to recognition of the enormous power of the meta-analysis approach for detecting causal genes in disease. However, these previous meta-analyses have been performed solely in European populations[13–16] and not in Asian ones. As multi-ancestry studies on validated rheumatoid arthritis susceptibility loci showed the existence of both population-specific and shared genetic components of rheumatoid arthritis[10,17], additional studies in Asian populations might provide useful insight into the underlying genetic architecture of rheumatoid arthritis, which would otherwise be difficult to capture using the studies in a single population. Here, we report a meta-analysis of GWAS and a replication study for rheumatoid arthritis in a Japanese population that was conducted by the Genetics and Allied research in Rheumatic diseases NETworking (GARNET) consortium[10,12]. We subsequently performed a multi-ancestry comparative analysis that incorporated results from a previously conducted meta-analysis of individuals of European ancestry[15].

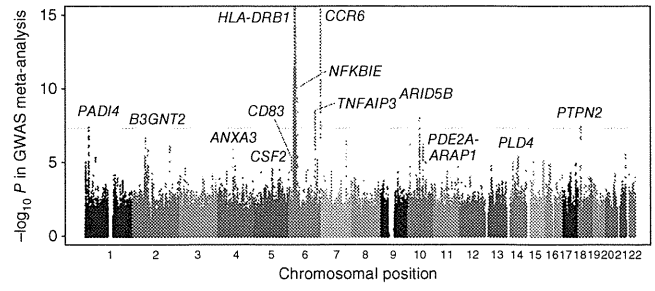

Figure 1 Manhattan plots of the GWAS meta-analysis for rheumatoid arthritis in the Japanese population. The genetic loci that satisfied the genome-wide significance threshold of $P < 5.0 \times 10^{-8}$ (gray line) in the meta-analysis or in the combined study of the meta-analysis and the replication study are presented. The *y* axis shows the $-\log_{10} P$ values of the SNPs in the meta-analysis. The SNPs for which the *P* values were smaller than 1.0×10^{-15} are indicated at the upper limit of the plot.

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Received 24 October 2011; accepted 1 March 2012; published online 25 March 2012; doi:10.1038/ng.2231

Table 1 Results of the GWAS meta-analysis and the replication studies for rheumatoid arthritis

					Associations in Japanese							Associations in Europeans[c]			
					GWAS meta-analysis			Replication study		Combined study		GWAS meta-analysis			
					Allele 1 freq.							Allele 1 Freq.			
rsID[a]	Chr.	Position (bp)	Cytoband	Gene(s)	Allele 1/2	RA	Control	OR (95% CI)[b]	*P*	OR (95% CI)[b]	*P*	OR (95% CI)[b]	*P*	RA	Control	OR (95% CI)[b]	*P*
SNPs with significant associations ($P < 5.0 \times 10^{-8}$ in the combined study)																	
rs11900673	2	62306165	2p15	*B3GNT2*	T/C	0.31	0.28	1.15 (1.08–1.21)	3.5×10^{-6}	1.09 (1.04–1.14)	6.0×10^{-4}	1.11 (1.07–1.15)	1.1×10^{-8}	0.13	0.13	1.05 (0.98–1.13)	0.17
rs2867461	4	79732239	4q21	*ANXA3*	A/G	0.46	0.44	1.13 (1.08–1.19)	4.7×10^{-6}	1.12 (1.08–1.17)	1.2×10^{-7}	1.13 (1.09–1.17)	1.2×10^{-12}	0.37	0.37	0.98 (0.92–1.04)	0.52
rs657075	5	131458017	5q31	*CSF2*	A/G	0.38	0.36	1.12 (1.06–1.18)	3.2×10^{-5}	1.11 (1.06–1.16)	3.8×10^{-6}	1.12 (1.08–1.15)	2.8×10^{-10}	0.10	0.10	1.04 (0.95–1.13)	0.37
rs12529514	6	14204637	6p23	*CD83*	C/T	0.16	0.14	1.19 (1.10–1.27)	6.8×10^{-6}	1.11 (1.05–1.18)	6.0×10^{-4}	1.14 (1.09–1.19)	2.0×10^{-8}	0.055	0.053	1.11 (0.99–1.24)	0.074
rs2233434	6	44340898	6p21.1	*NFKBIE*	G/A	0.24	0.21	1.23 (1.16–1.31)	9.2×10^{-11}	1.17 (1.11–1.23)	2.2×10^{-9}	1.19 (1.15–1.24)	5.8×10^{-19}	0.059	0.040	1.57 (1.11–2.21)	0.0099
rs10821944	10	63455095	10q21	*ARID5B*	G/T	0.39	0.36	1.17 (1.11–1.23)	1.0×10^{-8}	1.15 (1.10–1.20)	3.0×10^{-10}	1.16 (1.12–1.20)	5.5×10^{-18}	0.29	0.26	1.11 (1.05–1.17)	1.9×10^{-4}
rs3781913	11	72051144	11q13	*PDE2A-ARAP1*	T/G	0.71	0.69	1.11 (1.05–1.17)	3.2×10^{-4}	1.13 (1.08–1.18)	6.7×10^{-7}	1.12 (1.08–1.16)	5.8×10^{-10}	0.45	0.43	1.04 (0.99–1.09)	0.13
rs2841277	14	104462050	14q32	*PLD4*	T/C	0.72	0.69	1.11 (1.05–1.18)	2.8×10^{-4}	1.18 (1.13–1.24)	7.0×10^{-12}	1.15 (1.11–1.19)	1.9×10^{-14}	0.47	0.46	1.02 (0.96–1.09)	0.54
rs2847297	18	12787694	18p11	*PTPN2*	G/A	0.37	0.33	1.16 (1.11–1.23)	3.5×10^{-8}	1.06 (1.01–1.11)	0.013	1.10 (1.07–1.14)	2.2×10^{-8}	0.36	0.34	1.10 (1.05–1.15)	9.2×10^{-5}
SNPs with suggestive associations ($5.0 \times 10^{-8} \leq P < 5.0 \times 10^{-6}$ in the combined study)																	
rs4937362	11	127997949	11q24	*ETS1-FLI1*	T/C	0.71	0.68	1.13 (1.07–1.19)	2.0×10^{-5}	1.07 (1.02–1.12)	0.0061	1.09 (1.06–1.13)	7.5×10^{-7}	0.46	0.44	1.06 (1.01–1.11)	0.015
rs3783637	14	54417868	14q22	*GCH1*	C/T	0.76	0.74	1.13 (1.07–1.20)	6.5×10^{-5}	1.07 (1.02–1.13)	0.0062	1.10 (1.06–1.14)	2.0×10^{-6}	0.88	0.88	0.99 (0.88–1.11)	0.87
rs1957895	14	60978085	14q23	*PRKCH*	G/T	0.40	0.39	1.12 (1.06–1.18)	4.1×10^{-5}	1.07 (1.02–1.12)	0.0022	1.09 (1.05–1.13)	3.6×10^{-7}	0.093	0.089	1.01 (0.95–1.07)	0.73
rs6496667	15	88694672	15q26	*ZNF774*	A/C	0.38	0.35	1.13 (1.07–1.19)	4.7×10^{-5}	1.07 (1.02–1.11)	0.0050	1.09 (1.05–1.13)	1.4×10^{-6}	0.21	0.20	1.07 (1.01–1.13)	0.031
rs7404928	16	23796341	16p12	*PRKCB1*	T/C	0.65	0.62	1.13 (1.07–1.19)	1.5×10^{-5}	1.05 (1.01–1.10)	0.026	1.08 (1.05–1.12)	4.0×10^{-6}	0.75	0.75	1.01 (0.94–1.09)	0.79
rs2280381	16	84576134	16q24	*IRF8*	T/C	0.86	0.84	1.16 (1.08–1.25)	1.0×10^{-4}	1.09 (1.03–1.15)	0.0049	1.12 (1.07–1.17)	2.4×10^{-6}	0.62	0.60	1.05 (0.99–1.11)	0.081
SNPs in previously reported rheumatoid arthritis susceptibility loci ($P < 5.0 \times 10^{-8}$ in the GWAS)																	
rs766449	1	17547439	1p36	*PADI4*	T/C	0.44	0.40	1.17 (1.11–1.24)	4.6×10^{-8}	–	–	–	–	0.38	0.37	1.09 (1.03–1.05)	0.0022
rs2157337	6	32609122	6p21.3	*HLA-DRB1*	C/T	0.59	0.44	1.99 (1.88–2.11)	2.6×10^{-118}	–	–	–	–	0.69	0.46	2.50 (2.39–2.62)	$< 1.0 \times 10^{-300}$
rs6932056	6	138284130	6q23	*TNFAIP3*	C/T	0.092	0.073	1.35 (1.23–1.49)	3.2×10^{-9}	–	–	–	–	0.044	0.034	1.41 (1.24–1.60)	1.3×10^{-7}
rs1571878	6	167460832	6q27	*CCR6*	C/T	0.54	0.48	1.31 (1.24–1.39)	3.2×10^{-19}	–	–	–	–	0.47	0.43	1.13 (1.08–1.19)	5.9×10^{-7}

Chr., chromosome; Freq., frequency; RA, rheumatoid arthritis; OR, odds ratio; CI, confidence interval.
[a]SNPs with $P < 5.0 \times 10^{-6}$ in the combined study of the GWAS meta-analysis and the replication study or SNPs with $P < 5.0 \times 10^{-8}$ in the GWAS meta-analysis are annotated according to forward strand and NCBI Build 36.3. Full results of the replication study are provided in **Supplementary Table 3**. [b]Odds ratio of allele 1. [c]Associations in the previous meta-analysis in European populations[15].

The meta-analysis included 4,074 rheumatoid arthritis cases (with 81.4% and 80.4% of the subjects being positive for antibody to cyclic citrullinated peptide (anti-CCP) and rheumatoid factor, respectively) and 16,891 controls from three GWAS of Japanese subjects (from the BioBank Japan Project[10,18], Kyoto University[12] and the Institute of Rheumatology Rheumatoid Arthritis (IORRA)[19]; **Supplementary Table 1**). After the application of stringent quality control criteria, including principal-component analysis (PCA; **Supplementary Fig. 1**) for each GWAS, the meta-analysis was conducted by evaluating ~2.0 million autosomal SNPs with minor allele frequencies (MAFs) ≥0.01, which were obtained through whole-genome imputation of genotypes on the basis of the HapMap Phase 2 East Asian panels (Japanese in Tokyo (JPT) and Han Chinese in Beijing (CHB)). The inflation factor of the test statistics in the meta-analysis λ_{GC} was as low as 1.036, suggesting no substantial effects of population structure (**Supplementary Table 2**). The quantile-quantile plot of *P* values showed a marked discrepancy in the values in its tail from those anticipated under the null hypothesis that there is no association—even after removal of the SNPs located in the human leukocyte antigen (HLA) region, the major rheumatoid arthritis susceptibility locus—thereby showing the presence of significant associations in the meta-analysis (**Supplementary Fig. 2**).

We identified seven loci in the current meta-analysis that satisfied the genome-wide significance threshold of $P < 5.0 \times 10^{-8}$. These included previously known rheumatoid arthritis susceptibility loci, such as *PADI4* at 1p36, *HLA-DRB1* at 6p21.3, *TNFAIP3* at 6q23 and *CCR6* at 6q27 (refs. 1,3,6,10,15) (the smallest $P = 2.6 \times 10^{-118}$ was found at the *HLA-DRB1* locus; **Fig. 1** and **Table 1**). To our knowledge, the other three loci identified, *NFKBIE* at 6p21.1, *ARID5B* at 10q21 and *PTPN2* at 18p11, are newly associated ($P = 9.2 \times 10^{-11}$, 1.0×10^{-8} and 3.5×10^{-8}, respectively).

To validate the associations identified in the meta-analysis, we conducted a replication study of two independent Japanese rheumatoid arthritis case-control cohorts (cohort 1: 3,830 rheumatoid arthritis cases and 17,920 controls, cohort 2: 1,447 rheumatoid arthritis cases and 3,764 controls; **Supplementary Table 1**). To increase the number of subjects and enhance statistical power, genotype data obtained from other GWAS projects conducted for non-autoimmune diseases in Japanese using Illumina platforms were used for the replication control panels. For each of the 46 loci that exhibited $P < 5.0 \times 10^{-4}$ in

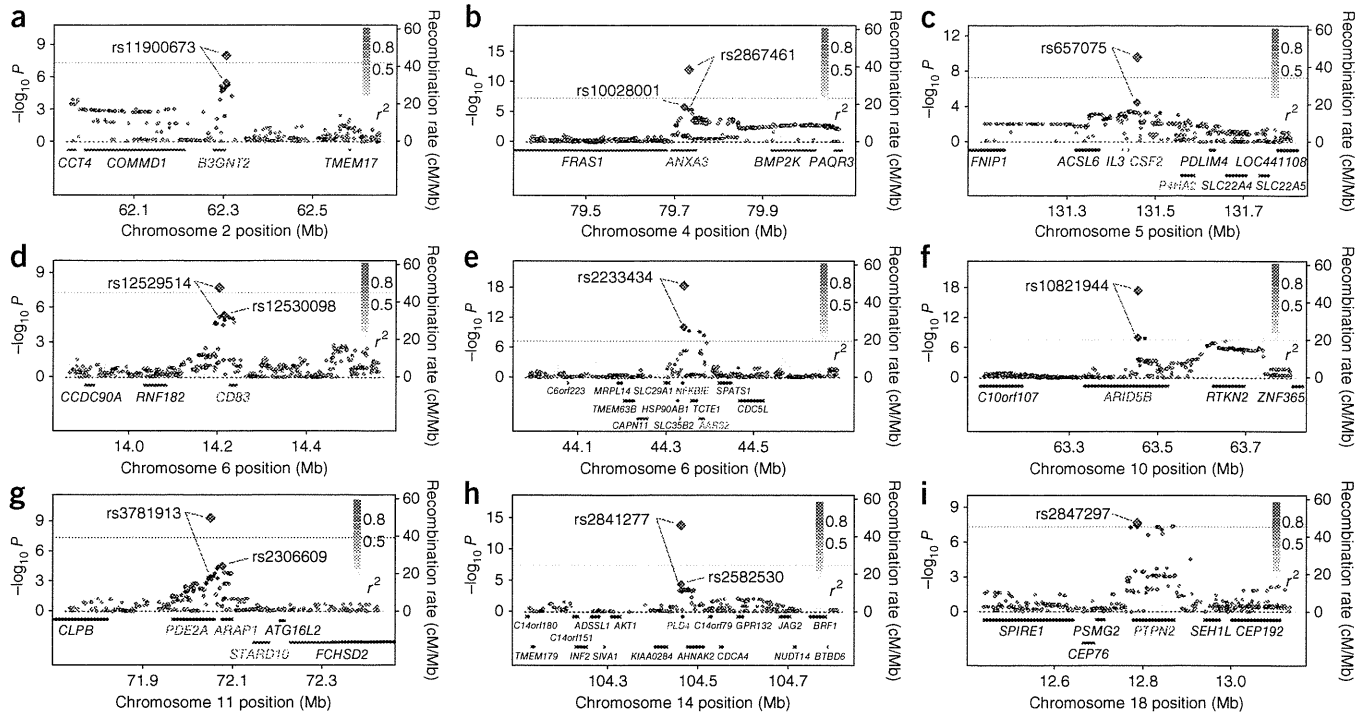

Figure 2 Regional plots of the loci newly associated with rheumatoid arthritis at the genome-wide significance threshold of $P < 5.0 \times 10^{-8}$ in the combined study of the meta-analysis and the replication study. (**a–i**) Regional plots are shown at *B3GNT2* (**a**), *ANXA3* (**b**), *CSF2* (**c**), *CD83* (**d**), *NFKBIE* (**e**), *ARID5B* (**f**), *PDE2A-ARAP1* (**g**), *PLD4* (**h**) and *PTPN2* (**i**). Diamonds represent the $-\log_{10} P$ values of the SNPs, and the red diamonds represent the $-\log_{10} P$ values of the SNPs in the meta-analysis. Red color for the smaller circles represents the r^2 value with the most significantly associated SNP (larger red circle). The purple circle represents the *P* value in the combined study. The blue line shows the recombination rates given by the HapMap Phase 2 east Asian populations (release 22). RefSeq genes at the loci are indicated below. Genes nearest to the marker SNPs at the loci are colored blue (**Supplementary Note**), and genes implicated in eQTL analysis are colored red (**Supplementary Table 4**). At 11q13, two genes (*PDE2A* and *ARAP1*) that are nearest to the SNP selected for the replication study and the most significant SNP in the meta-analysis are highlighted. The plots were drawn using SNP Annotation and Proxy Search (SNAP) version 2.2.

the meta-analysis and had not been reported as rheumatoid arthritis susceptibility loci[1–16], we selected a marker SNP for the replication study (Online Methods and **Supplementary Table 3**).

In the combined analyses of the meta-analysis and the replication study, including a total of 9,351 rheumatoid arthritis cases and 38,575 controls, we identified six newly associated loci, in addition to the *NFKBIE*, *ARID5B* and *PTPN2* loci, that satisfied the significance threshold of $P < 5.0 \times 10^{-8}$, including *B3GNT2* at 2p15, *ANXA3* at 4q21, *CSF2* at 5q31, *CD83* at 6p23, *PDE2A-ARAP1* at 11q13 and *PLD4* at 14q32 (**Figs. 1** and **2** and **Table 1**). Of these loci, *NFKBIE* had the smallest *P* value (5.8×10^{-19}). Although association with rheumatoid arthritis has been described for the *CSF2* and *PTPN2* loci[11,15,16,20,21], ours is the first report to our knowledge validating these associations with a threshold of $P < 5.0 \times 10^{-8}$. Suggestive associations were also observed in *ETS1-FLI1* at 11q24, *GCH1* at 14q22, *PRKCH* at 14q23, *ZNF774* at 15q26, *PRKCB1* at 16p12 and *IRF8* at 16q24 ($5.0 \times 10^{-8} \leq P < 5.0 \times 10^{-6}$). A summary of the genes in the newly associated loci and the results of *cis* expression quantitative trait locus (*cis* eQTL) analysis of the marker SNPs are provided (**Supplementary Table 4** and **Supplementary Note**).

Previous studies have reported associations of rheumatoid arthritis susceptibility loci with other autoimmune diseases[4,10,15,16]. Therefore, we assessed the association of these newly identified susceptibility loci with systemic lupus erythematosus (SLE) by examining the results of an SLE GWAS in the Japanese population (891 cases and 3,384 controls)[22] and in Graves' disease by genotyping 1,783 cases[10] (the controls from the SLE analysis were used for testing for Graves' disease). We observed significant associations of the *ANXA3* locus with SLE and of the *B3GNT2* and *ARID5B* loci with Graves' disease, which showed the same directional effects of the alleles as in rheumatoid arthritis ($P < 0.05/9 = 0.0056$, Bonferroni correction of the number of loci; **Supplementary Table 5**). It should be noted that relatively small sample sizes in the SLE and Graves' disease cohorts might yield limited statistical power, and further evaluations enrolling larger numbers of subjects would be desirable.

To highlight genetic backgrounds of rheumatoid arthritis that are common and divergent in different ancestry groups, we conducted a multi-ancestry comparative analysis of the present study in Japanese and a previous GWAS meta-analysis in Europeans that included 5,539 rheumatoid arthritis cases and 20,169 controls[15] (**Fig. 3a–c**). First, we compared associations in the reported[1–16] or newly identified rheumatoid arthritis susceptibility loci (**Fig. 3a** and **Supplementary Table 6**). Of the 46 rheumatoid arthritis risk variants evaluated, 6 were monomorphic in Japanese, and all were polymorphic in Europeans. We observed significant associations at 22 loci in Japanese and at 36 loci in Europeans (false discovery rate (FDR) < 0.05, $P < 0.0030$), with 14 loci being shared between the populations. Of the newly associated rheumatoid arthritis susceptibility loci identified in our Japanese meta-analysis, significant associations were also observed in the European meta-analysis at the *ARID5B* and *PTPN2* loci ($P = 1.9 \times 10^{-4}$ and 9.2×10^{-5}, respectively; **Table 1**). Significant positive correlation of odds ratios was observed between the studies ($r = 0.822$, $P = 8.1 \times 10^{-11}$; **Fig. 3b**), suggesting that a substantial proportion of genetic factors are shared between

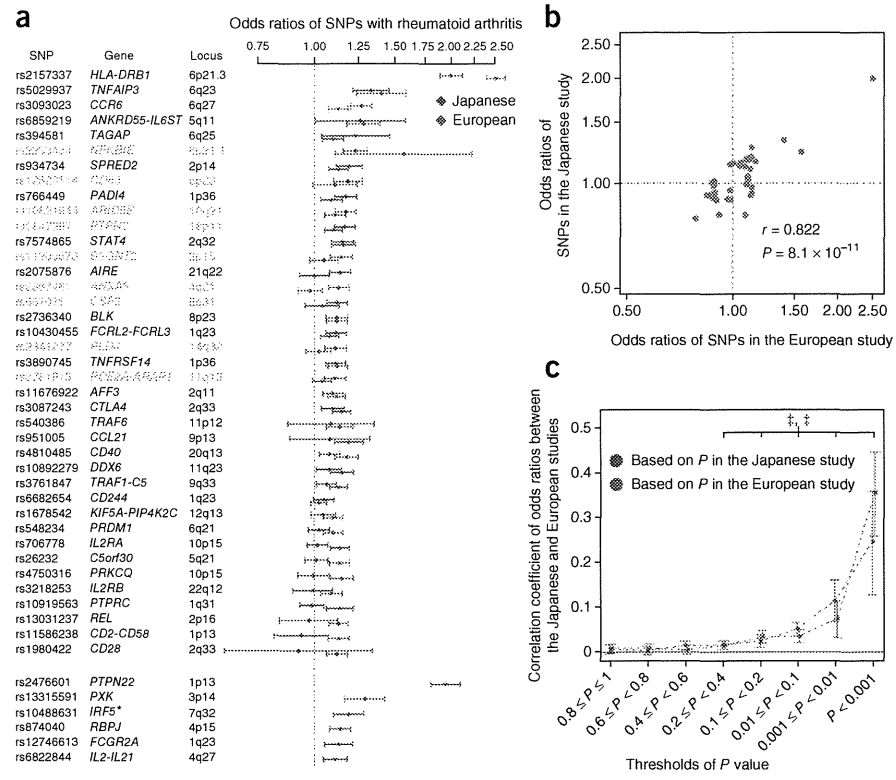

Figure 3 Overlap of the associations with rheumatoid arthritis between Japanese and European populations. (**a**) Forest plots of SNPs in the rheumatoid arthritis susceptibility loci (**Supplementary Table 6**). We selected the genetic loci that have been validated to be associated with rheumatoid arthritis susceptibility by showing associations in the reports of multiple cohorts or satisfying the genome-wide significant threshold ($P < 5.0 \times 10^{-8}$) in previous studies, including in the meta-analysis and replication phases[1–16]. For each of the loci, the most significant SNP among those reported in the previous or present study were selected[1–16]. SNPs in the newly identified rheumatoid arthritis susceptibility loci are colored green. Odds ratios and 95% confidence interval (CI) values are based on rheumatoid arthritis risk alleles, and the SNPs are ordered according to the odds ratios in the Japanese study. Several SNPs were monomorphic in the Japanese population. The odds ratios of these SNPs in the European study are presented below. The asterisk indicates that an association of another variant at the *IRF5* locus was reported in the Japanese population[24]. (**b**) Correlation of the odds ratios of the SNPs in the validated rheumatoid arthritis susceptibility loci between the two populations. SNPs that were polymorphic in both populations were used; odds ratios were based on the minor allele in the Japanese population. (**c**) Correlation of the odds ratios of the genome-wide SNPs, excluding the rheumatoid arthritis susceptibility loci. Correlations were evaluated for sets of SNPs stratified by the thresholds based on the meta-analysis *P* values in each population after pruning of the SNPs by LD ($r^2 < 0.3$). Correlation coefficient and 95% CI are indicated on the *y* axis. Significant correlation of the odds ratios was observed (‡, $P < 0.005$), even for the SNPs that showed moderate associations with rheumatoid arthritis (meta-analysis $P < 0.4$ in each population).

the two ancestry groups[17]. When the rheumatoid arthritis cases of the Japanese GWAS meta-analysis were stratified into anti-CCP–positive or rheumatoid factor–positive cases (n = 3,209) and controls (n = 16,891), similar results were observed (data not shown). Nevertheless, most of the SNPs assessed here are not necessarily causal variants, and further fine mapping of the loci is warranted to precisely evaluate the shared genetic predisposition between the populations.

Next, we compared regional associations within each of the loci and identified unique patterns in the *ARID5B* locus at 10q21 (**Supplementary Fig. 3**). In Japanese, three peaks of association were observed ($P = 1.0 \times 10^{-8}$ at rs10821944, $P = 5.7 \times 10^{-8}$ at rs10740069 and $P = 8.5 \times 10^{-6}$ at rs224311). These three variants were in weak linkage disequilibrium (LD) in Japanese ($r^2 < 0.10$), indicating independent associations with each of the other SNPs that satisfied a region-wide significance threshold of $P < 3.5 \times 10^{-5}$ (conditional $P = 4.3 \times 10^{-6}$, 1.7×10^{-5} and 1.8×10^{-5}, respectively) (**Supplementary Fig. 3**). In contrast, there was only one peak of association in Europeans ($P = 1.2 \times 10^{-6}$ at rs12764378; $r^2 = 0.59$ with rs10821944 in Europeans), and no additional association was observed in conditional analysis with rs12764378 (the smallest conditional $P = 2.2 \times 10^{-4}$), suggesting that the number of independent associations may be different at this locus in the two populations.

Finally, we conducted polygenic assessment for common variants showing modest associations to rheumatoid arthritis (those not meeting the genome-wide association threshold). This approach has been recognized to be a means to explain a substantial proportion of genetic risk[23]. For the SNPs that were shared between the two meta-analyses but not included in the validated rheumatoid arthritis susceptibility loci, we adopted LD pruning of the SNPs ($r^2 < 0.3$). We then evaluated the correlation of odds ratios of the SNPs between the two meta-analyses and observed a significant positive correlation ($r = 0.023$, $P < 1.0 \times 10^{-300}$). When the SNPs were stratified according to the *P* values in each meta-analysis, significant positive correlations of odds ratios were observed for the SNPs, even for those showing modest association ($P < 0.4$ in the meta-analysis of Japanese or Europeans; $r = 0.014$–0.36 for each *P* value range, $P < 0.005$ for each correlation test) (**Fig. 3c**). Correlations (r) of odds ratios observed herein suggest substantial overlap of the genetic risk of rheumatoid arthritis between the two populations, not only in the validated rheumatoid arthritis susceptibility loci but also at the loci showing nonsignificant associations. This suggests the usefulness of a meta-analysis approach involving multiple ancestry groups in identifying additional susceptibility loci.

In summary, we identified multiple new loci associated with rheumatoid arthritis through a large-scale meta-analysis of GWAS in Japanese. Multi-ancestry comparative analysis provided evidence of significant overlap in the genetic risks of rheumatoid arthritis between Japanese and Europeans. Thus, findings from the present study should contribute to the further understanding of the etiology of rheumatoid arthritis.

URLs. GARNET consortium, http://www.twmu.ac.jp/IOR/garnet/home.html; The BioBank Japan Project (in Japanese), http://biobankjp.org/; International HapMap Project, http://www.hapmap.org/; PLINK, http://pngu.mgh.harvard.edu/~purcell/plink/; EIGENSTRAT, http://genepath.med.harvard.edu/~reich/Software.htm; MACH and mach2dat, http://www.sph.umich.edu/csg/abecasis/MACH/index.

html; R statistical software, http://cran.r-project.org/; SNAP, http://www.broadinstitute.org/mpg/snap/index.php; NCBI GEO database, http://www.ncbi.nlm.nih.gov/geo/.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Note: Supplementary information is available on the Nature Genetics website.

ACKNOWLEDGMENTS

The authors acknowledge the essential role of the GARNET consortium in developing the study. In this study, the following GARNET members are included: CGM of RIKEN, University of Tokyo, the BioBank Japan Project, Kyoto University and IORRA. We would like to thank all the doctors and staff who participated in sample collection for the RIKEN cohort and the BioBank Japan Project. We thank K. Kobayashi and M. Kitazato for their technical assistance. We thank T. Raj for calculation of composite of multiple signals (CMS). We thank M. Kokubo for DNA extraction, GWAS genotyping and secretarial assistance. We would also like to thank H. Yoshifuji, N. Yukawa, D. Kawabata, T. Nojima, T. Usui and T. Fujii for collecting DNA samples. We thank Y. Katagiri for her technical efforts. We also appreciate the contribution of E. Inoue and other members of the Institute of Rheumatology, Tokyo Women's Medical University, for their efforts on the IORRA cohort. This study was supported in part by grants-in-aid from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) in Japan, the Ministry of Health, Labour and Welfare (MHLW) in Japan, the Japan Society for the Promotion of Science (JSPS), Core Research for Evolutional Science and Technology (CREST), Solution-Oriented Research for Science and Technology (SORST), INSERM and the Okawa Foundation for Information and Telecommunications.

AUTHOR CONTRIBUTIONS

Y. Okada, C.T., K.I., Y. Kochi and K.O. designed the study and drafted the manuscript. Y. Okada, C.T., K.I., T.K., H.O., N.N., M.T., M.L., K. Tokunaga and M.K. managed genotyping and manipulation of GWAS data. Y. Okada, Y. Kochi, C.T. and K.I. managed genotyping of replication cohorts. Y. Okada, T.K., H.O., E.A.S., A. Takahashi and R.Y. performed statistical analysis. Y. Kochi, A.S., K. Myouzen, T. Sawada, Y. Nishoka, M.Y., T. Matsubara, S.W., R.T. and S.T. collected samples and managed phenotype data for the rheumatoid arthritis cohorts from the BioBank Japan Project and CGM, RIKEN. C.T., K.O., T.K., M.T., K. Takasugi, K.S., A.M., S.H., K. Matsuo, H. Tanaka, K. Tajima and M.L. collected samples and managed phenotype data for the rheumatoid arthritis cohorts from Kyoto University. K.I., T. Suzuki, T.I., Y. Kawamura, H. Tanii, Y. Okazaki and T. Sakaki collected samples and managed phenotype data for the rheumatoid arthritis cohorts from IORRA. Y. Kochi managed the data for the SLE and Graves' disease cohorts. A.S., C.T. and K.I. analyzed the sera of subjects with rheumatoid arthritis. E.A.S., F.A.S.K., P.K.G., J.W., K.A.S., L.P. and R.M.P. managed the data for the rheumatoid arthritis cohorts in European populations. A. Taniguchi, A. Takahashi, K. Tokunaga, M.K., Y. Nakamura, N.K., T. Minori, R.M.P., H.Y., S.M., R.Y., F.M. and K.Y. supervised the overall study.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Subjects. The Japanese participants in the meta-analysis (4,074 rheumatoid arthritis cases and 16,891 controls) and the replication study (5,277 rheumatoid arthritis cases and 21,684 controls) were obtained through the collaborations of the GARNET consortium (**Supplementary Table 1**)[10,12]. The meta-analysis was conducted on three independent GWAS (from the BioBank Japan Project[18] with 2,414 rheumatoid arthritis cases and 14,245 controls[10], Kyoto University with 1,237 rheumatoid arthritis cases and 2,087 controls[12] and IORRA[19] with 423 rheumatoid arthritis cases and 559 controls). The replication study consisted of two independent cohorts (cohort 1 included 3,830 rheumatoid arthritis cases and 17,920 controls, and cohort 2 included 1,447 rheumatoid arthritis cases and 3,764 controls). We employed a case-control cohort of SLE (891 cases and 3,384 controls)[22] and 1,783 cases with Graves' disease[10]. Details of 5,539 rheumatoid arthritis cases and 20,169 controls included in the meta-analysis in European populations were described elsewhere[15]. All participants provided written informed consent for participation in the study, as approved by the ethical committees of the institutional review boards. Detailed descriptions of the participating subjects are provided (**Supplementary Note**).

Genotyping and quality control in the GWAS. Genotyping platforms and quality control criteria for the GWAS, including cutoff values for sample call rates, SNP call rates, MAF and Hardy-Weinberg *P* values, are given (**Supplementary Table 2**). For the subjects enrolled in each of three GWAS, we excluded closely related subjects with first- or second-degree kinship, which was estimated using PLINK version 1.06 (see URLs). We also excluded the subjects determined to be ancestry outliers from East Asian populations using PCA performed by EIGENSTRAT version 2.0 (see URLs) along with HapMap Phase 2 panels (release 24; **Supplementary Fig. 1**). Genotype imputation was performed on the basis of the HapMap Phase 2 East Asian populations, using MACH version 1.0.16 (see URLs) in a two-step procedure as described elsewhere[25]. We excluded imputed SNPs with MAF < 0.01 or *Rsq* < 0.5 from each of the GWAS. Associations of the SNPs with rheumatoid arthritis were assessed by logistic regression models assuming additive effects of the allele dosages of the SNPs using mach2dat software (see URLs).

Meta-analysis. We included 1,948,139 autosomal SNPs that satisfied quality control criteria in all three GWAS (**Supplementary Table 2**). SNP information was based on a forward strand of the NCBI build 36.3 reference sequence. The meta-analysis was performed using an inverse variance method assuming a fixed-effects model from the study-specific effect sizes (logarithm of odds ratio) and the standard errors of the coded alleles of the SNPs determined with the Java source code implemented by the authors[25]. Genomic control corrections[26] were carried out on test statistics of the GWAS using the study-specific inflation factor (λ_{GC}) and was applied or reapplied to the results of our current meta-analysis (**Supplementary Fig. 2**).

Replication study. We selected a SNP for the replication study from each of the loci that exhibited $P < 5.0 \times 10^{-4}$ in the meta-analysis that had not previously been reported as rheumatoid arthritis susceptibility loci[1–16] (**Supplementary Table 3**). For control subjects, we used genotype data obtained from additional GWAS for non-autoimmune diseases or healthy controls, genotyped using Illumina HumanHap550 BeadChips or HumanHap610-Quad BeadChips, and the cases for rheumatoid arthritis and Graves' disease were genotyped with the TaqMan genotyping system (Applied Biosystems; **Supplementary Table 1**). Selection of the SNP was conducted according to the following criteria: if the SNP with the most significant association in the locus was genotyped in the replication control panel, then that SNP was selected; otherwise, a tag SNP in the replication control panel with the strongest LD was selected (mean $r^2 = 0.89$). For the three SNPs that yielded low call rates (<90%), we alternatively selected proxy SNPs with the second strongest LD. As a result, average genotyping call rates of the SNPs were 99.9% and 99.0% for the controls and cases, respectively. We then evaluated concordance rates between the assayed genotypes by applying these two different methods to samples from 376 subjects who were randomly selected. This procedure yielded high concordance rates of ≥99.9%. Associations of the SNPs were evaluated using logistic regression assuming an additive-effects model of genotypes in R statistical software version 2.11.0 (see URLs). The combined study of the meta-analysis and replication study was performed using an inverse variance method assuming a fixed-effects model[25].

***Cis* eQTL analysis.** For each marker SNP of the newly identified rheumatoid arthritis susceptibility locus, correlations between SNP genotypes and expression levels of genes located 300 kb upstream or downstream of the SNP measured in B-lymphoblastoid cell lines (GSE6536) were evaluated using data from the HapMap Phase 2 east Asian populations[27].

Multi-ancestry analysis of the meta-analyses in Japanese and Europeans. We evaluated the associations of the variants in the validated rheumatoid arthritis susceptibility loci by comparing the results from the current meta-analysis in Japanese with those from a previous meta-analysis in Europeans[15]. We assessed two variants in the *IRF5* locus, where different causal variants were identified in the two populations[24]. For the conditional analysis of the regional associations in the *ARID5B* locus (**Supplementary Fig. 3**), we repeated the meta-analysis at that locus by incorporating genotypes of the referenced SNP(s) as additional covariate(s). For comparison of the odds ratios of the SNPs, we first selected SNPs that were shared between the meta-analyses in Japanese and Europeans. Next, we removed the SNPs located more than 1 Mb away from each of the marker SNPs in the validated rheumatoid arthritis susceptibility loci, except for in the HLA region, where we removed the SNPs located between 24,000,000 bp to 36,000,000 bp on chromosome 6 because of the existence of long-range haplotypes with rheumatoid arthritis susceptibility in this region[28]. LD pruning of the SNPs was conducted for the SNP pairs that were in LD ($r^2 \geq 0.3$) in both HapMap Phase 2 East Asian and Utah residents of Northern and Western European ancestry (CEU) populations (release 24). Correlations of the odds ratios were evaluated using R statistical software version 2.11.0.

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Mod Rheumatol (2012) 22:186–194
DOI 10.1007/s10165-011-0510-z

ORIGINAL ARTICLE

Structural damages disturb functional improvement in patients with rheumatoid arthritis treated with etanercept

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Received: 20 May 2011 / Accepted: 1 August 2011 / Published online: 8 September 2011

Abstract Tumor necrosis factor (TNF) inhibitors have produced improvements in clinical, radiographic, and functional outcomes in rheumatoid arthritis (RA) patients. However, it remains unclear whether factors affecting physical functions remain following TNF therapy. The objective of our study was to assess factors affecting improvement of physical functions and to shed light on relations to disease activity and structural changes in patients with RA treated with etanercept. The study enrolled 208 patients, all of whose composite measures regarding clinical, radiographic, and functional estimation both at 0 and 52 weeks after etanercept therapy were completed. Mean disease duration of 208 patients was 9.6 years, mean Disease Activity Score for 28 joints (DAS28) was 5.4, and mean van der Heijde modified total Sharp score (mTSS) was 94.6. Mean Health Assessment Questionnaire Disability Index (HAQ-DI) improved from 1.4 at 0 weeks to 1.0 at 52 weeks after etanercept therapy, a 31% reduction, which was much less than changes in DAS28 and mTSS. By multivariate analysis, HAQ-DI and mTSS at baseline were significantly correlated HAQ remission. Median HAQ-DI improved in 100 versus 20% of the HAQ-DI $\leq$0.6 versus $\geq$2.0 groups, respectively. The mTSS cutoff point at baseline to obtain HAQ remission was 55.5. During etanercept treatment in the mTSS <55.5 versus >55.5 groups, median HAQ-DI improved in 70 versus 39%; remission was achieved in 59 versus 33%; and there was no improvement in14 versus 30%, respectively. HAQ-DI improvement was significantly correlated with that of DAS28 but not of mTSS. In conclusion, higher HAQ and mTSS at baseline inhibits HAQ-DI improvement within 1 year of etanercept treatment, and the cutoff point necessary for mTSS to improve physical functions in patients with RA was 55.5.

Keywords Rheumatoid arthritis · Anti-TNF · Treatment · Disease activity · Physical function

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Introduction

Rheumatoid arthritis (RA) is a systemic inflammatory disease that causes significant morbidity and mortality. Tumor necrosis factor (TNF) plays a pivotal role in the pathological processes of RA through accumulation of inflammatory cells and self-perpetuation of inflammation, leading to joint destruction. The combined use of biologics targeting TNF and methotrexate (MTX) has revolutionized RA treatment, producing significant improvements in clinical, radiographic, and functional outcomes that were not previously observed, as well as producing the emerging outcome of clinical, structural, and functional remission [1–5]. Among them, the most important endpoint is

improvement and maintenance of physical functions and functional remission, but the relevance of clinical and structural factors affecting physical functions and limiting improvement of physical functions remain unclear.

The safety and efficacy of the representative TNF inhibitor etanercept, a fully human TNF soluble receptor Fc fusion protein, have been reported in patients with active RA regardless of treatment with MTX [6–10]. One of the most important reports regarding long-term safety, maintenance, and efficacy of etanercept for RA was reported by Weinblatt et al. [11–13]. In their studies, the Health Assessment Questionnaire Disability Index (HAQ-DI) score assessing physical functions decreased rapidly, and the HAQ-DI reductions were clinically significant and maintained for >10 years in all RA patients treated with etanercept. Also, greater median reductions in HAQ-DI scores occurred in patients with early (mean duration 1 year) compared with longstanding (mean duration 12 years) RA, and that difference was sustained at each observation point for 10 years, implying that HAQ-DI improvement is limited in longstanding RA patients. Furthermore, HAQ-DI decreased rapidly within 1 year, and the reduction maintained for 10 years and median HAQ-DI responses at year 11 were 0.4 for the early and 0.9 for longstanding RA patients, suggesting that HAQ-DI score at 10 years after initiation of etanercept therapy depends on HAQ-DI changes at during the first year of treatment [11]. Hence, it appears that physical function after a decade of etanercept therapy depends on the degree of HAQ-DI reduction within the first year of treatment initiation.

However, factors affecting reduced physical function at the initial 1 year remain unclear. Based on this background, the multicenter study reported here was undertaken to assess factors at baseline affecting improvement of physical functions, shedding light on not only disease activity but also on structural values to evaluate progression of articular destruction.

Materials and methods

Patients and methods

Data and information on RA patients that fulfilled the diagnostic criteria of the American College of Rheumatology (ACR) [14] were collected from the major rheumatology centers in Japan, including the First Department of Internal Medicine, School of Medicine, University of Occupational and Environmental Health Japan, Kitakyushu; the Division of Rheumatology and Clinical Immunology, Department of Internal Medicine, Saitama Medical Center, Saitama Medical University, Saitama; Department of Rheumatology and Clinical Immunology, School of Medicine, Keio University; and the Institute of Rheumatology, Tokyo Women's Medical University. This retrospective study (the ENRICH study) enrolled 208 patients with RA, all of whose information collection regarding composite disease activities, functional ability, and physical functions both at 0 and 52 weeks after initiation of etanercept therapy was completed. All patients who received etanercept treatment (25 mg twice a week in 203 patients and 25 mg once a week in five patients) by March 2009 were registered. The study design was approved by each institution, and informed consent was obtained from each patient before etanercept treatment was undertaken. Demographic data, including disease duration and concomitant therapy, were collected from medical charts. The following parameters were evaluated before and at 52 weeks after the initial etanercept therapy: tender joint count (TJC) 28, swollen joint count (SJC) 28, patient's global assessment of disease activity (PGA), and C-reactive protein (CRP). Disease activity of individual patients was assessed by the Disease Activity Score for 28 joints (DAS28) erythrocyte sedimentation rate (ESR) or DAS28-CRP, calculated according to the authorized formula (http://www.das-score.nl/). Concomitant use of MTX was instituted in all patients, although dose was determined by each attending physician. Joint damage was assessed by van der Heijde modified total Sharp score (mTSS). X-ray images of hands and feet at baseline, study entry, and 1 year after the study were available and evaluable for 120 patients due to loss of radiographs and/or low-quality of X-ray images. Two expert readers independently scored articular damage and progression in a blinded fashion according to the mTSS scoring method. Difference of the two readers' scores for each patient's radiographs was <1% of the maximum mTSS score—that is, 448 [15–17]. Patient demographic indicators and baseline disease characteristics are summarized in Table 1.

Statistical analysis

Patient's baseline characteristics are summarized in Table 1 using the mean values for continuous variables. All multivariate analyses were conducted using the variables of gender, age, disease duration, DAS28-ESR score, DAS28-CRP score, tender joint count (TJC) (0–28), swollen joint count (SJC) (0–28), PGA (0–100 mm, visual analogue scale), ESR, CRP, HAQ-DI, rheumatoid factor (RF), MTX dose, and prednisolone (PSL) dose at baseline. Spearman's correlation analyses were performed to evaluate the association between multivariables at baseline and at 52 weeks after initiation of etanercept therapy (last observation carried forward) of 208 patients. Logistic regression analysis was carried out to estimate HAQ-DI at 52 weeks as dependent variables (probability), and by mTSS at 0 weeks

Table 1 Demographic indicators and baseline disease characteristics

	Mean	Standard deviation	Maximum	Median	Minimum
Age	54.6	13.4	84.0	56.0	18.0
Sex	$f = 83.1\%$				
Duration (year)	9.6	8.2	41.0	8.0	1.0
MTX	w/= 65%				
CS	w/= 68%				
Prior biologics	w/= 20%				
RF	210	346	3510	116	0
MMP-3	278	311	2400	178	8
SJC	7.5	5.2	28.0	6.5	0.0
TJC	7.5	6.3	28.0	6.0	0.0
CRP (mg/dl)	2.9	3.1	23.4	1.9	0.0
ESR (mm/1 h)	51.9	25.6	140.0	49.0	2.3
GH (mm/100 mm)	56	23	100	60	1
DAS28-ESR	5.5	1.1	8.2	5.6	2.9
DAS28-CRP	4.9	1.2	7.8	4.9	2.2
HAQ-DI	1.4	0.8	3.0	1.4	0.0
mTSS	94.6	79.6	378.0	74.0	6.0
EJ	47.9	47.5	233.0	37.5	0.0
JSN	46.7	33.9	145.0	38.6	0.0
ΔmTSS	15.2	16.1	133.8	11.3	0.5

Data are number of patients (%) for categorical data and means for continuous data. Statistical difference was assessed by nonparametric Wilcoxon *t* test and *P* (Prob > ChiSq) values are shown. Data supplied for 208 patients with RA

HAQ-DI Health Assessment Questionnaire Disability Index, *DAS28* Disease Activity Score for 28 tender and 28 swollen joints, *CS* corticosteroid, *RF* rheumatoid factor, *MMP-3* matrix metalloprotease-3, *SJC* swollen-joint count, *TJC* tender-joint count, *GH* Global Health Assessment, *CRP* C-reactive protein, ESR erythrocyte sedimentation rate, *EJ* erosive joint, *JSN* joint-space narrowing, *mTSS* van der Heijde modified total Sharp score, Δ estimated yearly progression

as independent variables. A receiver operating characteristic (ROC) curve was developed based on logistic analysis, and the significant cutoff point was determined from the curve. For categorical response parameters, group comparisons were made using a nonparametric Wilcoxon *t* test. Statistical analyses were performed using JMP software version 8 (SAS Institute, Cary, NC, USA). All reported *P* values are two sided; $P < 0.05$ was considered significant.

Results

Changes in DAS28, ΔmTSS, and HAQ-DI in patients with RA before and after etanercept treatment

Demographic indicators and baseline characteristics of the 208 patients were: mean age 54.6 years; mean disease duration 9.6 years; mean HAQ-DI 1.4; mean DAS28-ESR 5.5, implying that most patients had highly active disease; and mean mTSS 94.6, indicating that the population included patients with long-established RA (Table 1). Mean DAS28-CRP was 4.9 at baseline, but DAS28-CRP at 52 weeks after initiation of etanercept treatment was 2.6 ($P < 0.0001$ by nonparametric Wilcoxon *t* test), producing a 46% reduction in DAS (Fig. 1a). Furthermore, as shown in the probability plot, score improvement was observed in the majority of patients, and 55% reached DAS remission, showing values of DAS28 < 2.6 (Fig. 1d). Estimated yearly mTSS progression (ΔmTSS) at 0 weeks was 15.3, whereas that at 52 weeks after etanercept therapy was 2.0 ($P < 0.0001$ by nonparametric Wilcoxon *t* test), producing a 87% reduction rate in joint destruction (Fig. 1b). In addition, progression was completely inhibited in 48% of patients (Fig. 1e). In contrast, after initiation of etanercept treatment, the HAQ-DI at 52 weeks was not markedly improved, and patients who showed higher HAQ-DI appeared to remain unchanged (Fig. 1c), although the mean HAQ-DI improved from 1.4 at 0 weeks to 1.0 at 52 weeks. The reduction in HAQ-DI from 0 to 52 weeks was 31%, which was much less than changes of DAS28-CRP and ΔmTSS; a similar probability curve was observed before and after initiation of etanercept treatment (Fig. 1f).