PATIENTS AND METHODS

Patients

Data and information on patients with RA fulfilling the diagnostic criteria of the American College of Rheumatology were collected from 26 centres of remission induction by Remicade in RA (RRR) investigator groups in Japan.[17] Disease activity of individual patients was assessed by Disease Activity Score, including a 28-joint count (DAS28)-erythrocyte sedimentation rate (ESR) or DAS28-C reactive protein (CRP) that was calculated according to the authorised formula (http://www.das-score.nl/, accessed 15 February 2010).[18] Since none of the patients, except for one, achieved LDA measured by DAS28 despite MTX or a combination of MTX and other disease-modifying antirheumatic drugs for at least 3 months, infliximab treatment (3 mg kg, every 8 weeks) was administered in the investigators' institutions, according to the treatment guideline proposed by the Japan College of Rheumatology.[17] Joint damage was assessed by the van der Heijde-modified total Sharp score (mTSS)[19] and for 102 patients, x-ray images of the hands and feet at baseline, RRR-study entry and 1 year after the study were available; these were evaluable for 49 patients owing to loss of the radiographs and/or low quality of the x-ray images.[20 21] Two blinded expert readers independently scored articular damage and progression according to the mTSS scoring method. The difference between the two readers' scores for each patient's radiographs was <1% of the maximum mTSS score—that is, 448.[9 20 21] To confirm that the x-ray results of the 49 patients represented the outcomes of the whole group, we compared multiple background characteristics and changes of each characteristic from baseline to RRR-study entry between 49 patients with evaluable x-ray images and 53 patients without them and no significant difference was seen between the two groups.

After patients had achieved DAS28 (ESR)<3.2 (LDA) for >24 weeks, informed consent to discontinue infliximab was obtained from 126 patients. Other criteria were that patients were controlled with <5 mg/day of oral prednisolone (PSL) and were >18 years old. Concomitant use of MTX was started in all patients, and the dose of MTX was determined by each attending doctor. Twelve patients dropped out at the screening period, and 114 patients were enrolled in the study and discontinued infliximab (figure 1). The demographic indicators and baseline disease characteristics of the 114 patients enrolled are summarised in table 1.

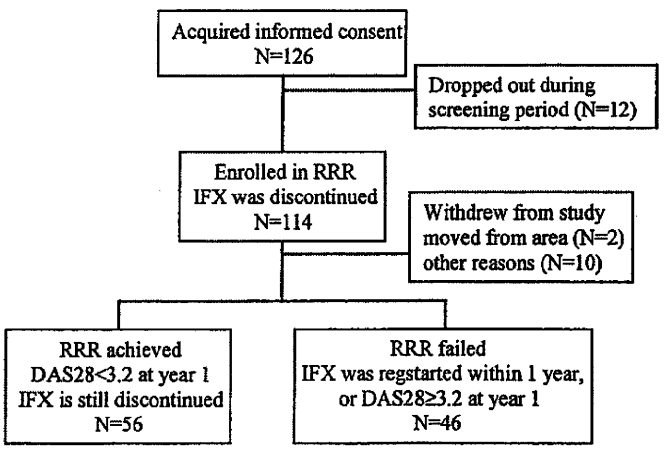

Figure 1 Study design and profile. DAS28, Disease Activity Score, including a 28-joint count; IFX, infliximab; RRR, remission induction by Remicade in rheumatoid arthritis.

Table 1 Demographic indicators and baseline disease characteristics

	Enrolled patients (N=114)	RRR-achieved (N=56)	RRR-failed (N=46)	p (probability >χ^2)
Women	87 (76%)	42 (75%)	38 (83%)	0.4691
Age (years)	51.4 (20.0–73.0)	49.5±12.6	56.1±12.2	*0.0053*
Disease duration (years)	5.9 (0.1–38.0)	4.8±5.9	7.8±7.7	*0.0238*
Tender joint count (0–28)	8.2±6.7	8.6±7.0	7.5±5.8	0.5798
Swollen joint count (0–28)	9.0±7.2	10.1±7.7	7.6±5.8	0.1674
PaGA (0–100 mm, VAS)	50.0±23.0	50.0±24.2	49.3±23.1	0.9520
CRP (mg/dl)	2.5±3.0	2.6±2.6	2.7±3.7	0.5531
ESR (mm/h)	46.2±26.9	43.1±24.2	54.1±30.1	0.1555
DAS28 (ESR) score	5.5±1.2	5.5±1.4	5.6±1.1	0.9112
DAS28 (CRP) score	4.9±1.2	5.1±1.3	4.8±1.3	0.5486
HAQ-DI	1.0±0.7	0.9±0.6	1.2±0.7	0.1112
mTSS*	63.3 (1.0–314.0)	46.9±46.5	97.2±86.9	*0.0207*
RF (U/ml)	201.9±496.5 (68.5%)	225.7±583.3	197.9±427.8	0.5190
MTX (mg/week)	7.7±2.3	7.9±1.9	7.8±2.8	0.3232
PSL (mg/day)	2.5±3.4 (45.6%)	2.4±3.5	2.8±3.5	0.5223

Data are number of patients (%) for categorical data and the means for continuous data. Statistical difference was assessed by non-parametric Wilcoxon t test and p (probability >χ^2) values are shown. Values in italic indicate a significant difference ($p<0.05$).
*Data supplied for 33 patients who achieved RRR and 16 patients for whom RRR failed.
CRP, C-reactive protein; DAS28, Disease Activity Score, including a 28-joint count; ESR, erythrocyte sedimentation rate; HAQ-DI, Health Assessment Questionnaire-Disability Index; mTSS, modified total Sharp score; MTX, methotrexate; PaGA, patient global assessment of disease activity; PSL, prednisolone; RF, rheumatoid factor; RRR, remission induction by Remicade in rheumatoid arthritis; VAS, visual analogue scale.

Procedures

Study protocol was a simple observation after discontinuation of infliximab. The follow-up observation was monitored by symptoms, signs and DAS28 (ESR) every 4–13 weeks for 2 years. The dose of concomitant MTX was basically consistent, but tapering of non-steroidal anti-inflammatory drugs and glucocorticoid was allowed during the study period. The primary end points were that after discontinuing infliximab, DAS28 remains <3.2 (LDA) for 1 year and (B) yearly progression of mTSS remains <0.5 (structural remission) for 1 year. Secondary end points were DAS28 remains <2.6 (clinical remission) for 1 year, DAS28 remains <3.2 for 2 years, yearly progression of mTSS remains <0.5 for 2 years and no rescue with infliximab for 1 or 2 years is needed, after discontinuing infliximab. When a flare-up occurred in patients after the discontinuation, restart of infliximab was allowed and patients were categorised into the 'RRR-failed' group. For the restart of infliximab, the same dose (3 mg/kg) and the same premedication as used before the study entry were used.

Statistical analysis

Baseline characteristics of patients are summarised in table 1 using the mean values for continuous variables. All multivariate analyses were conducted using the variables gender, age, duration of disease, DAS28 (ESR) score, DAS28 (CRP) score, tender joint count (0–28), swollen joint count (0–28), patient global assessment of disease activity (PaGA, 0–100 mm, visual analogue scale), ESR, CRP, Health Assessment Questionnaire-Disability Index (HAQ-DI), rheumatoid factor (RF), MTX dose and PSL dose at baseline. Spearman correlation analyses were performed to evaluate the

association between multivariables at RRR-study entry and DAS28 at the primary end point (last observation carried forward) of 102 patients. Logistic regression analysis was carried out to estimate DAS28 at the primary end point as dependent variables (probability) by DAS28 at RRR entry as independent variables. A receiver operating characteristic (ROC) curve was developed based on the logistic analysis and the significant cut-off point was determined from the curve. For categorical response parameters, group comparisons were made using a non-parametric Wilcoxon t test. Statistical analyses were performed using JMP software version 7 (SAS Institute, Cary, North Carolina, USA). All reported p values are two sided and p values <0.05 were considered significant.

RESULTS

Study end points

The demographic indicators and baseline characteristics of the 114 patients enrolled were as follows: mean age 51.4 years, mean disease duration 5.9 years and mean mTSS 63.3, indicating that the population included patients with long-established disease, and the mean DAS28 (ESR) score was 5.5, implying that most patients had highly active disease (table 1). Figure 1 shows the study profile. After maintaining DAS28<3.2 (LDA) for >24 weeks by infliximab treatment, infliximab was discontinued in 114 patients. Twelve patients withdrew because they moved from area (n=2) and for other reasons (n=10), and thus DAS28 could be evaluated in 102 patients at year 1.

Of the 102 patients, 56 patients achieved the primary end point having a DAS28<3.2 and remaining without infliximab for 1 year after the discontinuation (figure 2A). Thus, 55% of the enrolled patients met the primary end point that LDA was maintained for 1 year after discontinuing infliximab. Furthermore, 44 patients (43%) reached DAS<2.6 after the discontinuation. On the other hand, 29 patients flared within 1 year (mean duration was 6.4 months) after the discontinuation and in 17 patients DAS28 was >3.2 at year 1 and thus RRR failed for 46 patients (45%) at year 1. Re-treatment with infliximab in 32 patients was effective and the majority of patients reached DAS28<3.2 within 24 weeks (figure 2B). Minimal adverse reactions at infusion of the agent were seen in five patients only at the first or second infusion.

To clarify the background factors related to the RRR-achievement, multiple clinical parameters at baseline were compared between patients for whom RRR was achieved and those for whom it failed. Patients for whom RRR was achieved were younger (49.5 vs 56.1), their disease duration was shorter (4.8 vs 7.8) and mTSS was lower (46.9 vs 97.2) than for those for whom RRR failed. Among 56 patients who achieved RRR, 10 patients had early RA (disease duration <1.0 year) and eight long-established disease (>10 years). Of 46 patients for whom RRR failed, eight had early RA and 12 established disease. These results imply that infliximab can be discontinued in patients with long-established RA. In contrast, no significant difference was seen in gender, DAS including DAS28, tender or swollen joint count, ESR and CRP, HAQ-DI, RF and the dose of MTX and PSL. Since these factors interact with one another, we analysed the relationship between RRR-achievement and a series of clinical parameters at baseline using multivariate analysis after adjusting for confounding variables. No significant relations

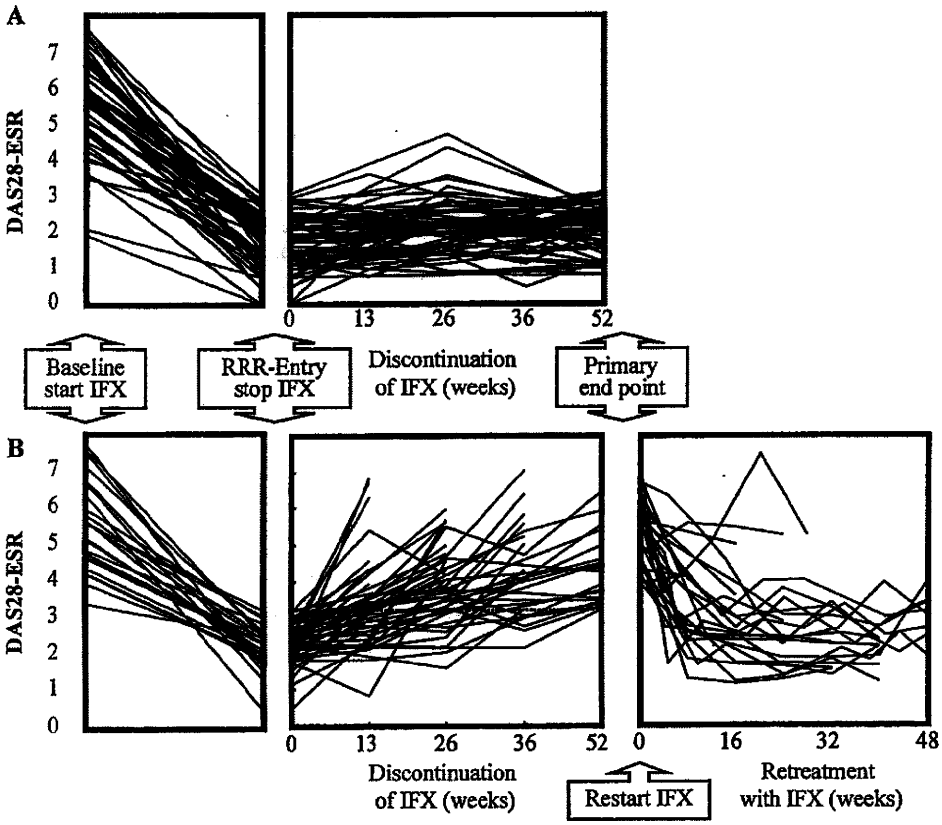

Figure 2 Changes of Disease Activity Score, including a 28-joint count (DAS28) in patients with remission induction by Remicade in rheumatoid arthritis-achieved (RRR-achieved) and patients for whom RRR failed (RRR-failed). (A) Changes of Disease Activity Score, including a 28-joint count (DAS28) at baseline when infliximab (IFX) was administered, at RRR-study entry when infliximab was discontinued and at the primary end point at week 52 after discontinuing IFX in 56 patients who were still satisfied with DAS28 (erythrocyte sedimentation rate (ESR)) <3.2 at week 52, RRR-achieved'. (B) Changes of DAS28 at baseline, at RRR entry and the end point in 46 patients whose disease activity flared after the discontinuation of IFX or DAS28 >3.2 at week 52, 'RRR-failed'. The lower right panel shows changes of DAS28 after the restarting IFX in 32 patients for whom RRR failed.

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between RRR-achievement and age, gender, DAS28 (ESR) score, PaGA and CRP were found, whereas a significant correlation was found with disease duration (p=0.0019) and serum levels of RF (p=0.0128) in RRR-achievement.

To determine the correlation of DAS28 at the primary end point with clinical parameters at RRR-study entry, univariate analysis of multiple variables was carried out. No significant correlations between DAS28 (ESR) at the primary end point and a series of clinical parameters were found, whereas DAS28 (ESR) and DAS28 (CRP) at RRR-entry were significantly correlated with DAS28 (ESR) at the primary end point. Subsequently, logistic regression analysis to estimate the probability of DAS28<3.2 at the primary end point as dependent variable by DAS28 at RRR-entry as independent variable was assessed. A significant logistic regression curve was drawn between the dependent and independent variables (p=0.0005) (figure 3A). Thus, DAS28 at RRR-study entry had the most marked correlation with the maintenance of LDA for 1 year after the discontinuation. By reciprocal statistics, DAS28 at RRR-study entry was estimated as 2.22, to attain DAS28<3.2 at the end point in 50% of the 102 patients (figure 3A). Furthermore, 71.4% of patients whose DAS28 at study entry was <2.225, a cut-off point calculated from the ROC curve, continued to have DAS28<3.2 for 1 year, whereas only 32.6% of patients whose DAS28 at RRR-entry was 2.225–3.2 continued to have DAS28<3.2 (figure 3B), indicating that 'deep remission' was required to maintain lower disease activity for 1 year after discontinuation of infliximab.

Structural and functional changes

From the 102 patients enrolled in the study, 49 patients were selected in whom both hand and feet x-ray data were available and evaluable; experts examined the structural damage before and after the infliximab treatment. When the baseline characteristics of the 49 patients in the study were compared with the rest of the patients in the study with insufficient x-ray data (n=53), no significant difference was seen. Next, the baseline characteristics of the 33 patients who achieved RRR and 16 patients for whom RRR failed were compared. As described in table 1, disease duration was shorter and mTSS was lower in patients who achieved RRR than in patients for whom RRR failed, but yearly progression of mTSS (ΔmTSS) was comparable between two groups (table 2). ΔmTSS at RRR entry was also comparable between two groups. However, means (0.3 vs 1.6) and medians (0.0 vs 1.5) of ΔmTSS were lower in the RRR-achieved group than in the RRR-failed group and more patients in the RRR-achieved group (67%) achieved ΔmTSS<0.5, radiographic remission, than patients in the RRR-failed group (44%). Thus, another primary end point for structural remission was achieved for 1 year after the discontinuation. Furthermore, HAQ-DI at baseline and RRR entry was comparable between patients for whom RRR was achieved and those for whom it failed, whereas HAQ-DI at the primary end point in patients who achieved RRR was significantly lower than that in patients for whom RRR failed (0.174 vs 0.614) (figure 4).

DISCUSSION

This multicentre study was undertaken to determine the possibility of discontinuing infliximab treatment in patients with RA after acquiring DAS-guided LDA, including those with long-established disease. Among 102 patients who could be evaluated at year 1, 56 patients (55%) satisfied the primary end point by maintaining DAS28<3.2 (LDA) and 44 patients (43%) reached DAS<2.6 (remission), remaining without infliximab at year 1 after the discontinuation. Of the 102 patients, 83 (81.4%) were in clinical remission at study entry and after discontinuing infliximab, 39/83 patients (47%) remained in remission and 10/83 patients (12%) progressed to LDA at the primary end point.

These data are similar to those of the BeSt study. However, the greatest difference between the patient populations enrolled in the two studies is mean disease duration—0.4 years in the BeSt study versus 5.9 years in our RRR study.[12–16] Joint destruction also differed between the two studies—mean mTSS 7.0 in the BeSt study versus 63.3 in our RRR study—suggesting that discontinuation of infliximab after reaching LDA is possible in patients with early RA and also in patients with long-established disease.[13–15] On the other hand, among multiple clinical parameters at baseline, disease duration was statistically related to RRR-achievement by multivariate analysis and disease duration was shorter (4.8 vs 7.8) and mTSS was lower (46.9 vs 97.2) in patients who achieved

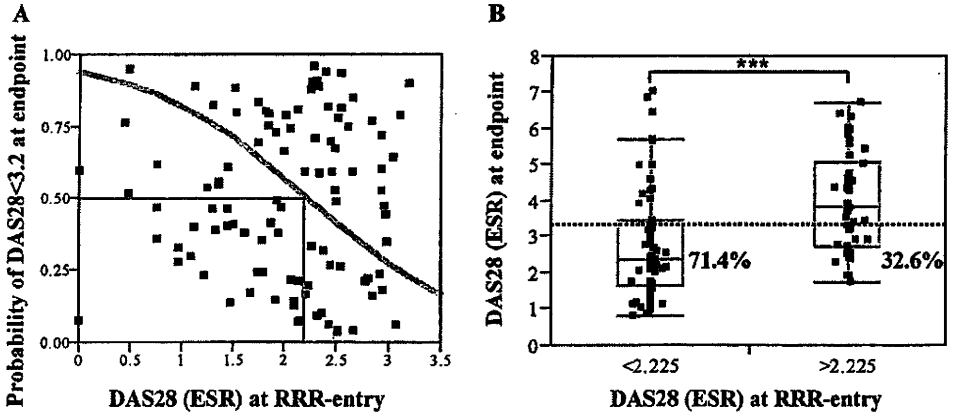

Figure 3 Logistic analysis of probability of Disease Activity Score, including a 28-joint count (DAS28) was <3.2 at primary end point by DAS28 at remission induction by Remicade in rheumatoid arthritis entry (RRR entry). (A) Logistic regression analysis to estimate DAS28 at primary end point as dependent variables by DAS28 at RRR entry as independent variables. The y-axis shows the probability of DAS28 <3.2 at the primary end point after the 52 weeks discontinuation of infliximab and a scatter diagram of an individual patient and logistic regression curve (solid line) are shown. To attain DAS28 <3.2 at the end point in 50% of the 102 patients, DAS28 at RRR study entry was estimated by reciprocal statistics. (B) From the receiver operating characteristic curve based on the logistic regression analysis above, the cut-off point of DAS28 at RRR-study entry was 2.225. Subsequently, one-way analysis of DAS28 at the primary end point by DAS28 at study entry, <2.225 versus between 2.225 and 3.2, was performed and the statistical difference of the two groups was sought by non-parametric Wilcoxon t test (***p<0.001). ESR, erythrocyte sedimentation rate.

Table 2 Radiographic indicators and baseline disease characteristics

	RRR-achieved (N=33)	RRR-failed (N=16)	p (probability $>\chi^2$)
Baseline			
Disease duration (years)	4.7 (0.5–14.0)	8.6 (0.5–25.0)	*0.0280*
DAS28 (ESR) score	5.5 (1.9–7.6)	5.7 (4.2–6.8)	0.6976
HAQ-DI	1.0 (0.0–2.3)	1.1 (0.0–1.8)	0.6271
mTSS	46.9 (1.0–216.5)	97.2 (6.0–314.0)	*0.0207*
Bone erosion score	23.7 (0.0–127.5)	55.5 (1.5–192.5)	*0.0119*
Joint space narrowing score	23.2 (1.0–89.0)	41.6 (4.5–121.5)	*0.0621*
Yearly progression of mTSS	13.1 (0.8–51.3)	15.0 (1.0–47.8)	0.5794
RRR-entry			
Yearly progression of mTSS	1.0 (−2.9 to 10.5)	0.7 (−2.0 to 6.7)	0.5788
Primary end point			
Yearly progression of mTSS	0.3 (−3.6 to 8.5)	1.6 (−3.6 to 7.0)	0.1087
Median of yearly progression of mTSS	0.0	1.5	-
Yearly progression of mTSS <0.5 (%)	67	44	0.2161

Data are number of patients (%) for categorical data and the means for continuous data. Statistical difference was assessed by non-parametric Wilcoxon t test and p (probability $>\chi^2$) values were shown. Values in italic indicate a significant difference ($p<0.05$). DAS28, Disease Activity Score based on assessments of 28 tender and 28 swollen joints; ESR, erythrocyte sedimentation rate; HAQ-DI, Health Assessment Questionnaire-Disability Index; mTSS, modified total Sharp score; RRR, remission induction by Remicade in rheumatoid arthritis.

RRR than in those for whom RRR failed. These results imply that remission free from biological agents can be more easily obtained in patients with shorter disease duration than in those with more established disease, but discontinuation of infliximab is still possible even in patients with long-established RA, because eight patients whose disease duration was >10 years successfully remained without infliximab for 1 year.

Among 102 evaluated patients, disease in 29 patients flared within 1 year, 17 patients had DAS28≥3.2 at year 1 after discontinuing infliximab and 32 patients had already been re-treated with infliximab. One of the major concerns of restarting infliximab is the possibility of an infusion reaction after the long-term discontinuation, partly owing to human anti-chimeric antibodies.[22] However, minimal adverse reactions at infusion of the agent were seen only in five patients at the first or second infusion. Another concern is the progress of joint damage after discontinuation of infliximab. However, although the yearly progression of mTSS at RRR-study entry was also comparable between two groups, means (0.3 vs 1.6) and medians (0.0 vs 1.5) of ΔmTSS were shorter in the RRR-achieved group than in the RRR-failed group. Furthermore, at year 1 after the discontinuation of infliximab, more patients in the RRR-achieved group (67%) tended to satisfy ΔmTSS<0.5—that is, structural remission, than those in the RRR-failed group (44%) and HAQ-DI in patients who achieved RRR was significantly lower than that in patients for whom RRR failed (0.174 vs 0.614). These results indicate that both structural remission and functional remission were maintained for 1 year in patients with LDA even after discontinuing infliximab.

This study also shows the significance of DAS-guided tight control of RA in order to maintain remission free from biological agents. There was a significant correlation between DAS28 (ESR) or DAS28 (CRP) at RRR entry and DAS28 (ESR) at year 1 after the discontinuation of infliximab by univariate analysis of multiple variables and a logistic regression analysis. Thus, DAS28 at RRR-study entry had the greatest correlation with maintenance of LDA for 1 year after discontinuation. Also, DAS28 at study entry was mainly influenced by PaGA and ESR among the composite measures. By reciprocal statistics, the estimated DAS28 (ESR) at RRR-study entry was 2.22 (1.85–2.70), to attain DAS28<3.2 at the primary end point in 50% of the 102 patients studied. Also, 71.4% of patients whose DAS28 at study entry was <2.225, a cut-off point calculated from ROC curve, remained DAS28<3.2 for 1 year, whereas only 32.6% of patients whose DAS28 at RRR-entry was 2.225–3.2 remained DAS28<3.2. These results indicate that 'deep remission' appears to be required to maintain lower disease activity for 1 year after discontinuation of infliximab.

About 55% of the 102 patients, who were in an LDA state for >24 weeks with infliximab and MTX treatment, could discontinue infliximab for >1 year without progression of radiological articular destruction or functional disturbance. These data may have significant implications for the optimal use of expensive biological treatments: (*a*) re-treatment with infliximab is efficient and tolerable in the patients for whom RRR failed; (*b*) DAS-guided monitoring is valuable to keep tight control; (*c*) 'deep

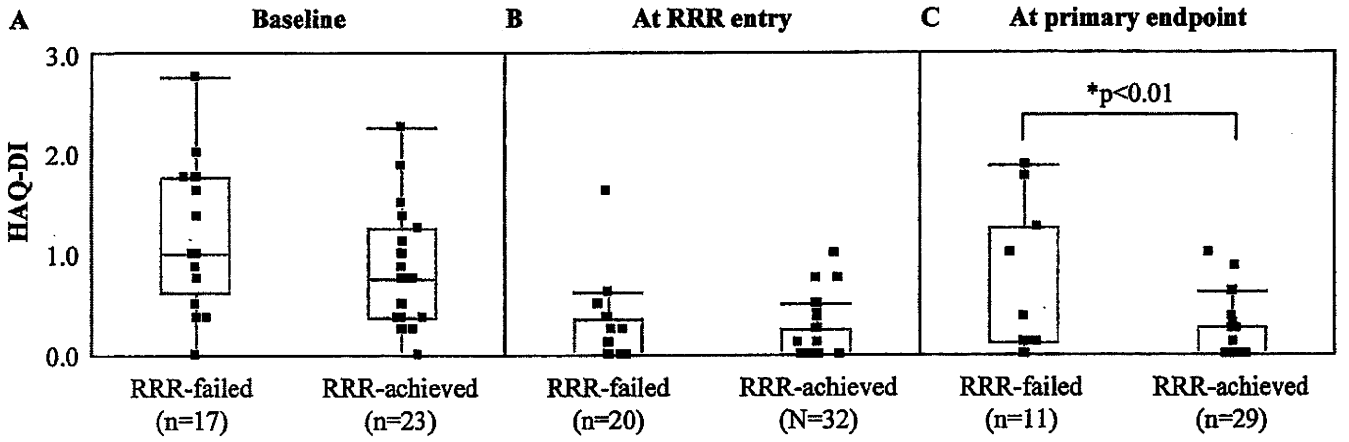

Figure 4 Health Assessment Questionnaire-Disability Index (HAQ-DI) in patients for whom remission induction by Remicade in rheumatoid arthritis failed (RRR-failed)' and in patients for whom 'RRR was achieved (RRR-achieved)' at (A) baseline, (B) RRR entry and (C) the primary end point. The line in the box represents the median value and the upper and lower ends of the box indicate the 25th and 75th centiles of the population. Statistical difference was assessed by non-parametric Wilcoxon t test.

remission' by tight control is required to maintain discontinuation of infliximab; (*d*) remission free from biological agents may be easier to attain in patients with early RA, but is possible for patients with long-established disease; (*e*) treatment aimed at reaching a target of LDA is pivotal to the approach to remission free from biological agents. Finally, TNFα is not a cause of RA, but if appropriate treatment with infliximab can lead to drug-free remission, TNF inhibitors may shut down pathological processes and may change or modify the disease course in RA. Thus, a clinical and basic research approach to the 'process-driven disease' of RA is warranted.

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Competing interests YT has received consultant fees from Mitsubishi-Tanabe Pharma, Pfizer Inc; lecture fees from Mitsubishi-Tanabe Pharma, Takeda Pharmaceutical Co Ltd, Abbott, Eisai Pharma, Chugai Pharma. TT has received consultant fees from Mitsubishi-Tanabe Pharma, Wyeth Japan, Abbott, Eisai Pharma, Janssen Pharma, Chugai Pharma, Bristol-Myers-Squibb, Novartis; lecture fees from Mitsubishi-Tanabe Pharma, Takeda Pharmaceutical Co Ltd, Abbott, Eisai Pharma, Chugai Pharma. HK has received lecture fees from Mitsubishi-Tanabe Pharma, Centocor, Wyeth Japan, Takeda Pharmaceutical Co Ltd, Abbott, Eisai Pharma, Chugai Pharma. NM has received consultant fees from Mitsubishi-Tanabe Pharma; Abbott, Eisai Pharma, Janssen Pharma, Chugai Pharma, Bristol-Myers-Squibb; lecture fees from Mitsubishi-Tanabe Pharma, Takeda Pharmaceutical Co Ltd, Wyeth Japan, Abbott, Eisai Pharma, Chugai Pharma. TK has received consultant fees from Bristol-Myers-Squibb, Abbott; lecture fees from Mitsubishi-Tanabe Pharma, Takeda Pharmaceutical Co Ltd, Wyeth Japan, Abbott, Eisai Pharma, Chugai Pharma.

Patient consent Obtained.

Ethics approval This study is an observational study and is registered with the University Hospital Medical Information Network-Clinical trials Registry, number R000002571. Also, ethics committees of the participating centres approved the study protocol.

Provenance and peer review Not commissioned; externally peer reviewed.

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ORIGINAL ARTICLE

Change of Synovial Vascularity in a Single Finger Joint Assessed by Power Doppler Sonography Correlated With Radiographic Change in Rheumatoid Arthritis: Comparative Study of a Novel Quantitative Score With a Semiquantitative Score

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Objective. To investigate the relationship between synovial vascularity assessed by quantitative power Doppler sonography (PDS) and progression of structural bone damage in a single finger joint in patients with rheumatoid arthritis (RA).
Methods. We studied 190 metacarpophalangeal (MCP) joints and 190 proximal interphalangeal (PIP) joints of 19 patients with active RA who had initial treatment with disease-modifying antirheumatic drugs (DMARDs). Patients were examined by clinical and laboratory assessments throughout the study. Hand and foot radiography was performed at baseline and the twentieth week. Magnetic resonance imaging (MRI) was performed at baseline. PDS was performed at baseline and the eighth week. Synovial vascularity was evaluated according to both quantitative and semiquantitative methods.
Results. Quantitative PDS was significantly correlated with the enhancement rate of MRI in each single finger joint. Comparing quantitative synovial vascularity and radiographic change in single MCP or PIP joints, the level of vascularity at baseline showed a significant positive correlation with radiographic progression at the twentieth week. The change of vascularity in response to DMARDs, defined as the percentage change in vascularity by the eighth week from baseline, was inversely correlated with radiographic progression in each MCP joint. The quantitative PDS method was more useful than the semiquantitative method for the evaluation of synovial vascularity in a single finger joint.
Conclusion. The change of synovial vascularity in a single finger joint determined by quantitative PDS could numerically predict its radiographic progression. Using vascularity as a guide to consider a therapeutic approach would have benefits for patients with active RA.

INTRODUCTION

In recent years, the therapeutic goal for rheumatoid arthritis (RA) has moved far beyond the traditional factors of clinical remission, defined by the American College of Rheumatology (ACR) core data set or the European League Against Rheumatism (EULAR) Disease Activity Score in 28 joints (DAS28) remission criteria (1,2). To halt the progression of bone destruction, there has been a great need for a reliable predictive indicator of radiographic progression. Modern imaging techniques such as power Doppler sonography (PDS) and magnetic resonance imaging (MRI) have the potential to predict bone destruction (3–6). However, the relationship between therapeutic efficacy and image responses of these techniques has not been established.

PDS has several advantages in terms of medical cost and safety compared with other modern imaging techniques; therefore, it is more practical to use it repeatedly for monitoring disease activity. PDS detects the abnormal synovial vascular flow related to inflammation and has the potential to evaluate the level to represent this as a measurable

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Table 1. Clinical and laboratory characteristics of patients at baseline and the eighth and twentieth weeks*

	Baseline	8th week	20th week
Age, mean (range) years	54 (24–87)		
Sex, female/male	17/2		
RF positive, yes/no	15/4		
Prior use of DMARDs, yes/no	3/16		
Duration of symptoms, months	5 (3–11)		
Swollen joint count	3 (2–7)	1 (0–2)	0 (0–2)
Tender joint count	6 (2.5–13)	2 (1–5)	1 (1–1.5)
Patient's global assessment by VAS	60 (45–60)	29 (20.5–43.5)	25 (15.5–30)
ESR, mm/hour	43 (27–80)	24 (16–58)	27 (16–35)
CRP level, mg/dl	0.5 (0.25–2.82)	0.12 (0.1–1.1)	0.1 (0.1–1.4)
DAS28-ESR, mean ± SD mm/hour	5.21 ± 1.39	4.08 ± 1.60	3.56 ± 1.31

* Values are the median (interquartile range) unless otherwise indicated. RF = rheumatoid factor; DMARDs = disease-modifying antirheumatic drugs; VAS = visual analog scale; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein; DAS28 = Disease Activity Score in 28 joints.

parameter (7,8). With growing interest in the ability to define remission in RA, it has been reported that abnormal synovial vascular flow still remains in individual joints after achievement of clinical remission, and therefore bone destruction would progress at a high rate in such cases (3,9). In this sense, direct assessment of synovial vascular flow in a single joint would be of use. Semiquantitative scoring has been widely used to evaluate synovial vascularity (10,11). The scoring was divided into 4 steps that were judged subjectively by the observer, and represented, accordingly, as a semiquantitative approach. The relationship between synovial vascular changes and progression of structural bone damage in a single joint has been the focus of much investigation, but only a few studies have been successful despite the intensive attempts of many researchers (3,12–14). In our preliminary study, we established quantitative PDS for synovial vascularity in each finger joint (15,16). The measurement was able to assess vascularity as quantitative data, objectively determined by the ultrasonographic program, and to detect small changes in individual finger joints. We investigated the relationship between synovial vascular changes and progression of structural bone damage in a single finger joint using the quantitative PDS measurement. We further defined the vascularity in response to disease-modifying antirheumatic drugs (DMARDs) by imaging and investigated its clinical significance in patients with active RA.

PATIENTS AND METHODS

Patients. Nineteen new patients with RA were enrolled in the study. All of the patients satisfied the ACR (formerly the American Rheumatism Association) 1987 diagnostic criteria (17). All of the patients were diagnosed as having the active state of RA according to the DAS28–erythrocyte sedimentation rate (ESR; >2.7 mm/hour). Demographic, clinical, and laboratory characteristics of the patients are shown in Table 1. Three patients were already receiving DMARDs at the initial diagnosis, but they were having no therapeutic effect (1 patient with sulfasalazine [SSZ], 2 patients with auranofin). After baseline examinations, all of the patients were given one of the new DMARDs. Initial treatments were continued throughout the study, but additional treatment and escalating doses of DMARDs were permitted in cases with disease exacerbation after the eighth week. We performed clinical and imaging examinations as mentioned in each section.

The study was conducted in accordance with the Helsinki Declaration. Informed consent to the protocol approved by the ethics committee of the hospital was obtained from all of the patients.

Ultrasonography and assessment. Ultrasonography was performed at baseline and the eighth week by 1 of the 3 ultrasonographers (MH, FS, AN) specialized in musculoskeletal ultrasonography who were blinded to other clinical information. A 13-MHz linear array transducer was used (HITACHI EUP-L34P). Pulse Doppler settings were standardized for the detection of synovial blood flow by adjusting color gain, pulse repetition, and flow optimization parameters according to a previous study (15). Power Doppler settings (75 dB dynamic range, medium persistence, medium frame rate, low wall filter, 1,300 Hz pulse repetition frequency, flow optimization: medium vein, 1,300 Hz speed velocity) were identical throughout the examinations. Room temperature was kept at 25°C. The patients were positioned comfortably, and the examinations were then started after 10 minutes of stabilization of the pulse rate. The scanning technique on each finger joint was standardized and fixed as follows: scanning of the first through fifth metacarpophalangeal (MCP) joints and the first through fifth proximal interphalangeal (PIP) joints was performed in the longitudinal plane over the dorsal surface of the joint with light skin pressure. The basic scanning technique followed the EULAR guidelines (18). The synovial vascular area with the most pronounced power Doppler activity was identified from the cine-loop and stored. The PDS images were recorded in the hard disk of the ultrasonographic machine. All of the examinations were completed within 15 minutes. Semiquantitative scoring has been described in previous studies (0 = absence of signal, 1 = single vessel dots, 2 = vessel dots over less than

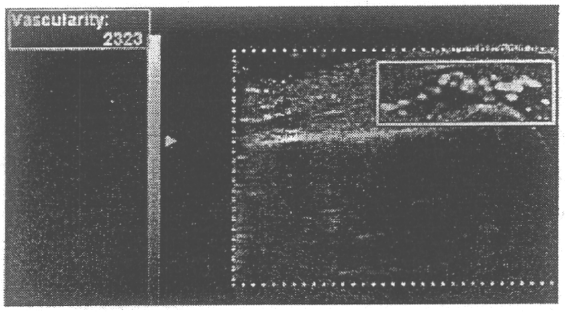

Figure 1. An image of finger joint ultrasonography (right 5th metacarpophalangeal joint). Each joint was scanned as described in the Patients and Methods section. The white line box indicates the region of interest (ROI) that was located at synovial vascular flow. Pixels of vascular flow inside the ROI were measured by the ultrasonographic program and displayed at the upper left corner of the monitor.

half of the synovium area, 3 = vessel dots over greater than half of the synovium area) (10,11,19). A synovial vascularity value, measured by quantitative PDS, was defined as P-vasc, which is the number of vascular flow pixels in the region of interest (ROI). The ROI was a standardized box type (5 mm × 10 mm) that was located to contain as many of the vascular flow pixels as possible. Vascular flow pixels in the ROI were measured automatically using the program's Vascularity mode in the ultrasonographic machine (HITACHI EUB-6500) (Figure 1).

Radiography and assessment. Plain radiographs of the hands, wrists, and feet were obtained at baseline and the twentieth week. Radiologic assessments were examined according to the Genant-modified Sharp score (GSS) by a rheumatologist (YK) who was blinded to other clinical information (20).

MRI and assessment. MRIs of both finger joints were taken at baseline using the 1.5T system (Signa Excite, version 12) with a cardiac coil. During the examination, patients were placed in the supine position with both hands on the abdomen, and these were covered by the anterior segment of the cardiac coil. Dynamic 3-dimensional forkhead activin signal transducer spoiled gradient-recalled acquisition in the steady state T1-weighted coronal images (time to recovery 500 msec, time to echo 11 msec, field of view 30 cm, matrix 256 × 160, 20 slices, slice thickness 3 mm, gap 0.4 mm, imaging time 10, 17 loops with an interval time of 5 seconds) were obtained for both hands in addition to the other images with different scan sequences. A bolus injection of gadopentate dimeglumine (Gd-DTPA; 0.1 mmole/kg body weight) was administered at 1 ml/second via a 21-gauge indwelling needle inserted into an antecubital fossa vein during acquisition of the baseline images (first loop) of the dynamic study. Data were transferred from the MRI console to a Digital Imaging and Communication in Medicine viewer and then a workstation (Advantage Windows workstation) for quantitative analysis. The severity of synovitis has been previously assessed by the rate of enhancement (E-rate) in a dynamic study by injection of Gd-DTPA (21). The E-rate indicates the index of enhancement by plotting the signal intensity against time in a selected ROI (~20–30 mm^2 in area) of the site of maximum enhancement in the above-mentioned 20 joints. Image analysis was carried out by an experienced radiologist (TK) who was blinded to other clinical information.

Statistical analysis. Statistical analyses were calculated with the use of the Excel program and the MedCalc program, version 10.4.5.0. Differences between the 2 groups were examined using either Student's *t*-test or a nonparametric test (Wilcoxon's signed rank test, Mann-Whitney U test), as applicable. A correlation between 2 variables was examined using either a parametric test (Pearson's correlation test) or a nonparametric test (Spearman's rank correlation test) according to the distribution of values. Intra-and interobserver reliability of the semiquantitative PDS score was estimated using calculations of weighted kappa statistics and overall agreement. Intra-and interobserver reliability of P-vasc was estimated using calculations of intraclass correlation coefficients (ICCs). The smallest detectable change for the radiographic score change was calculated according to a previous study (22).

RESULTS

Clinical disease activity. The mean ± SD DAS28-ESR at baseline was 5.21 ± 1.39 mm/hour. The mean ± SD DAS28-ESR at the eighth week was 4.08 ± 1.60 mm/hour, which was significantly decreased from baseline (P = 0.0001). There was no significant difference in the DAS28-ESR between the eighth week and the twentieth week (P = 0.0741). At the twentieth week, 13 patients were receiving monotherapy (9 with methotrexate [MTX], 2 with SSZ, and 2 with bucillamine) and 6 patients were receiving combination therapy of DMARDs (3 with MTX plus bucillamine, 1 with MTX plus SSZ, 1 with SSZ plus bucillamine, and 1 with SSZ plus tacrolimus). Thirteen patients were receiving oral prednisolone (3–10 mg/day) at the twentieth week.

Intra-and interobserver reliability for PDS. All PDS images for MCP joints and PIP joints were blindly evaluated twice according to the semiquantitative score for each joint by 2 ultrasonographers (MH, AN). The obtained intraobserver kappa values of the semiquantitative score were 0.944 for MCP joints and 0.930 for PIP joints. The intraobserver overall agreement for these joints was 96% and 95.4%, respectively. The obtained interobserver kappa values of the semiquantitative score were 0.950 for MCP joints and 0.923 for PIP joints. The interobserver overall agreement for these joints was 95.7% and 97.1%, respectively.

Representative images for 20 MCP joints and 20 PIP joints were randomly chosen, and P-vasc was measured 3 times each by 3 ultrasonographers (MH, FS, AN). The obtained intraobserver ICC values were 0.990 for MCP joints and 0.990 for PIP joints. The interobserver ICC values were 0.990 for MCP joints and 0.990 for PIP joints.

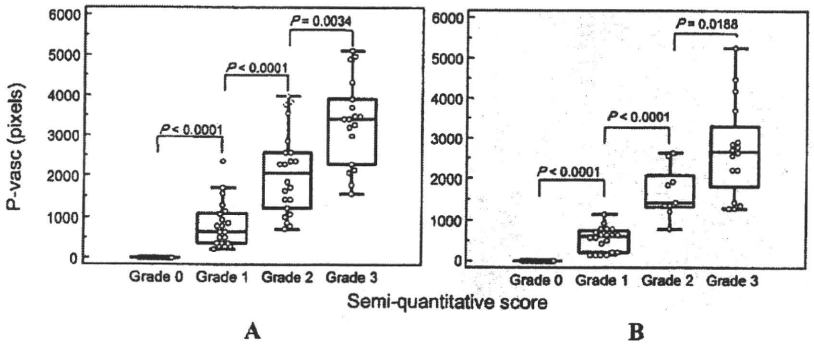

Figure 2. Relation between quantitative measurement and semi-quantitative scoring for synovial vascularity. The levels of synovial vascularity value (P-vasc) were plotted against semi-quantitative scores in MCP joints (**A**) and PIP joints (**B**).

Relationship of quantitative PDS measurement (P-vasc) to semiquantitative scoring for synovial vascularity and to the E-rate of MRI. The PDS images for 190 MCP joints and 190 PIP joints at baseline were evaluated using both the semiquantitative score and the P-vasc. The P-vasc significantly increased as the semiquantitative score increased in both MCP joints and PIP joints (Figure 2).

One patient was unable to undergo MRI because of claustrophobia. One hundred eighty MCP joints and 180 PIP joints of 18 patients were evaluated using both the P-vasc and E-rate. The P-vasc had a significant positive correlation with the E-rate of MRI in both MCP and PIP joints (Pearson's r = 0.739, $P < 0.0001$ and Pearson's r = 0.537, $P < 0.0001$, respectively) (Figure 3).

Association between vascularity and radiographic progression in a single joint. The median local GSS at baseline for MCP and PIP joints were 0 (interquartile range [IQR] 0–1) and 0.5 (IQR 0–1.5), respectively. The median local GSS at the twentieth week for MCP and PIP joints were 0.5 (IQR 0–1.5) and 0.75 (IQR 0–1.5), respectively. The median total GSS was 16.5 (IQR 11.3–37.3) at baseline. The median total GSS at the twentieth week was 30.0, which was significantly higher than the baseline score ($P = 0.001$).

We next focused on changes of single-joint P-vasc and local GSS. We investigated the association between the level of vascularity at baseline and radiographic progression at the twentieth week in each single finger joint. One hundred ninety MCP joints and 190 PIP joints at baseline were evaluated. The level of P-vasc at baseline significantly correlated with progression of the local GSS in both MCP and PIP joints (Spearman's $\rho = 0.466$, $P < 0.0001$ and Spearman's $\rho = 0.362$, $P < 0.0001$, respectively) (Figures 4A and B). The association between the semiquantitative score and the progression of the local GSS had the same tendency (data not shown). We took note of the positive PDS joints at baseline and calculated their improvement rate (IR), defined as the percentage change in P-vasc by the eighth week from baseline. The IR was calculated as follows: (P-vasc value at baseline − P-vasc value at eighth week)/P-vasc value at baseline × 100 (%). At baseline, 61 MCP joints and 44 PIP joints had positive PDS signals. The IR of P-vasc had a significant inverse correlation with local GSS progression in each single MCP joint (Spearman's $\rho = -0.340$, $P = 0.00386$) (Figure 5A). There was no significant correlation between the IR of P-vasc and local GSS progression in each single PIP joint (Spearman's $\rho = -0.223$, $P = 0.1430$) (Figure 5B). In the case of assessment by semiquantitative score, there was no significant correla-

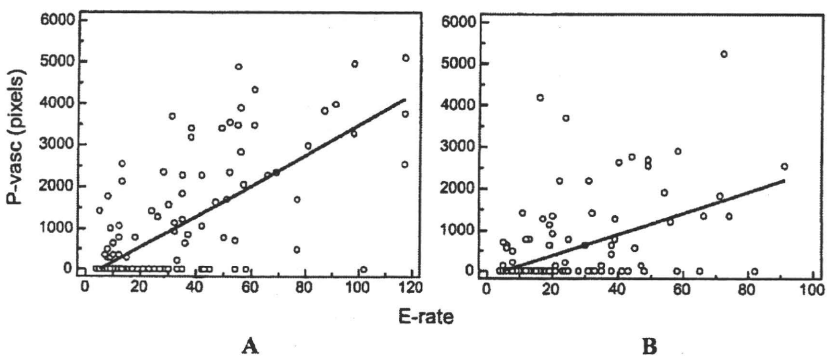

Figure 3. Relationship between quantitative measurement of synovial vascularity with power Doppler sonography and the index of synovial enhancement of magnetic resonance imaging (MRI). Scatter diagrams and regression lines of synovial vascularity value (P-vasc) against the enhancement rate (E-rate) of MRI in metacarpophalangeal joints (**A**) or proximal interphalangeal joints (**B**) are shown.

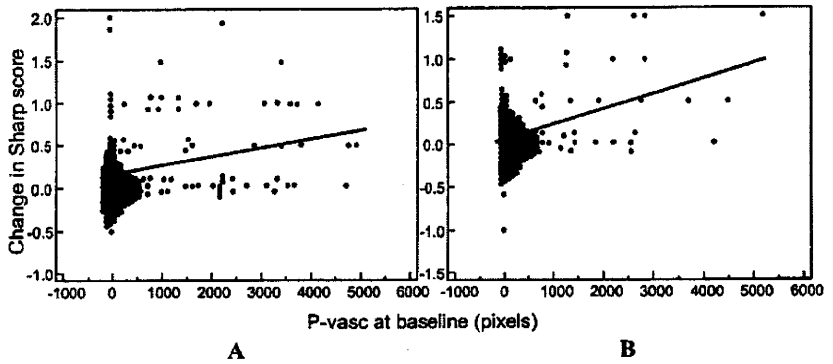

Figure 4. Relationship between the level of synovial vascularity and radiographic progression in each single finger joint. Scatter diagrams and regression lines of the synovial vascularity value (P-vasc) at baseline against progression of the local Genant-modified Sharp score from baseline to the twentieth week in metacarpophalangeal joints (A) or proximal interphalangeal joints (B) are shown.

tion to MCP or PIP joints (Spearman's $\rho = -0.256$, $P = 0.0579$ and Spearman's $\rho = -0.105$, $P = 0.5179$, respectively) (data not shown). The smallest detectable change values were calculated for the radiographic erosion score, joint space narrowing score, and combined score for single MCP and PIP joints (0.21–0.48). All of the calculated smallest detectable changes did not exceed the smallest unit of the scoring (0.5).

DISCUSSION

In this study, we quantitatively evaluated synovial vascularity in a single finger joint. In each finger joint, we found that a level of vascularity at baseline correlated with the radiographic progression. We also demonstrated that the change of vascularity in response to DMARD therapy could numerically predict the radiographic progression in each single finger joint.

We defined a standardized box type ROI and quantitatively evaluated synovial vascularity, as mentioned in the Patients and Methods section. All of the kappa values and ICCs calculated for intra-and interobserver reliability during the PDS measurements were acceptable in this study.

To demonstrate the validity of our quantitative PDS method, we first examined a relationship between the P-vasc and semiquantitative scoring. The P-vasc significantly increased in parallel with semiquantitative scoring. The E-rate of MRI is an index of Gd-DTPA enhancement indicating the inflammatory level (21,23,24), and was used for comparing with quantitative ^{99m}Tc-labeled nanocolloid scintigraphy for assessing RA (25). We next examined the relationship between the E-rate and P-vasc. Although the P-vasc was not detected in some joints with a high E-rate, a positive significant correlation was shown between the E-rate and P-vasc, suggesting that synovial vascularity determined by our quantitative PDS reflects, in part, the inflammatory level. The main reason of discrepancy between a joint with a high E-rate and negative PDS should be explained by the fact that MRI covered inflammation from all sites of synovial tissue, whereas PDS detected only from the dorsal side of synovial tissue. In addition, the difference of sensitivity of PDS and that of the E-rate might be a problem. Because the PDS is one of the advancing modalities for imaging rheumatic joints, there would be many more points to be improved in the

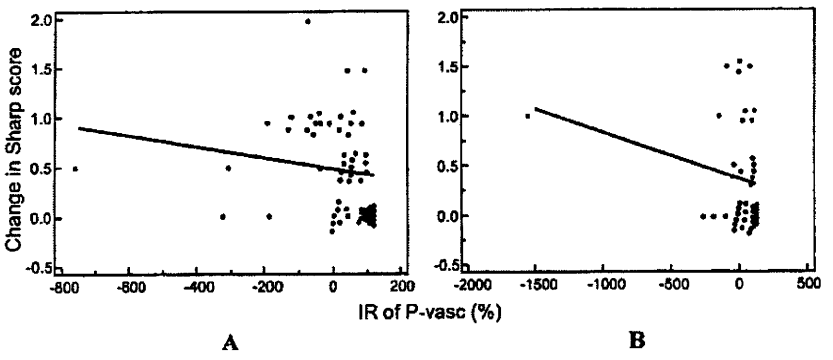

Figure 5. Relationship between improvement of synovial vascularity and radiographic progression in each single finger joint. Scatter diagrams and regression lines of the improvement rate (IR) for synovial vascularity value (P-vasc) between baseline and the eighth week against progression of the local Genant-modified Sharp score from baseline to the twentieth week in metacarpophalangeal joints (A) or proximal interphalangeal joints (B) are shown.

technological aspects, and such a process will promise to overcome the current problems in the future.

We used the P-vasc for investigating the relationship between the change of synovial vascularity and radiographic progression in a single finger joint. We found that, for each single finger joint, the baseline P-vasc significantly correlated with progression of the local GSS over 20 weeks. Our study, for the first time, has quantitatively confirmed the recent reports of Brown et al and Naredo et al that the presence of vascularity using PDS in a qualitative way correlated with the bone destruction in each single joint (3,13).

We next focused on PDS-positive finger joints at baseline and calculated each IR from baseline to the eighth week. The IR of P-vasc had a significant inverse correlation with radiographic progression in each single MCP joint. It was a novel finding that improvement in the rate of vascularity resulted in the suppression of radiographic progression. The semiquantitative score failed to demonstrate the same tendency due to its low sensitivity at detecting small changes in vascularity. On the other hand, the IR of P-vasc in PIP joints was not significantly correlated with radiographic progression, presumably due to either the sample size or the ROI setting. Further refinement of ROIs specific to PIP joints may improve the accuracy of the technique.

According to the 2002 ACR guidelines for the treatment of RA, therapeutic evaluation of first-line DMARDs was assessed at 8–12 weeks using clinical indices (26). Naredo et al reported that the time-integrated value of the PDS parameters correlated with the radiographic progression over 1 year (13), suggesting that rapid reduction in the PDS signal could predict a better radiologic prognosis. The IR of P-vasc, a change rate of 2 points, could be a useful index for preventing bone destruction. A quantitative PDS method was more useful than a semiquantitative method to detect change of synovial vascularity in each single finger joint.

Although this is a preliminary study with a small number of patients, it is noteworthy that the clinical implications of our results include the potential of synovial vascularity to numerically predict an outcome of bone destruction in each single finger joint. Furthermore, we found that the change of vascularity influenced radiographic progression. The IR of synovial vascularity should be an index of therapeutic efficacy, and therefore be of value in making judgments about additional treatment with DMARDs or to change to early biologic agent therapy. Using vascularity as guide to make therapeutic decisions at early stages would have benefits for patients with active RA. Larger and longitudinal studies would indicate the efficacy of PDS for the better management of affected patients.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be submitted for publication. Dr. Fukae 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 conception and design. Fukae, Kon, Tanimura, Kamishima, Atsumi, Koike.

Acquisition of data. Fukae, Kon, Henmi, Sakamoto, Narita, Shimizu, Tanimura, Matsuhashi, Kamishima.

Analysis and interpretation of data. Fukae, Kon, Henmi, Sakamoto, Narita, Shimizu, Tanimura, Matsuhashi, Kamishima, Atsumi, Koike.

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RESEARCH ARTICLE Open Access

Association of TNFAIP3 interacting protein 1, TNIP1 with systemic lupus erythematosus in a Japanese population: a case-control association study

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Abstract

Introduction: *TNFAIP3* interacting protein 1, *TNIP1* (ABIN-1) is involved in inhibition of nuclear factor-κB (NF-κB) activation by interacting with TNF alpha-induced protein 3, A20 (*TNFAIP3*), an established susceptibility gene to systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). Recent genome-wide association studies revealed association of *TNIP1* with SLE in the Caucasian and Chinese populations. In this study, we investigated whether the association of *TNIP1* with SLE was replicated in a Japanese population. In addition, association of *TNIP1* with RA was also examined.

Methods: A case-control association study was conducted on the *TNIP1* single nucleotide polymorphism (SNP) rs7708392 in 364 Japanese SLE patients, 553 RA patients and 513 healthy controls.

Results: Association of *TNIP1* rs7708392C was replicated in Japanese SLE (allele frequency in SLE: 76.5%, control: 69.9%, $P = 0.0022$, odds ratio [OR] 1.40, 95% confidence interval [CI] 1.13-1.74). Notably, the risk allele frequency in the healthy controls was considerably greater in Japanese (69.9%) than in Caucasians (24.3%). A tendency of stronger association was observed in the SLE patients with renal disorder ($P = 0.00065$, OR 1.60 [95%CI 1.22-2.10]) than in all SLE patients ($P = 0.0022$, OR 1.40 [95%CI 1.13-1.74]). Significant association with RA was not observed, regardless of the carriage of human leukocyte antigen DR β1 (*HLA-DRB1*) shared epitope. Significant gene-gene interaction between *TNIP1* and *TNFAIP3* was detected neither in SLE nor RA.

Conclusions: Association of *TNIP1* with SLE was confirmed in a Japanese population. *TNIP1* is a shared SLE susceptibility gene in the Caucasian and Asian populations, but the genetic contribution appeared to be greater in the Japanese and Chinese populations because of the higher risk allele frequency. Taken together with the association of *TNFAIP3*, these observations underscore the crucial role of NF-κB regulation in the pathogenesis of SLE.

Introduction

TNFAIP3 (tumor necrosis factor α-induced protein 3) encodes a ubiquitin-editing protein, A20, known as an inhibitor of nuclear factor-κB (NF-κB). Several adaptor molecules are thought to associate with A20 and be involved in inhibition of NF-κB [1]. TNIP1 (TNFAIP3 interacting protein 1), also known as ABIN (A20-binding inhibitor of NF-κB)-1, is one such adaptor molecule binding to A20. *TNIP1* mRNA is strongly expressed in peripheral blood lymphocytes, spleen and skeletal muscle, and the expression is also detected in kidney [2]. *TNIP1* expression is induced by NF-κB, and in turn, overexpression of *TNIP1* inhibits NF-κB activation by TNF [1], although deficiency of *TNIP1* has few effects on NF-κB inhibition [3]. Thus, TNIP1 appears to play a

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role in NF-κB inhibition, at least partly by interacting with A20. In addition, TNIP1 was shown to inhibit TNF-induced apoptosis independently of A20 [3].

TNFAIP3, located at 6q23, has been identified as a susceptibility gene for both systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) in Caucasian and Asian populations [4-8]. Recently, Shimane et al. [9] replicated association of *TNFAIP3* single nucleotide polymorphisms (SNPs) with SLE and RA in a Japanese population. We also detected association of *TNFAIP3* rs2230926 with Japanese SLE patients in an independent study [10].

Recently a genome-wide association study (GWAS) reported association of *TNIP1* (5q32-q33.1) as well as *TNFAIP3* SNPs with psoriasis in the Caucasian populations [11]. Subsequently, two recent GWAS revealed association of *TNIP1* intronic SNPs rs7708392 and rs10036748, which are in strong linkage disequilibrium (LD) with SLE in the Caucasian (European-American and Swedish) and Chinese Han populations, respectively [8,12]. These observations underscored the role of the pathway involving *TNFAIP3-TNIP1* in the genetic predisposition to SLE. The association of *TNIP1* with SLE needs to be further confirmed.

Recently, it has become increasingly clear that SLE and RA share a number of susceptibility genes. *TNFAIP3* [4-10], *STAT4* [13,14] and *BLK* [15,16] represent such shared susceptibility genes. *TNIP1* has been shown to be upregulated in synovial tissues from RA [17], raising a possibility that *TNIP1* may also play a role in the pathogenesis of RA. To date, association of RA with *TNIP1* has not been reported.

This study was conducted to examine whether *TNIP1* was associated with SLE and RA in a Japanese population.

Materials and methods

Patients and controls

Three hundred sixty-four Japanese patients with SLE (21 males and 343 females, mean ± SD age, 42.8 ± 13.9 years), 553 patients with RA (43 males and 510 females, mean ± SD age, 58.0 ± 11.3 years) and 513 healthy controls (238 males and 275 females, mean ± SD age, 34.1 ± 9.9 years) were recruited at University of Tsukuba, Juntendo University, Sagamihara National Hospital, Matsuta Clinic and the University of Tokyo (Table 1). All patients and healthy individuals were native Japanese living in the central part of Japan. All patients with SLE and RA fulfilled the American College of Rheumatology criteria for SLE [18] and RA [19], respectively. Consecutive patients ascertained in rheumatology specialty hospitals or clinics were recruited. The patients with SLE were classified into subgroups according to the presence or absence of renal disorder, neurologic disease and serositis based on the definition of ACR criteria [18], anti-dsDNA and anti-Sm antibodies, and age of onset (< 20 yr). The numbers of the missing data were 5 (renal disorder), 3 (neurologic disease), 21 (serositis), 19 (anti-dsDNA antibody), 22 (anti-Sm antibody) and 6 (age of onset). Patients with RA and healthy controls were stratified by the presence or absence of human leukocyte antigen DR β1 (*HLA-DRB1*) shared epitope. The numbers of the missing data were 6 (RA) and 15 (controls).

Table 1 Characteristics of the patients and healthy controls studied

	SLE	RA	Healthy controls
n	364	553	513
Male/female	21/343	43/510	238/275
Age*	42.8 ± 13.9	58.0 ± 11.3	34.1 ± 9.9

*Mean ± SD.

The control group consisted of healthy volunteers without any signs or symptoms of autoimmune diseases recruited at the same institutes.

This study was carried out in compliance with the Helsinki Declaration. The study was reviewed and approved by the research ethics committees of the University of Tsukuba, Sagamihara National Hospital and Juntendo University. Informed consent was obtained from all study participants.

Genotyping

Genotyping of *TNIP1* rs7708392 was carried out using the TaqMan genotyping assay (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions, using a TaqMan probe C__29349759_10. *HLA-DRB1* was genotyped at the sequence level using a polymerase chain reaction (PCR) microtiter plate hybridization assay as previously described [20].

Statistical analysis

A case control association study was conducted by χ^2 test using 2 × 2 contingency tables. The null hypotheses tested in this study were that there was no difference in the genotype or allele frequencies between all SLE patients and healthy controls, between SLE subsets and healthy controls, between all RA patients and all healthy controls, or between RA patients and healthy controls stratified by the presence or absence of *HLA-DRB1* shared epitope.

The power to detect association was calculated on the basis of the frequency of the rs7708392C allele in Japanese healthy controls (69.9%). The sample size of this study (364 SLE patients, 553 RA patients and 513 controls) had the power of 80% to detect association when the genotype relative risk was1.36 (SLE) and 1.32 (RA) or greater, respectively [21].

To adjust for the gender difference between patients and healthy controls (Table 1), multiple logistic

regression analyses were employed. The following were used as independent variables: for the genotypes of rs7708392, C/C = 1, C/G = 0, G/G = 0 under the recessive model for the C allele, C/C = 2, C/G = 1, G/G = 0 under the codominant model, and for gender, male = 0, female = 1.

The interaction between *TNIP1* rs7708392 and *TNFAIP3* rs2230926, which we recently replicated to be associated with SLE [10], was examined in 308 SLE, 372 RA and 449 healthy controls, using logistic regression analysis. Codominant (risk allele homozygotes $x_i = 2$, heterozygotes $x_i = 1$, nonrisk allele homozygotes $x_i = 0$), dominant (risk allele homozygotes $x_i = 1$, heterozygotes $x_i = 1$, nonrisk allele homozygotes $x_i = 0$) and recessive (risk allele homozygotes $x_i = 1$, heterozygotes $x_i = 0$, nonrisk allele homozygotes $x_i = 0$) models for gene i were tested. The logistic regression model for interaction between gene i and gene j was given by $\text{logit}(P) = \beta_0 + \beta_i x_i + \beta_j x_j + \beta_{ij} x_i x_j$. The deviation from 0 was evaluated for b_{ij} by the Wald test. Population attributable risk percentage (PAR%) was estimated by the formula:

$$\text{PAR\%} = \text{Pe}\,(\text{RR}-1)/(\text{Pe}\,[\text{RR}-1]+1)\times 100,$$

where Pe represents the risk genotype frequency in the population and RR represents the relative risk of the risk genotype [22]. Although RR cannot be determined from the case-control study design, it can be approximated by odds ratio (OR) when the incidence of the disease is sufficiently low. Because the incidence of SLE has been reported to be 3.0 in Japan and 1.8-7.6 in the United States per 100,000 persons per year [23] and is sufficiently small, OR can be adequately used for an approximation for RR. The PAR% in the Caucasian populations were calculated using the raw genotype count data for the previously reported study (cases: C/C 293, C/G 1,389, G/G 1,632, controls: C/C 735, C/G 4,510, G/G 7,050) [12].

Results

Replication of *TNIP1* association with SLE in Japanese

The association of *TNIP1* rs7708392 with SLE, recently demonstrated in the Caucasian (European-American and Swedish) populations [12], was examined in a Japanese population. Departure from Hardy-Weinberg equilibrium was observed neither in the cases nor in the controls ($P > 0.3$). As shown in Table 2, rs7708392C allele was significantly increased in Japanese SLE patients (76.5%) compared with healthy controls (69.9%, $P = 0.0022$, OR 1.40, 95% confidence interval [95% CI] 1.13-1.74), confirming the association in the Caucasians. The association was also detected under the recessive model for the rs7708392C allele ($P = 0.0023$, OR 1.52, 95% CI 1.16-2.00). Notably, the risk allele frequency was considerably greater in the Japanese (69.9%) than in the Caucasian healthy controls (24.3%) [12]. In the Japanese, PAR% was estimated to be 20.4% under the recessive model for the C allele (OR 1.52, population frequency of C/C 48.9%) and 31.0% under the dominant model (OR 1.50, population frequency of C/C + C/G 90.8%). These estimates were substantially greater than in the Caucasian populations, where the PAR% was 3.0% under the recessive model (OR 1.53, population frequency of C/C 6.0%) and 14.1% under the dominant model (OR 1.39, population frequency of C/C + C/G 42.7%).

Because the female-to-male ratio was different between SLE patients and healthy controls (Table 1), we carried out multiple logistic regression analysis to examine the association after adjustment for gender. The association with SLE remained significant both under the recessive model for rs7708392C ($P = 0.030$, OR 1.40, 95% CI 1.03-1.89) and under the codominant model ($P = 0.033$, OR 1.30, 95% CI 1.02-1.65).

Association of *TNIP1* with Clinical Subsets of SLE

We next analyzed whether *TNIP1* was associated with clinical subsets such as presence or absence of renal disorder, neurological disease, serositis, anti-dsDNA antibody, anti-Sm antibody, as well as the age of onset (< 20 yr). When the association was tested between patients having each phenotype and healthy controls, a tendency of stronger association was observed in the subsets with renal disorder and anti-dsDNA antibody as compared with all SLE (Table 2). These associations remained significant after adjustment for gender using logistic regression analysis (nephropathy positive versus controls: $P = 0.0070$, OR 1.50, 95% CI 1.12-2.01 under the codominant model and $P = 0.011$, OR 1.59, 95% CI 1.11-2.26 under the recessive model; anti-dsDNA positive versus controls: $P = 0.033$, OR 1.32, 95% CI 1.02-1.71 under the codominant model and $P = 0.024$, OR 1.45, 95% CI 1.05-2.00 under the recessive model).

On the other hand, significant association was not observed in the patient subsets having neurologic disease, serositis, anti-Sm antibody, and the patients with the age of onset <20 yr.

Lack of Association with RA

We next tested association of *TNIP1* rs7708392 with RA. Although a slight tendency toward association was observed, significant association with RA was not detected (Table 3). Significant association was not detected after the adjustment for gender ($P = 0.847$, OR 1.02, 95% CI 0.83-1.26 under the codominant model, $P = 0.753$, OR 1.04, 95% CI 0.80-1.36 under the recessive model), nor after stratification according to the presence or absence of *HLA-DRB1* shared epitope (Table 3).

Table 2 Association study of *TNIP1* rs7708392 with SLE in a Japanese population

	Genotype			Allele	Allelic association		Recessive model*	
	C/C	C/G	G/G	C	*P*	OR (95%CI)	*P*	OR (95%CI)
All SLE (*n* = 364)	216 (59.3)	125 (34.3)	23 (6.3)	557 (76.5)	0.0022	1.40 (1.13-1.74)	0.0023	1.52 (1.16-2.00)
SLE subsets								
Renal disorder + (*n* = 203)	126 (62.1)	68 (33.5)	9 (4.4)	320 (78.8)	0.00065	1.60 (1.22-2.10)	0.0015	1.71 (1.23-2.38)
Neurologic disorder + (*n* = 53)	28 (52.8)	21 (39.6)	4 (7.5)	77 (72.6)	0.55	1.14 (0.73-1.79)	0.59	1.17 (0.66-2.05)
Serositis + (*n* = 55)	33 (60.0)	18 (32.7)	4 (7.3)	84 (76.4)	0.16	1.39 (0.88-2.20)	0.12	1.57 (0.89-2.75)
Anti-dsDNA Ab + (*n* = 280)	169 (60.4)	93 (33.2)	18 (6.4)	431 (77.0)	0.0026	1.44 (1.14-1.83)	0.0021	1.59 (1.18-2.13)
Anti-Sm Ab + (*n* = 67)	37 (55.2)	26 (38.8)	4 (6.0)	100 (74.6)	0.26	1.27 (0.84-1.91)	0.33	1.29 (0.77-2.15)
Onset <20 yr (*n* = 86)	46 (53.5)	34 (39.5)	6 (7.0)	126 (73.3)	0.37	1.18 (0.82-1.70)	0.43	1.20 (0.76-1.90)
Healthy controls (*n* = 513)	251 (48.9)	215 (41.9)	47 (9.2)	717 (69.9)		reference		reference

OR: odds ratio, 95% CI: confidence interval. Genotype and allele frequencies are shown in parentheses (%).

Association was tested by χ^2 analysis using 2 × 2 contingency tables. All SLE group as well as each SLE subset was compared with healthy controls.

*Association was tested under the recessive model for rs7708392C allele.

Lack of Evidence for Genetic Interaction between *TNFAIP3* and *TNIP1*

Finally, we examined whether genetic interaction exists between *TNFAIP3* and *TNIP1* SNPs, because molecular interaction is known between the protein products of these genes. Although all combinations of the codominant, dominant and recessive models for each gene were examined, statistically significant gene-gene interaction was not detected (P >0.05).

Discussion

In the present study, we replicated the association of *TNIP1* rs7708392 with SLE in a Japanese population, which indicated that *TNIP1*, as well as *TNFAIP3*, is a susceptibility gene to SLE shared by the Caucasian and Asian populations. Because both TNIP1 and A20 are thought to be involved in the inhibition of NF-κB activation, genetic association of these genes implicates a causal role of NF-κB regulation pathway in the pathogenesis of SLE.

Kalergis et al. [24] demonstrated that expression of IκB-α, an inhibitor of NF-κB, was decreased in Fcγ receptor IIb-deficient mice which present lupus-like symptoms, and the symptoms were reduced by treatment with NF-κB inhibitors. Previous studies demonstrated that *TNFAIP3* risk allele rs2230926G (127Cys) leads to reduced inhibitory activity of NF-κB activation [6] or reduced mRNA level of *TNFAIP3* [10]. In view of these observations, it is speculated that the risk allele of *TNIP1* may also be associated with reduced inhibitory activity of *TNFAIP3-TNIP1* pathway.

TNIP1 rs7708392 is located in intron 1. Expression analysis using the GENEVAR [25] and the International HapMap databases [26] as previously described [27] did not show significant effect of rs7708392 genotypes on the mRNA level of *TNIP1* (data not shown). Although the direct molecular mechanism of the risk allele to cause SLE remains unclear, it is possible that the risk allele may be associated with the selection of splicing

Table 3 Association study of *TNIP1* rs7708392 with RA in a Japanese population

	Genotype			Allele	Allelic Association		Recessive Model*	
	C/C	C/G	G/G	C	*P*	OR (95%CI)	*P*	OR (95%CI)
All RA (*n* = 553)	292 (52.8)	215 (38.9)	46 (8.3)	799 (72.2)	0.23	1.12 (0.93-1.35)	0.21	1.17 (0.92-1.49)
All healthy controls (*n* = 513)	251 (48.9)	215 (41.9)	47 (9.2)	717 (69.9)		reference		reference
HLA-DRB1 shared epitope positive								
RA (*n* = 376)	203 (54.0)	142 (38.7)	31 (8.2)	548 (72.9)	0.76	1.04 (0.80-1.37)	0.57	1.11 (0.78-1.56)
Healthy controls (*n* = 202)	104 (51.5)	83 (41.1)	15 (7.4)	291 (72.0)		reference		reference
HLA-DRB1 shared epitope negative								
RA (*n* = 171)	86 (50.3)	70 (40.9)	15 (8.8)	242 (70.8)	0.67	1.07 (0.80-1.43)	0.68	1.08 (0.74-1.58)
Healthy controls (*n* = 296)	143 (48.3)	125 (42.2)	28 (9.5)	411 (69.4)		reference		reference

OR: odds ratio, 95% CI: confidence interval. Genotype and allele frequencies are shown in parentheses (%).

Association was tested by χ^2 analysis using 2 × 2 contingency tables. Comparisons were made between all RA and all healthy controls, HLA-DRB1 shared epitope positive RA and controls, and shared epitope negative RA and controls.

*Association was tested under the recessive model for rs7708392C allele.

variant. To date, at least 11 splice variants of *TNIP1* have been identified [1]. Presence of alternative exon 1A and 1B, as well as splice variants lacking exon 2, has been described. Because rs7708392 is located between exon 1B and exon 2, it is possible that this SNP may influence the usage of the splicing isoform. It is also possible that other causative SNPs in tight LD with rs7708392 may exist. Such a possibility would be addressed by resequencing the entire *TNIP1* gene.

Interestingly, in sharp contrast to the Caucasian populations, the risk rs7708392C constituted the major allele in the Japanese population. This resulted in substantially higher PAR% in the latter. We previously reported similar findings in *STAT4* and *BLK* SNPs [14,15]. In Chinese, a SNP rs10036748, which is in tight LD with rs7708392 in Japanese (r^2 = 0.81, HapMap database [26]), has been shown to be associated with SLE. The frequencies of rs10036748 risk allele in Chinese (cases 79.7%, controls 66.1%) [8] are similar to those of rs7708392 in Japanese (Table 2). It should be noted that, because the information used to estimate the PAR % was based on the data from a variant that has not been shown to be the causal variant in *TNIP1*, and the estimates of the allele frequency and OR (as an approximation for RR) were taken from a rather small case-control study, the PAR% values shown here represent rough estimates. Nevertheless, the data suggest that the significance of *TNIP1* in the genetic background of SLE may be substantially greater in the Asian than in the Caucasian populations.

In the association analysis with the clinical subsets, none of the case-only comparisons (cases with each clinical phenotype versus those without) reached statistical significance, partly because of the insufficient statistical power caused by the small sample size due to stratification. However, preferential association of *TNIP1* with renal disorder and anti-dsDNA antibody was suggested by comparison with healthy controls. In our subjects, preferential association with renal disorder was also observed for *TNFAIP3* [10].

On the other hand, association was not observed with the SLE subsets having neurological disease, serositis, anti-Sm antibody and age of onset <20. It is interesting to note that renal disorder and presence of anti-dsDNA are significantly correlated in SLE, while neurologic disorders are not, suggesting that these clinical features might represent different clinical subsets of SLE [28]. In view of this, our findings could be interpreted such that polymorphisms in *TNIP1-TNFAIP3* pathway might play a significant role in the subset of SLE characterized by renal disorder and anti-dsDNA antibody, but not in the subset with neurologic disease. Such a hypothesis should be validated in future large-scale studies.

No strong evidence for association of rs7708392 with RA was obtained in this study. The sample size in this study (553 RA patients and 513 controls) provides 80% power to detect associations with genotype relative risk of 1.32 or greater, but we cannot rule out a possibility of weak association. Recently published meta-analysis of GWAS in Caucasians also failed to demonstrate statistically significant association of *TNIP1* SNP with RA, although similarly to our observation, a tendency for association was detected [29]. Thus, while a role of *TNFAIP3* is observed both in SLE and RA genetics, *TNIP1* appears to play a major role in SLE, but not in RA. Such a difference might possibly imply that the molecular mechanism of *TNIP1* association might not be fully explained by A20 modification. In support of this possibility, TNIP1 has been shown to block TNF-induced programmed cell death in *TNFAIP3* deficient cells, indicating that TNIP1 does not always require A20 to perform its anti-apoptotic function [3]. Thus, further analysis on the molecular mechanisms involving these molecules is required.

Conclusions

Association of *TNIP1* with SLE was confirmed in a Japanese population. *TNIP1* is a shared SLE susceptibility gene in the Caucasian and Asian populations, but the genetic contribution appeared to be greater in the Asians because of the higher risk allele frequency in the population. Taken together with the association of *TNFAIP3*, these observations underscore the crucial role of NF-κB regulation in the pathogenesis of SLE.

Abbreviations

95%CI: 95% confidence interval; ABIN-1: A20-binding inhibitor of NF-κB -1; CI: confidence interval; GWAS: genome-wide association studies; HLA-DRB1: human leukocyte antigen DR β1; LD: linkage disequilibrium; NF-κB: nuclear factor-κB; OR: odds ratio; PAR%: population attributable risk percentage; PCR: polymerase chain reaction; RA: rheumatoid arthritis; RR: relative risk; SLE: systemic lupus erythematosus; SNP: single nucleotide polymorphism; TNFAIP3: tumor necrosis factor α-induced protein 3; TNIP1: TNFAIP3 interacting protein 1.

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Authors' contributions

AK participated in the study design, carried out all genotyping and statistical analyses, and wrote the manuscript. JO carried out statistical analysis with AK and helped in the manuscript preparation. SI, HF, TH, DG, IM, MK, KM, ST, YT, HH and TS recruited Japanese patients with SLE and collected clinical information. RRG and TWB provided Caucasian data. NT designed and coordinated the study and helped in the manuscript preparation. All authors read and approved the final manuscript.

Competing interests

RRG and TWB are employees of Genentech, Inc. (South San Francisco, CA, USA). The other authors declare that they have no competing interests.

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The Japanese experience with biologic therapies for rheumatoid arthritis

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Abstract | The unique genetic, environmental and medical backgrounds of people in Japan might influence the effectiveness and safety of biologic agents in patients with rheumatoid arthritis (RA). Indeed, clinical trials revealed higher response rates to some biologic agents (including infliximab, etanercept and tocilizumab) in patients with RA in Japan than patients treated with the same agents in Western countries, although response rates to adalimumab were comparable in both populations. The reasons why response rates to some biologic agents differ in Japanese individuals is currently under investigation. Post-marketing surveillance data have been collected for all patients with RA who were treated with biologic agents in Japan to monitor drug safety. These data clearly demonstrated that only ~5% of these patients experienced adverse drug reactions to biologic agents, which were well tolerated. Pneumonia, tuberculosis, *Pneumocystis jirovecii* pneumonia and interstitial pneumonitis are considered important severe adverse reactions and risk factors for these adverse effects have been identified. Adverse drug reactions could exaggerate the risks associated with biologic therapy in Japanese patients with RA. Attempts have, therefore, been made to predict clinical response and adverse effects to enable personalized therapy with biologic agents and to optimize the outcomes of these patients.

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Introduction

Given the notable genetic and environmental differences between the populations of Eastern and Western countries, rheumatologists in countries in the Far East, such as Japan, have continually faced challenges concerning the use of biologic agents. Many factors (such as genetic background) can influence the effectiveness and safety of these therapies. For example, within Eastern countries, the frequency of HLA-B27 positivity (which is associated with rheumatic diseases such as ankylosing spondylitis) in the general population of Japan is <1%, whereas in China and Korea the corresponding value is about 5%,[1] which suggests that the Japanese population has a distinct genetic background even compared with other Eastern populations.

Striking differences in environmental factors exist between Japan and Western countries, such as the USA. For example, the incidence of tuberculosis (TB) in Japan in 2002 was 25.8 cases per 100,000 individuals, which was fourfold higher than the corresponding incidence in the USA.[2] This discrepancy raised concerns about the possible increased risk of TB in Japan just as therapies that target tumor necrosis factor (TNF) were introduced to the Japanese market. This concern is particularly relevant as patients receiving anti-TNF therapy are potentially at an increased risk of TB.[2] Smoking can be associated with an increased risk of developing rheumatoid arthritis (RA). In 2008, the proportion of individuals who smoke in Japan was 39.4% in men and 11.0% in women; smoking rates for men are higher in Japan than they are in the USA, whereas the converse pattern is true in women.[3] In addition, the average body weight of Japanese patients with RA is ~54 kg,[2,4–6] which is only 60–70% that of American patients with RA. This difference, therefore, must be taken into consideration when interpreting the results of clinical trials that used fixed doses of biologic agents, such as etanercept and adalimumab. Finally, treatment profiles and doses of DMARDs and glucocorticoids used in Japan are somewhat different from those used in Western countries (discussed below).[7] The Japanese experience of biologic agents for RA provides a unique view on the biologic agents in use for RA compared to those obtained in Western countries. In addition, the extensive post-marketing surveillance (PMS) for all patients with RA who were treated with biologic agents in Japan is robust and could provide safety information to rheumatologists worldwide.

In this Review, we focus on PMS data and the results of clinical trials of biologic agents for patients with RA in Japan, with particular attention to the distinct genetic, environmental and medical backgrounds of Japanese patients. The effectiveness and safety of biologic agents in Japanese patients with RA are also discussed. Clearly, as outlined in this article, multiple factors

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Competing interests
T. Takeuchi declares associations with the following companies: Abbott, Bristol-Myers Squibb, Chugai Pharmaceutical, Eisai Pharmaceuticals, Janssen Pharmaceutica, Mitsubishi Tanabe Pharma, Novartis, Takeda Pharmaceuticals, Wyeth Japan. H. Kameda declares associations with the following companies: Abbott, Centocor Ortho Biotech, Chugai Pharmaceutical, Eisai Pharmaceuticals, Mitsubishi Tanabe Pharma, Takeda Pharmaceuticals, Wyeth Japan. See the article online for full details of the relationships.