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# A genome-wide association study identifying the SNPs predictive of rapid joint destruction in patients with rheumatoid arthritis

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**Abstract.** Rheumatoid arthritis (RA) is a common chronic autoimmune disease leading to joint destruction. The aim of the present study was to identify the genomic factors predictive of susceptibility to joint destruction in patients with RA by performing a genome-wide association study of genetic variants, including single nucleotide polymorphisms (SNPs). The study sample included 228 patients with a diagnosis of RA in the past 5 years. Patients were classified into rapid (total Sharp score/years of RA, $\geq$50) and slow (total Sharp score/years of RA, <50) joint destruction groups for analysis. The association between the genome-wide SNP analysis and joint destruction was evaluated. The following SNPs were strongly associated with rapid radiographic joint destruction: rs2295926 ($P<1x10^{-7}$), belonging to the *N-acetylgalactosaminyltransferase 12* (*GALNT12*) gene and rs11958855 ($P<1x10^{-6}$), belonging to the *KCNN2* gene (associated with the potassium calcium-activated channel subfamily). The identification of genetic predictors of rapid joint destruction in RA (*GALNT12* and *KCNN2*) may provide information regarding potential therapeutic targets, and this information may be used to assist in the management RA disease progression, thereby improving the functional outcomes for patients.

## Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation of the synovial lining of the joint (1), with progressive joint destruction and systemic complications being commonly observed (2). The prevalence rate of RA has been estimated to range between 0.5 and 1.0% (3). As an autoimmune disease, 70-80% of the patients with RA possess auto-antibodies, such as rheumatoid factor or anti-citrullinated protein antibodies (ACPA) (4). Early aggressive disease-modifying antirheumatic drug therapy can improve the clinical outcomes of patients with RA, including a decrease in long-term radiographic progression, and anti-TNF therapy can alter the natural progression of RA (5). Therefore, identifying patients at high risk of severe RA is important, such that more suitable treatments can be offered earlier on, thereby improving their disease prognosis (6).

Genetic polymorphisms may serve as useful markers of RA disease prognosis (7,8). To date, polygenic risk scores have largely been used to predict RA disease progression in humans (7), although other genomic predictive approaches using animal models or statistical methods have also been described (9,10). However, the International HapMap Project, which defines variations in the map of the human genome (11), has allowed genome-wide association screening (GWAS) of genetic variants, including single nucleotide polymorphisms (SNPs) (12). As an example, through GWAS, *PADI4* was identified as a non-major-histocompatibility complex genomic locus predictive of RA in a Japanese population (13). GWAS meta-analyses provided further evidence of a shared genetic background amongst patients with RA across different populations (14,15). A large-scale GWAS meta-analysis in a Japanese population of patients with RA identified the following new RA risk loci: *B3GNT2, ANXA3, CSF2, CD83, NFKBIE, ARID5B, PDE2A*-i *PLD4* and *PTPN2* (16).

Clinically, radiological damage provides an objective measure of RA severity; with joint destruction quantified using radiographic scores, such as the Sharp/van der Heijde score (SHS), which reflects the inflammatory status of the joint (17). Only a few studies to date have evaluated the association between radiographic joint destruction and genetic analysis. Rodriguez-Rodriguez *et al* (18) reported a probable association between the prostaglandin E receptor 4 variant, rs76523431 and radiographic joint destruction in Caucasian patients with RA. Suzuki *et al* (19) reported an association between the *PADI4* risk allele and radiographic joint destruction amongst Japanese patients with RA. However, GWAS has not previously been used to identify risk factors of radiographic joint destruction. Therefore, the purpose of the present study was

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to identify genomic factors predictive of susceptibility to joint destruction in patients with RA by performing a GWAS of genetic variants, including SNPs.

## Patients and methods

*Ethics statement and patient consent.* The methods used in the present study were approved by the Research Institute of Joint Disease Kobe on June 11, 2008. All patients included in the study satisfied the American College of Rheumatology 1987 revised criteria for RA (20) and completed the genetic analysis in the Patient Registry. A total of 240 patients were recruited, all of whom provided written informed consent in Matsubara Mayflower Hospital between October 2008 and December 2012. Additionally, another group of 228 patients (median age, 55 years old; age range, 23-84 years; 49 males and 179 females) were recruited between January 2013 and September 2017. Data collection, including blood examination and x-rays of the 240 patients (median age 60 years old; age range 21-83 years; 45 males and 195 females), was performed in the Matsubara Mayflower Hospital between January 2009 and December 2012. In the second group of 228 patients, data collection was performed between January 2013 and December 2017. All DNA samples were included in the Japanese GWAS analysis.

*Radiographic evaluation.* Radiographs of the hands and feet were scored according to the Sharp method (17). All radiographic data included in the analysis were obtained within 5 years of RA diagnosis, and clinical data and blood samples were collected on the same day as the diagnosis. In total, 228 radiographs (228 patients) were scored by a single experienced examiner who was blinded with respect to the clinical and genetic data. A total of 16 and 6 areas were considered for assessing erosions and joint space narrowing (JSN) for the hands and feet, respectively (Fig. 1). Erosions were scored as follows: 1, discrete but clearly present; 2 or 3, larger, dependent on the surface area of the joint involved. A score of 3 was given if the erosion was large and extended over the imaginary middle of the bone. A score of 5 was given if a complete collapse of the joint was observed or if the entirety of the joint was affected. In each joint, individual erosions were totaled, up to a maximum of 5 (Fig. 1). For JSN, a normal joint space was scored 0. A score of 2 indicated focal narrowing of the joint. A score of 1 was not used when the reviewer was unsure whether there was joint space narrowing. A generalized narrowing leaving >50% of the original joint space present was scored as 2. A generalized narrowing leaving <50% of the original joint space present was scored as 3. A subluxation of the joint was also scored as 3. A bony ankylosis or a complete luxation of the joint was scored as 4 (Fig. 1). The maximum erosion score of the hands and wrists was 160 and that of the feet was 120 (maximum total erosion score, 280). Accordingly, the maximum JSN score of the hands and wrists was 120 and that of the feet was 48 (maximum total JSN score, 168). The sum of the erosion and JSN scores was the total SHS (maximum, 448). Radiographic joint destruction was quantified as the total SHS score divided by the duration of RA.

To avoid bias in the RA duration, radiographic joint destruction was quantified as the total SHS score divided by the duration of RA. A previous study defined the remission



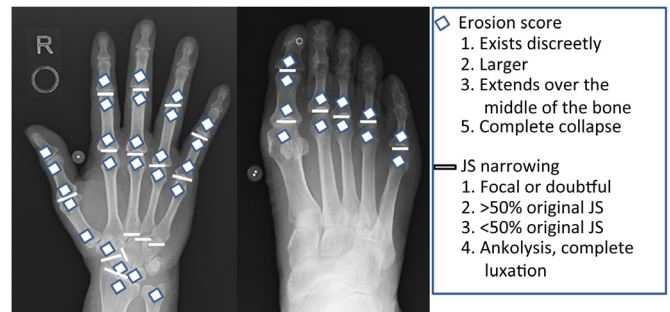Figure 1. Sharp/van der Heijde score scoring system. JS, joint space.

of joint destruction as changes from baseline of <0.5 points per year and rapid joint destruction as changes from baseline to >5 points per year (21). However, the SHS score change from baseline because was not determined as radiographical data was collected at the time of blood sample collection. The ATTRACT trial showed that an SHS of >90 points indicated a high degree of joint destruction within 3 years of RA diagnosis (22). Furthermore, the patient populations in the present study with high disease activity based on DAS 28 (C-reactive protein) >5.1 (15.6%) and populations with over 50 points SHS/year (13.2%) were similar to that observed in the ATTRACT trial. Therefore, >50 points of SHS/year was defined as rapid joint destruction. Patients were assigned to either the rapid joint destruction group (defined as total SHS/year ≥50) or the slow joint destruction group (defined by a total SHS/year <50).

*Genome-wide SNP analysis and association study.* Samples of 7 ml venous blood were drawn into glass tubes and stored at 4°C until required for DNA extraction at Mitsubishi BCL, Inc. DNA samples were prepared using a Gentra Puregene DNA isolation kit according to the manufacturer's protocol (cat. no. 158489; Qiagen, GmbH). DNA integrity was assessed using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Inc.) to determinate the OD260/280 ratio (acceptable range, 1.82-1.92) and the concentration of the total DNA (acceptable range, 8-28 $\mu$g/ml), and all the samples were deemed to be of sufficient quality (2.0>OD260/280>1.8; DNA concentration >5 $\mu$g/ml). Furthermore, the integrity of the DNA was assessed by loading ~100 ng per sample on a 0.75% agarose gel and comparing separation of the full length DNA band.

Genome-wide SNP genotyping was performed by deCode Genetics Inc. using the Illumina HumanHap300K chip technology (Illumina, Inc.). The genotyping of 317,503 SNPs was performed, including a quality control analysis. SNP genotyping was performed using an Infinium OmniExpressExome-8 Bead Chip kit (cat. no. 20024676; Illumina, Inc.) according to the manufacturer's protocol. SNP genotyping calling and quality control for samples and SNPs were performed using Illumina GenomeStudio version 2011 (Illumina, Inc.) and a cluster file. Genotypes were scored using GenomeStudio using a GenCall threshold of 0.15. Samples were accepted when their call rates were >98%. SNPs were excluded if they: i) Had an R mean value in at least 1 of 3 clusters <0.25; ii) Cluster Sep values <0.4; or iii) the number of no calls value was >2 on all chromosomes except for the Y chromosome.

Table I. Clinicopathological characteristics.

| Factor | TSS ≥50, n=30 | TSS <50, n=198 | P-value |
|---|---|---|---|
| Sex, N (%) | | | |
| Male | 4 (13) | 45 (22.8) | |
| Female | 26 (87) | 153 (77.2) | 0.243 |
| Age, years | 58.1±12.5 | 54.1±13.0 | 0.227 |
| RA duration (years) | 1.4±1.1 | 3.6±1.2 | <0.001[c] |
| IgM RF titer, IU/ml | 62.6±65.8 | 63.1±60.9 | 0.992 |
| ACPA titer, IU/ml | 81.3±66.0 | 83.9±69.2 | 0.982 |
| Stage, N (%) | | | |
| 1 | 2 (6.7) | 35 (17.7) | 0.127 |
| 2 | 6 (20.0) | 72 (36.4) | 0.078 |
| 3 | 12 (40.0) | 56 (28.3) | 0.196 |
| 4 | 10 (33.3) | 35 (17.7) | 0.045[a] |
| Class (%) | | | |
| 1 | 4 (13.3) | 61 (16.7) | 0.143 |
| 2 | 17 (56.7) | 115 (58.0) | 0.884 |
| 3 | 9 (4.3) | 22 (10.1) | 0.005[b] |
| 4 | 0 (0.0) | 0 (0.0) | 1 |
| DAS 28, C-reactive protein | 4.1±1.9 | 4.6±1.6 | 0.298 |
| Total sharp score/year | 101.3±69.6 | 20.3±18.3 | <0.001[c] |

[a]P<0.05, [b]P<0.01, [c]P<0.001. RA, rheumatoid arthritis; ACPA, anti-citrullinated protein antibodies; TSS, total SHS score/year.

Table II. Patients characteristics of the second cohort of 240 samples.

| Factor | TSS ≥50, n=32 | TSS <50, n=208 | P-value |
|---|---|---|---|
| Sex, N (%) | | | |
| Male | 7 (21.9) | 38 (18.3) | |
| Female | 25 (78.1) | 170 (81.7) | 0.306 |
| Age, years | 57.5±16.0 | 61.0±12.4 | 0.316 |
| RA duration (years) | 1.4±1.1 | 2.2±1.6 | 0.014[a] |
| IgM RF titer, IU/ml | 60.1±70.1 | 68.3±64.2 | 0.919 |
| ACPA titer, IU/ml | 86.1±60.9 | 79.1±61.8 | 0.816 |
| Stage, N (%) | | | |
| 1 | 2 (6.2) | 36, 17.3% | 0.111 |
| 2 | 4 (12.5) | 80, 38.5% | 0.004[b] |
| 3 | 15 (46.9) | 61, 29.3% | 0.047[a] |
| 4 | 11 (34.4) | 31, 14.9% | 0.007[b] |
| Class (%) | | | |
| 1 | 5 (15.6) | 22 (10.6) | 0.143 |
| 2 | 20 (62.5) | 149 (71.6) | 0.15 |
| 3 | 5 (15.6) | 37 (17.8) | 0.982 |
| 4 | 0 (0.0) | 0 (0.0) | 1 |
| DAS 28, C-reactive protein | 4.1±1.9 | 4.7±1.6 | 0.251 |
| Total sharp score/year | 115.6±59.1 | 19.4±11.4 | <0.001[c] |

[a]P<0.05, [b]P<0.01, [c]P<0.001. RA, rheumatoid arthritis; ACPA, anti-citrullinated protein antibodies; TSS, total SHS score/year.

After exclusion based on these criteria, 278,347 SNPs were retained in the case-control analysis.

*Statistical analysis.* Demographic and clinicopathological characteristics of patients assigned to the rapid and slow joint destruction groups were compared using a one-way ANOVA for continuous variables (such as age and RA duration). Bonferroni's correction was used for multiple comparisons. A contingency table for categorical data (Steinbrocker stage and class) was analyzed using a $\chi^2$ test. The frequency of minor allele homo combinations of SNPs were compared between the rapid and slow joint destruction groups for 277,339 SNPs using a Fisher's exact test in Plink (23). The frequency of the minor allele homo combinations between the rapid and slow joint destruction groups for another 240 samples using the genotyping data of 178,748 SNPs to confirm the results obtained for the 228 samples using genotyping data of 317,503 SNPs. P<0.05 was considered to indicate a statistically significant difference.

**Results**

*Patient characteristics.* Among the 228 patients enrolled in the study, 30 patients were included in the rapid joint destruction group (group A), and 198 patients were classified into the slow joint destruction group (group B). There was no significant difference in the distribution of sex, age, titer of IgM RF, titer of ACPA and DAS28 (C-reactive protein) between groups A and B (Table I). The RA duration, number of patients classed as Steinbrocker 3, and total SHS score/year (TSS) were significantly higher in group A compared with group B. Amongst the other 240 samples enrolled in the study, 32 patients were included in the rapid joint destruction group (group C), and 204 patients were classified into the slow joint destruction group (group D). The RA duration, number of patients classed as Steinbrocker 2, 3 or 4, as well as TSS were significantly higher in group C compared with group D (Table II). The patient's characteristics were similar between the two sets of patients.

*Analysis of genetic association with joint destruction.* Several SNPs were identified that were strongly associated with rapid radiographic joint destruction (Figs. 2 and 3). Using a Manhattan plot. SNPs that were significantly different between the rapid and slow joint destruction groups were identified; all of which had a different chromosomal locus (Figs. 2 and 3). A focus was placed on SNPs with smaller P-values <1x10⁻⁶. The selected SNPs, shown in Table III, were as follows: one minor allele homo SNP with a P-value difference between groups A and B of 1x10⁻⁷·²⁴ (rs2295926 located on chromosome 9; gene symbol name, *GALNT12*); gene location, intron), and another minor allele homo SNP with a P-value difference of 1x10⁻⁶·⁶⁴ (rs11958855 located on chromosome 5; gene symbol name, *KCNN2*; gene location, intron). The same minor allele homo
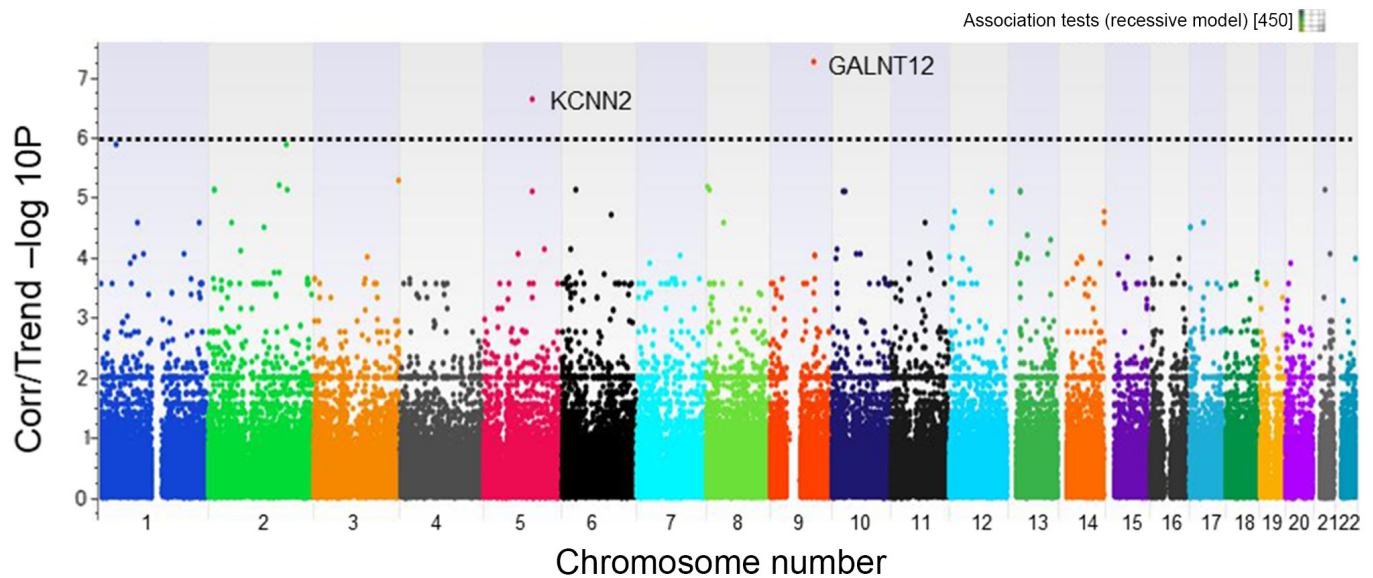
Figure 2. Manhattan plot, showing the difference in SNPs between the rapid (total SHS score/year, ≥50) and slow (total SHS score/year, <50) joint destruction groups. Dotted line indicates thresholds for significance (P<1x10⁻⁶). X-axis, chromosome number. Y-axis, $\log_{10}^{-P}$. SHS, Sharp/van der Heijde score; SNP, single nucleotide polymorphism.
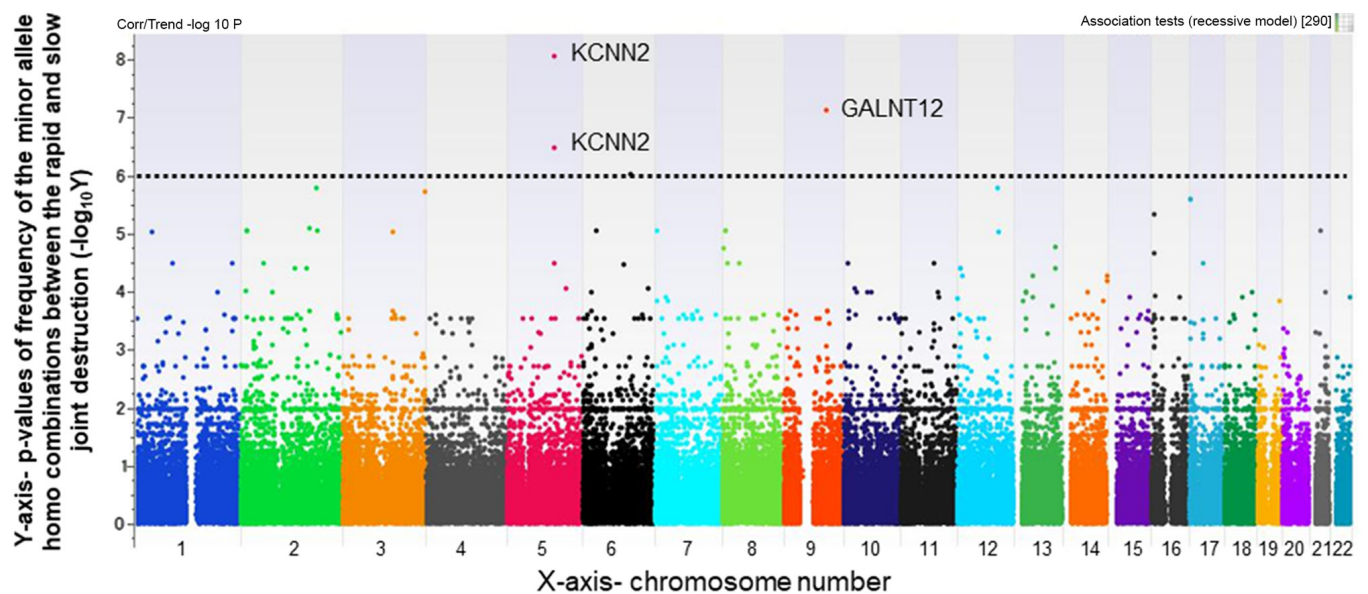


Figure 3. Manhattan plot of the 240 samples, showing the difference in SNPs between the rapid (total SHS score/year, ≥50) and slow (total SHS score/year, <50) joint destruction groups. Dotted line indicates thresholds for significance (P<1x10⁻⁶). X-axis, chromosome number. Y-axis, $\log_{10}^{-P}$. SHS, Sharp/van der Heijde score; SNP, single nucleotide polymorphism.

SNPs rs2295926 (*GALNT12*) and two SNPs of rs11958855 and rs36963 in *KCNN2* gene were detected between the groups C and D (rs2295926; P=1x10⁻⁷·¹¹, rs11958855; P=1x10⁻⁸·⁰⁵, rs36963; P=1x10⁻⁶·⁴⁶) in the genotyping results of 178,748 SNPs (Table IV).

**Discussion**

In the present study, various SNPs were first identified as novel risk loci which may be used to predict susceptibility to joint destruction in patients with RA. The risk loci identified were rs2295926, an intronic SNP of the *GALNT12* gene located

on chromosome 9, and rs11958855, an intronic SNP of the *KCNN2* gene located on chromosome 5.

*GALNT12* belongs to a family of hexosyltransferases, which are involved in the initial steps of the mucin-type O-glycosylation (24). Activity of these enzymes can lead to aberrant glycosylation, and this is associated with alterations in cell growth, differentiation, transformation, adhesion, metastasis and immune surveillance in several types of cancer (25). GALNT12 expression is upregulated in the digestive tract (24), and is frequently downregulated in colorectal cancer (26). Glycosaminoglycans are proteoglycans that are involved in the regulation of the diffusion of growth factors, and can also bind

Table III. Single nucleotide polymorphisms associated with rapid joint destruction in the first cohort of 228 patients.

| -Log$_{10}$(P-value) | Name | Chromosome | Gene symbol | Gene description | Gene location |
|---|---|---|---|---|---|
| 7.24 | rs2295926 | 9 | GALNT12 | Polypeptide N-acetylgalactosaminyltransferase 12 | Intron |
| 6.64 | rs11958855 | 5 | KCNN2 | Potassium calcium-activated channel subfamily N member 2 | Intron |
| 5.88 | rs4266846 | 1 | LOC105378657 | Uncharacterized LOC105378657 | Non-coding RNA |
| 5.88 | rs13029379 | 2 | SCHLAP1 | SWI/SNF complex antagonist associated with prostate cancer 1 | Intron |
| 5.28 | rs7629215 | 3 | LOC105374287 | Uncharacterized LOC105374287 | Non-coding RNA |
| 5.19 | rs355808 | 2 | COBLL1 | Cordon-blue WH2 repeat protein like 1 | Intron |
| 5.18 | rs7357519 | 8 | CSMD1 | CUB and Sushi multiple domains 1 | Intron |
| 5.11 | rs4669995 | 2 | TRIB2/FAM84A | Tribbles homolog 2 (Drosophila)/ family with sequence similarity 84, member A | Intergenic |
| 5.11 | rs10181834 | 2 | TRIB2/FAM84A | Tribbles homolog 2 (Drosophila)/ family with sequence similarity 84, member A | Intergenic |
| 5.11 | rs833126 | 2 | PDE1A | Phosphodiesterase 1A | Intron |

Table IV. Single nucleotide polymorphisms associated with rapid joint destruction in the second cohort of 240 patients.

| -Log$_{10}$(P-value) | Name | Chromosome | Gene symbol | Gene description | Gene location |
|---|---|---|---|---|---|
| 8.05 | rs11958855 | 5 | KCNN2 | Potassium calcium-activated channel subfamily N member 2 | Intron |
| 7.11 | rs2295926 | 9 | GALNT12 | Polypeptide N-acetylgalactosaminyltransferase 12 | Intron |
| 6.46 | rs36963 | 5 | KCNN2 | Potassium calcium-activated channel subfamily N member 2 | Intron |
| 6.02 | rs1539403 | 6 | LOC100128588/ RFPL4B | Uncharacterized LOC100128588/ PIN2/TERF1 interacting, telomerase inhibitor 1 pseudogene/ret finger protein-like 4B | Intergenic |
| 5.77 | rs13029379 | 2 | SCHLAP1 | SWI/SNF complex antagonist associated with prostate cancer 1 | Intron |
| 5.77 | rs1582341 | 12 | NEDD1/RMST | Neural precursor cell expressed, developmentally down-regulated 1/ rhabdomyosarcoma 2 associated transcript (non-protein coding) | Intergenic |
| 5.71 | rs7629215 | 3 | LOC105374287 | Uncharacterized LOC105374287 | Non-coding RNA |
| 5.57 | rs2644714 | 17 | VPS53/FAM57A | Vacuolar protein sorting 53 homolog (S. cerevisiae)/ family with sequence similarity 57, member A | Intergenic |
| 5.57 | rs2295479 | 17 | TLCD3A | TLC domain containing 3A | Intron |
| 5.33 | rs12447219 | 16 | RBFOX1 | RNA binding fox-1 homolog 1 | Intron |

different functional proteins, such as fibroblast growth factors and hedgehog proteins, thereby modulating various signaling pathways (27,28). Mucin-type O-glycosylation consists of glycans attached via O-linked *N*-acetyl-D-galactosamine

to serine and threonine residues, and it is one of the most abundant types of protein glycosylation in animals, where it is controlled by a large family of GALNT genes (29). GALNT12 and GALNT3 regulate mucin-type O-glycosylation (24). Therefore, GALNT12 may serve a role in cartilage homeostasis, and the rs2295926 SNP may serve a significant role in rapid joint destruction in patients with RA.

The SNP rs11958855 was identified as having a genome-wide significant association with rapid joint destruction in RA. rs11958855 is an intergenic SNP of the *KCNN2* gene located on chromosome 5. KCNN2 encodes an integral membrane protein that forms small conductance calcium-activated potassium (SK) channels. SK2 channels are voltage-independent $Ca^{2+}$-activated $K^+$ channels. In neurons of the central nervous system, activation of these channels modulates neuronal excitability by hyperpolarizing the membrane (30). Regarding cartilage homeostasis, low conductance-potassium calcium-activated channel transcript subtype SK1 (KCNN1, KCa2.1) and SK3 (KCNN3, KCa2.3) and intermediate potassium calcium-activated channel transcript subtypes (IK, KCNN4, SK4 and KCa3.1) have been shown to be expressed in OUMS-27 cells (a chondrosarcoma cell line). SK channels have been proposed to be involved in the response to osmotic change in chondrocytes (31). SK channels also provide a direct link between inflammation and chondrocyte function (31). SK2 was not expressed in normal cartilage tissues (31), but it may be expressed and have a function related to inflammation in cartilage tissues of patients with RA.

The present study has some limitations. First, the actual effects of these genetic variants were not assessed *in vitro* or *in vivo*. Further studies are necessary to fully elucidate the effects of these and other SNPs. Second, SHS was assessed by a single examiner, which may have led to a potential bias. The cohorts were comprised entirely of Japanese patients; SNPs may differ in subjects of different ethnicities. Finally, the SHS score change from the baseline was not evaluated as only radiographical data was collected at the time of blood sample collection; however, previous reports have defined rapid joint destruction as changes from baseline of >5 points per year.

In conclusion, the rs2295926 SNP variant in the GALNT12 gene and rs11958855 in the *KCNN2* gene may be associated with rapid joint destruction amongst Japanese patients with RA. At present, there are no means of managing RA with the aim of reducing joint destruction. Therefore, the identification of genetic predictors of rapid joint destruction in RA (*GALNT12* and *KCNN2*) may highlight potentially novel therapeutic targets to improve management of disease progression, thereby improving the quality of life of patients.

## Availability of data and materials

The datasets generated and/or analyzed during the present study are not publicly available due to an application for a patent in Japan (patent no. 4869834, registration date Nov 25, 2011) but are available from the corresponding author upon reasonable request.

## Authors' contributions

SH, RK and TsM designed the study. All authors were involved in drafting and revising the manuscript. KoF, SH, KeF, ToM, TK, MH and YT participated in data collection. SH, TsM and KeF confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

The methods used in the present study were approved by the Research Institute of Joint Disease Kobe on June 11, 2008. All patients provided written informed consent for participation.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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