Rheumatoid arthritis

ORIGINAL RESEARCH

Association of a FAM13A variant with interstitial lung disease in Japanese rheumatoid arthritis

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ABSTRACT

Background Interstitial lung disease (ILD) occasionally occurs in rheumatoid arthritis (RA) and confers a dismal prognosis. We previously reported that a single-nucleotide variant (SNV) of MUC5B was associated with ILD in RA. However, the pathogenesis of ILD in Japanese patients with RA could not be explained solely by this SNV because its frequency is extremely low in the Japanese population. Here, we examined whether a different idiopathic pulmonary fibrosis susceptibility SNV might be associated with ILD in Japanese patients with RA.

Methods Genotyping of rs2609255 (G/T) in FAM13A was conducted in 208 patients with RA and 420 without chronic lung disease using TaqMan assays.

Results A significant association with usual interstitial pneumonia (UIP) in RA was detected for rs2609255 under the allele model (p=0.0092, OR 1.53, 95% CI 1.12 to 2.11) and recessive model for the G allele (p=0.0003, OR 1.52, 95% CI 1.59 to 4.32). FAM13A rs2609255 was significantly associated with UIP in male patients with RA (p=0.0043, OR 3.65, 95% CI 1.52 to 8.73) under the recessive model.

Conclusions This study is the first to document an association of rs2609255 with ILD in Japanese patients with RA, implicating it in the pathogenesis of UIP, though studies on the function of rs2609255 are warranted.

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterised by the destruction of the synovial joints. It is occasionally complicated by the development of interstitial lung disease (ILD) characterised by interstitial inflammation of the lung detected in about 10% of patients with RA.1 The prognosis of patients with RA with ILD is quite poor.2 Although the aetiology of RA is unclear, it is thought that disease susceptibility is associated with genetic factors, many of which have been reported for RA or idiopathic interstitial pneumonia. In contrast, only a few genetic analyses have been conducted for susceptibility to ILD in RA.

We previously reported that a single-nucleotide variant (SNV) in the MUC5B promoter was associated with ILD in a multiethnic study of RA.3 This SNV has the strongest association with susceptibility to idiopathic pulmonary fibrosis.4–6 However, the pathogenesis of ILD in Japanese patients with RA cannot be explained by this SNV because its frequency is extremely low in the Japanese population.3

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ A single-nucleotide variant (SNV) of MUC5B was associated with interstitial lung disease (ILD) in rheumatoid arthritis (RA), but its frequency is extremely low in the Japanese population.

WHAT THIS STUDY ADDS

⇒ An association of FAM13A rs2609255 with ILD in Japanese RA was detected.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The pathogenesis of ILD in Japanese RA could be explained by the SNV in FAM13A, though studies on the function of the SNV are warranted.
with ILD in European RA. Additionally, it was reported that FAM13A is associated with chronic obstructive pulmonary disease. FAM13A is expressed in the lung and is thought to be involved in the Wnt signalling pathway, which is activated in idiopathic pulmonary fibrosis. Thus, FAM13A could be a candidate susceptibility gene for ILD in RA. Accordingly, the present study was conducted to determine whether SNVs in FAM13A are associated with ILD in Japanese RA.

**MATERIAL AND METHODS**

**Patients**

Patients with RA fulfilled American College of Rheumatology criteria for RA or Rheumatoid Arthritis Classification Criteria and were recruited at the institutes of research groups organised by Tokyo National Hospital and Sagamihara National Hospital. The patients with RA were native Japanese living in Japan. Patients with RA were diagnosed with usual interstitial pneumonia (UIP), non-specific interstitial pneumonia (NSIP) or no chronic lung disease (CLD) based on chest conventional or high-resolution CT, as follows: UIP: irregular linear opacities and honeycombing, NSIP: bilateral ground-glass attenuation patterns predominantly in subpleural and basal regions, and CLD (−): no abnormalities in CT images. A total of 208 patients with RA with ILD (94 with UIP and 114 with NSIP) and 420 patients with RA without CLD were enrolled (Table 1). The allele frequency of FAM13A rs2609255 in the Japanese population was extracted from the 38KJPN panel of the Japanese Multi Omics Reference Panel (https://jmorp.megabank.tohoku.ac.jp/).

**Genotyping**

Genotyping of rs2609255 (G/T) in the FAM13A gene was performed using a TaqMan assay (assay ID: C_15906608_10; Thermo Fisher Scientific, Waltham, Massachusetts, USA) on a 7500 Fast Real-Time PCR System (Thermo Fisher Scientific), according to the manufacturer’s instructions. Conditions for thermal cycling were denaturation at 95°C for 20 s, followed by 40 cycles of 95°C for 3 s and 60°C for 30 s.

**Statistical analysis**

Associations of the variants were analysed to compare RA with ILD to RA without CLD by Fisher’s exact test using 2x2 contingency tables under the allele model or the recessive model.
Table 3 Genotype frequencies of FAM13A rs2609255 in the subpopulations of patients with RA

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Male</th>
<th></th>
<th>Recessive model for G allele</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>(G/G)</td>
<td>(G/T)</td>
</tr>
<tr>
<td>UIP (+) RA, n (%)</td>
<td>42</td>
<td>19 (45.2)</td>
<td>17 (40.5)</td>
</tr>
<tr>
<td>ILD (+) RA, n (%)</td>
<td>79</td>
<td>25 (31.6)</td>
<td>38 (48.1)</td>
</tr>
<tr>
<td>CLD (−) RA, n (%)</td>
<td>66</td>
<td>13 (19.7)</td>
<td>37 (56.1)</td>
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</tbody>
</table>

Age >65

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Male, age &gt;65</th>
<th></th>
<th>Recessive model for G allele</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)[1]</td>
<td>(G/G)</td>
<td>(G/T)</td>
</tr>
<tr>
<td>UIP (+) RA, n (%)</td>
<td>76</td>
<td>29 (38.2)</td>
<td>25 (32.9)</td>
</tr>
<tr>
<td>ILD (+) RA, n (%)</td>
<td>152</td>
<td>48 (31.6)</td>
<td>57 (37.5)</td>
</tr>
<tr>
<td>CLD (−) RA, n (%)</td>
<td>189</td>
<td>36 (19.0)</td>
<td>102 (54.0)</td>
</tr>
</tbody>
</table>

Genotype frequencies are shown in parentheses (%). Associations were tested in comparison with the CLD(−) RA population by Fisher’s exact test using 2x2 contingency tables under the recessive model.

RESULTS

Association of FAM13A rs2609255 with UIP or ILD in RA

FAM13A rs2609255 was genotyped in the patients with RA. No deviation from the Hardy-Weinberg equilibrium was observed (p=0.7683). It was investigated whether FAM13A rs2609255 was associated with UIP, NSIP or ILD in RA. A significant association with UIP was detected for FAM13A rs2609255 under the allele model (p=0.0092, Pc=0.0276, OR 1.59 (UIP vs CLD (−)), 1.54 (NSIP vs CLD (−)) and 1.41 (ILD vs CLD (−)) or higher under the allele model. It was also calculated to be 2.19 (UIP vs CLD (−)), 2.08 (NSIP vs CLD (−)) and 1.82 (ILD vs CLD (−)) under the recessive model for the G allele of rs2609255 (http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize).25 Meta-analysis in the allele model for ILD in RA was performed with EZR under the fixed effect model (http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmed.html).26

DISCUSSION

Here, we found that rs2609255G is a risk allele for UIP or ILD in Japanese RA. It was also determined that FAM13A rs2609255 was significantly associated with UIP in male and older patients with RA, as well as an association of FAM13A rs2609255 generally with RA. This had already been reported in European populations,11 but to the best of our knowledge, the present study is the first in Asian populations.

MUC5B rs35705950 was reported to be associated with ILD in RA.3 However, the frequency of the rs35705950T risk allele is extremely low in Japanese. The predominant pathogenesis of ILD in Japanese RA therefore cannot be explained by this SNV. Other genetic factors...
were suspected to be associated with ILD in Japanese RA. *RPA3-U*AD1* rs12702634 was reported to be associated with ILD in the GWAS of Japanese RA, but the association described in that study was not confirmed in our replication study in other Japanese populations, suggesting heterogeneity of ILD in RA. The confirmation of the results of genetic association studies by replication is important to establish their validity. The present study indicated that *FAM13A* rs2609255 is associated with UIP in Japanese RA. *FAM13A* rs2609255 was apparently associated with UIP in male and older patients with RA. However, *FAM13A* rs2609255 was not associated with NSIP in Japanese RA. Additionally, older men are predominant in RA with UIP but not in RA with NSIP. These data suggest that the pathogenesis of ILD in RA is heterogeneous.

In a recent GWAS, *FAM13A* was associated with ILD in European RA. This SNV had already been tested in a previous study in European and Mexican populations; it was not associated with ILD in RA. In the present study, this association was confirmed in Japanese populations, establishing a clear genetic association. It was reported that *FAM13A* is expressed in the lungs and expression quantitative trait loci analysis in the Genotype-Tissue Expression portal database revealed an association of *FAM13A* rs2609255 with the expression of the gene in lung or tibial artery. It was reported that *FAM13A* modulated Wnt signalling, which is thought to be involved in the pathogenesis of idiopathic pulmonary fibrosis. Thus, *FAM13A* rs2609255 would be a candidate for pathogenicity in the development of ILD in RA.

To the best of our knowledge, this is the first report of an association of *FAM13A* rs2609255 with ILD in Asian RA. The study does have several limitations. The sample size was modest and the study was based on the results solely from Japanese populations. This study did not include replicates in other Asian populations, though this is the replication of the European report. Thus, larger scale multiethnic studies with other populations including other Asians should be performed to validate the etiology of ILD in RA. Future studies on the function of *FAM13A* rs2609255 on ILD in RA are warranted to reveal the causality. The associations of *FAM13A* rs2609255 with the severity, progression or prognosis of ILD in patients with RA should be focused in future analyses, though it was not able to be assessed in the present study. This study established an association of *FAM13A* rs2609255 with ILD in RA, especially in older men, suggesting an explanation for the pathogenesis of ILD in Japanese RA.

**Contributors** HF and ST designed the study, TH, SO and HF conducted the experiments. TH and HF analysed the data. HF, KSh, STs, SI, AO, MK, KSa, SM, KM, SN and STo contributed to the collection of clinical information and materials. TH, HF and STo wrote the manuscript. HF is the guarantor.

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**Competing interests** STo received honoraria from Pfizer Japan, Ots Pharmaceutical Co., Mitsubishi Tanabe Pharma Corporation, Chugai Pharmaceutical Co., Astellas Pharma, AbbVie GK and Asahi Kasei Pharma Corporation, and was supported by research grants from Teijin Pharma, Takeda Pharmaceutical Company, Pfizer Japan, Mitsubishi Tanabe Pharma Corporation, Merck Sharp and Dohme, Eisai Co., Chugai Pharmaceutical Co., Astellas Pharma and Abbott Japan Co. HF received honoraria from Takeda Pharmaceutical Company, Pfizer Japan, LumineX Japan Corporation, Dainippon Sumitomo Pharma Co., Daiichi Sankyo Co., Ayumi Pharmaceutical Corporation and Ajinomoto Co., and was supported by research grants from Bristol-Myers-Squibb Co., Mitsui Sumitomo Insurance Welfare Foundation established by Mitsui Sumitomo Insurance Co., DaWa Securities Health Foundation established by DaWa Securities Group, NakATOMI Foundation established by Hisamitsu Pharmaceutical Co., Takeda Science Foundation supported by Takeda Pharmaceutical Company and Japan Research Foundation for Clinical Pharmacology run by Daiichi Sankyo.

**Patient consent for publication** Not applicable.

**Ethics approval** This study involves human participants and was approved by the research ethics committee of Tokyo National Hospital (190010), Sagamihara National Hospital and all other institutes participating in the present study. Written informed consent was obtained from all participants. This study was conducted in accordance with the principles expressed in the Declaration of Helsinki.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** Data are available upon reasonable request. Data supporting the findings of this study are presented in the paper and the supplementary file. Other data are available from the authors upon reasonable request. However, the clinical information and genotype data of each participant are not available under the conditions of informed consent mandated by the Act on the Protection of Personal Information.

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