Lack of Association of rs12702634 in *RPA3-UMAD1* With Interstitial Lung Diseases in Japanese Rheumatoid Arthritis Patients

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Takashi Higuchi[1,2], Shomi Oka[1,3], Hiroshi Furukawa[1,3], Kota Shimada[3,4] and Shigeto Tohma[1,3] on behalf of the Rheumatoid Arthritis associated Interstitial Lung Disease (RA-ILD) Study Consortium†

[1]Department of Clinical Research, National Hospital Organization Tokyo National Hospital, Kiyose, Japan. [2]Department of Nephrology, Ushiku Aiwa General Hospital, Ushiku, Japan. [3]Clinical Research Center for Allergy and Rheumatology, National Hospital Organization Sagamihara National Hospital, Minami-ku, Sagamihara, Japan. [4]Department of Rheumatic Diseases, Tokyo Metropolitan Tama Medical Center, Fuchu, Japan.

ABSTRACT

BACKGROUND: Rheumatoid arthritis (RA) is occasionally complicated with interstitial lung disease (ILD). A recent genome-wide association study of ILD in RA reported an association with the polymorphism rs12702634 in *RPA3-UMAD1*. We conducted an association study of this variant with ILD in Japanese RA patients to replicate this association.

METHODS: Genotyping of rs12702634 was performed in 175 RA with ILD and 411 RA without chronic lung disease.

RESULTS: No association was detected for rs12702634 with ILD in RA ($P = .6369$, odds ratio [OR] 1.13, 95% confidence interval [CI] 0.72-1.78). Meta-analysis of these data combined with the data from the recent report showed no significant association ($P = .0996$, OR 1.52, 95% CI 0.92–2.49).

CONCLUSIONS: The present study demonstrated no association of *RPA3-UMAD1* rs12702634 with ILD in RA, suggesting the heterogeneity of the disease.

KEYWORDS: Rheumatoid arthritis, interstitial lung diseases, polymorphism, genetic association, extraarticular manifestation

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CORRESPONDING AUTHOR: Hiroshi Furukawa, Department of Clinical Research, National Hospital Organization Tokyo National Hospital, 3-1-1 Takeoka, Kiyose 204-8585, Japan. Email: furukawa-tky@umin.org.

Introduction

Rheumatoid arthritis (RA) is characterized by the distraction of the synovial joints and is occasionally complicated with the development of interstitial lung disease (ILD). ILD is detected in about 10% of RA cases[1] and precedes RA diagnosis in about 10% of RA cases with ILD.[2] The prognosis of RA patients with ILD is quite poor.[3] Although the etiology of RA is vague, it is thought that the disease susceptibility of RA is associated with genetic factors. Many genetic factors for RA or idiopathic interstitial pneumonia were reported; a few genetic analyses had been conducted for ILD in RA. A recent genome-wide association study (GWAS) of ILD in RA in a Japanese population identified a significant association with a single nucleotide polymorphism (SNP), rs12702634, in the *RPA3-UMAD1* gene.[4] An association of this SNP with ILD in Japanese RA patients was analyzed in the present study to replicate this association.

Materials and Methods

Patients

Japanese RA patients with available chest computed tomography images were recruited at outpatient departments or hospital wards of the rheumatology centers for this case control study; all

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the RA patents fulfilled American College of Rheumatology criteria for RA[5] or Rheumatoid Arthritis Classification Criteria.[6] Usual interstitial pneumonia (UIP), nonspecific interstitial pneumonia, (NSIP), or no chronic lung diseases (CLDs) complications in RA patients were diagnosed using chest conventional or high-resolution computed tomography images.[7] RA patients with other computed tomography findings, bronchiolitic airway disease, bronchiectatic airway disease, cryptogenic organizing pneumonia, mosaic perfusion, pleural effusion, pneumonia, or cancer were excluded.

175 RA with ILD (76 UIP and 99 NSIP) and 411 RA without CLD were enrolled in the present study. This study was reviewed and approved by Yokohama Minami Kyosai Hospital Research Ethics Committee (22-6-2), Tokyo National Hospital Ethics Committee (190010), Tama Medical Center Research Ethics Committee (H23-30), Sagamihara National Hospital Research Ethics Committee (2009061621), Niigata Rheumatic Center Research Ethics Committee (2017-018), Nagoya Medical Center Research Ethics Committee (2012-526), Nagasaki Medical Center Research Ethics Committee (22081), Miyakonojo Medical Center Research Ethics Committee, Kumamoto Center for Arthritis and Rheumatology Research Ethics Committee, Hyogo College of Medicine Research Ethics Committee (178), and all the institutes involved in the recruitment of the subjects. Written informed consent was obtained from all subjects. This study was conducted in accordance with the principles expressed in the Declaration of Helsinki.

Genotyping

Genotyping of rs12702634 [C/G] in *RPA3-UMAD1* was conducted with TaqMan genotyping assay (Assay ID: C__31572862_10, Thermo Fisher Scientific Inc., Waltham, MA) and 7500 Fast Real-Time PCR System (Thermo Fisher Scientific Inc.). Thermal cycling conditions comprised denaturation at 95°C for 20 seconds, followed by 40 cycles at 95°C for 3 seconds then at 60°C for 30 seconds.

Statistical analysis

The distribution of allele frequencies was compared between RA patients with ILD and those without CLD by Fisher's exact test using 2 × 2 contingency tables under the allele model. The 80% statistical power was estimated to be provided when the odds ratio (OR) was 1.84 or higher under the allele model (http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize)[8]; this threshold was lower than the previously reported OR.[4] Meta-analysis was conducted using EZR (http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmed.html)[9] with the DerSimonian-Laird method under the random effects model.[10]

Results

Demographic features of the RA patients

Demographic features of RA with ILD, UIP, or NSIP were compared with RA without CLD (Table 1). Age at onset, male percentage, and mean age at enrollment in RA patients with ILD, UIP, or NSIP were higher compared with RA patients without CLD. Rheumatoid factor was increased in RA with ILD or UIP. Surfactant protein-D and Krebs von den lungen-6 in RA with ILD, UIP, or NSIP were also increased. No difference was detected in anti-citrullinated peptide antibody, smoking status, and Steinbrocker stage.[11]

Lack of association of rs12702634 with ILD in RA patients

Genotyping of rs12702634 was conducted; deviation from Hardy-Weinberg equilibrium was not detected in RA with ILD ($P = .7829$) or RA without CLD ($P = .3243$). There was no significant association of rs12702634 with ILD in RA ($P = .6369$, OR 1.13, 95% confidence interval [CI] 0.72-1.78, Table 2). There was no significant association of rs12702634 with UIP or NSIP.

Meta-analysis of our data combined with the data from the previous report[4] was performed under the random effects model and did not reach the genome-wide significance threshold (Supplemental Figure 1; $P = .0996$, OR 1.52, 95% CI 0.92-2.49; the weight of this study in the random effects model was 42.2%). The lack of heterogeneity was not confirmed between our data and the previously reported data on rs12702634 ($I^2 = 74.6\%$, $\tau^2 = 0.0980$, $H = 1.99$, $Q = 3.94$, $P = .0470$); these data supported the analysis under the random effects model.

Discussion

Although the association of the *MUC5B* promoter variant rs35705950 with ILD was reported in RA, the allele frequency of the susceptible variant was low (0.2%) in Japanese populations[12], suggesting that this variant could not explain the predominant pathogenesis of ILD in Japanese RA patients. The disease-susceptible genes are occasionally different between different ethnic populations; *PTPN22*, a RA-susceptible gene in European populations, is not the susceptible gene for RA in Japanese populations.[13]

It was suspected that other genetic factors would be associated with ILD in Japanese RA. Hence, a genome-wide association study of ILD in RA was conducted,[4] and the association of rs12702634 in *RPA3-UMAD1* was reported. However, the results from the previous study were not reproduced in the present study and the results of meta-analysis did not indicate the association, suggesting the heterogeneity of the disease. The pathogenesis of ILD in RA would be heterogeneous, since male is dominant in RA with UIP but not in RA with NSIP.[7] Thus, different results of genetic analyses between different populations in the same ethnic group may provide the explanation for the heterogeneity of ILD in RA.

To the best of our knowledge, this is the first replication study on the association of rs12702634 with ILD in RA, but it failed to confirm the association. This study has some limitations. This study revealed a strong heterogeneity of ILD in RA patients, although our sample size was modest. Moreover,

Table 1. Characteristics of RA patients.

	ILD		UIP		NSIP		CLD(-)
Number	175		76		99		411
Mean age, years (SD)	68.8 (10.5)	1.61×10^{-12}	69.7 (10.2)	1.48×10^{-8}	68.1 (10.7)	1.34×10^{-7}	61.2 (12.7)
Male, n (%)	67 (38.3)	*4.74×10^{-9}	34 (44.7)	*6.84×10^{-8}	33 (33.3)	*0.0002	63 (15.4)
Age at onset, years (SD)	58.7 (13.6)	0.0002	63.0 (14.0)	0.0026	56.9 (13.1)	0.0083	50.2 (14.5)
Steinbrocker stage III and IV, n (%)	21 (39.6)	*0.8706	6 (42.9)	*0.7772	15 (38.5)	*1.0000	59 (37.8)
Smoker or past smoker, n (%)	24 (58.5)	*0.1544	3 (42.9)	*1.0000	21 (61.8)	*0.0882	60 (45.1)
RF, IU/ml (SD)	489.8 (1141.4)	0.0347	442.2 (489.3)	0.0146	527.5 (1469.1)	0.1445	233.7 (661.6)
ACPA, IU/ml (SD)	248.4 (275.9)	0.8009	268.8 (289.5)	0.8487	234.0 (267.6)	0.5916	258.5 (433.7)
KL-6, U/ml (SD)	1112.7 (1395.8)	3.90×10^{-7}	1268.3 (1563.4)	0.0003	985.0 (1241.4)	0.0004	353.1 (324.1)
SP-D, ng/ml (SD)	139.1 (122.4)	2.19×10^{-5}	148.7 (125.8)	0.0024	131.4 (121.6)	0.0029	49.3 (46.5)

Abbreviations: RA, rheumatoid arthritis; ILD, interstitial lung disease; UIP, usual interstitial pneumonia; NSIP, nonspecific interstitial pneumonia; CLD, chronic lung disease; RF, rheumatoid factor; ACPA, anti-citrullinated peptide antibody; KL-6, Krebs von den lungen-6, SP-D: surfactant protein-D. Number or average value of each group are shown. Standard deviations or percentages are shown in parentheses. Difference was tested in the comparison with the CLD(-) population by Fisher's exact test using 2×2 contingency tables or Student's *t*-test. *Fisher's exact test was employed.

Table 2. Allele frequencies of *RPA3-UMAD1* rs12702634 in the RA patients with ILD or without CLD.

	N	GENOTYPE	[C/G]	[G/G]	ALLELE	*P*	OR (95%CI)
		[C/C]			[C]		
ILD(+)RA, n (%)	175	1 (0.6)	28 (16.0)	146 (83.4)	30 (8.6)	0.6369	1.13 (0.72-1.78)
UIP(+)RA, n (%)	76	1 (1.3)	13 (17.1)	62 (81.6)	15 (9.9)	0.3332	1.32 (0.73-2.38)
NSIP(+)RA, n (%)	99	0 (0.0)	15 (15.2)	84 (84.8)	15 (7.6)	1.0000	0.99 (0.55-1.77)
CLD(-)RA, n (%)	411	1 (0.2)	61 (14.8)	349 (84.9)	63 (7.7)		

Abbreviations: RA, rheumatoid arthritis; ILD, interstitial lung disease; UIP, usual interstitial pneumonia; NSIP, nonspecific interstitial pneumonia; CLD, chronic lung disease; OR, odds ratio; CI, confidence interval. Genotype and allele frequencies are shown in parentheses (%). Association was tested by Fisher's exact test using 2×2 contingency tables under the allele model.

multi-ethnic studies with other populations should be conducted, since this study was performed only in Japanese. The genetic analyses should be continued in future larger scale studies to reveal the true etiology of ILD in RA.

Conclusions

The present study demonstrated no association of *RPA3-UMAD1* rs12702634 with ILD in RA. The results of meta-analysis could not confirm the association, suggesting the heterogeneity of the disease.

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Authors' Contributions

HF, KS, and ST designed the study. TH, SO, and HF conducted the experiments. TH and HF analyzed the data. HF, KS, and ST contributed to the collection of clinical information and materials. TH, HF, and ST wrote the manuscript.

Availability of Data and Material

All data are presented in the paper.

Ethics Approval and Consent to Participate

This study was reviewed and approved by Yokohama Minami Kyosai Hospital Research Ethics Committee, Tokyo National Hospital Ethics Committee, Tama Medical Center Research Ethics Committee, Sagamihara National Hospital Research Ethics Committee, Niigata Rheumatic Center Research Ethics Committee, Nagoya Medical Center Research Ethics Committee, Nagasaki Medical Center Research Ethics Committee, Miyakonojo Medical Center Research Ethics Committee, Kumamoto Center for Arthritis and Rheumatology Research Ethics Committee, Hyogo College of Medicine Research Ethics Committee, and all the institutes involved in the recruitment of the subjects. Written informed consent was obtained from all subjects. This study was conducted in accordance with the principles expressed in the Declaration of Helsinki.

ORCID iD

Hiroshi Furukawa https://orcid.org/0000-0003-1353-8056

Supplemental Material

Supplemental material for this article is available online.

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