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ORIGINAL ARTICLE

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The alpha 2 type IX collagen gene tryptophan polymorphism is not associated with rheumatoid arthritis in the Japanese population

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Abstract The aim of this study was to investigate whether the alpha 2 type IX collagen (COL9A2) polymorphism that introduces tryptophan residue into the collagen triple-helix is a marker of susceptibility to, or severity of, rheumatoid arthritis (RA). The study included 749 Japanese patients with RA. One hundred twenty-four unrelated healthy individuals served as the control subjects. The relationship between the COL9A2 gene polymorphism and clinical manifestations of RA was evaluated. For the number of subjects positive for *COL9A2* tryptophan polymorphism, there was no statistically significant difference between RA patients and normal controls. Furthermore, we did not detect any association of *COL9A2* tryptophan polymorphism with disease status, least erosive subset, more erosive subset, or mutilating disease. The lack of association of *COL9A2* tryptophan polymorphism with RA and the clinical findings in our study implies that the polymorphism may not function as a candidate gene marker for screening RA patients.

Keywords Polymorphism · Rheumatoid arthritis · Severity · Susceptibility · Type IX collagen

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Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease affecting about 1% of the population [1]. Environmental and genetic factors are thought to be involved in the onset of the disease. However, its genetic basis is largely unknown. Despite that the human leukocyte antigen (HLA) gene region has been shown significant association with RA, HLA association probably accounts for only one third of the overall genetic contribution [2]. This suggests that non-HLA genes are also involved in disease pathogenesis.

Type IX collagen, a heterotrimer of genetically distinct alpha 1(IX), alpha 2(IX), and alpha 3(IX) chains, is a structural component of the extracellular matrix of hyaline cartilage and functions as an interfacial protein through covalent cross-linkage to the surface of type II collagen fibrils [3]. Accumulating evidences have indicated that type IX collagen is important for the maintenance and longevity of the cartilaginous tissues in the locomotory system. Mutations in the collagen genes cause early development of osteoarthritis in humans [4] and progressive degeneration of articular cartilage and intervertebral discs in mice [5, 6]. In a Finnish population, polymorphic variants of type IX collagen genes that encode tryptophan at position 326 of the alpha 2(IX) chain (Trp2) or at position 103 of the alpha 3(IX) chain (Trp3) were linked to an increased risk of lumbar disc disease and chronic sciatica [7, 8]. Recent analysis suggested that Trp2 and Trp3 polymorphisms predispose carriers to the development of symptomatic spinal stenosis associated with spondylolisthesis [9].

Given the critical roles in cartilage biology and pathology, polymorphisms in the genes for type IX collagen are good candidates for arthritic diseases. Indeed, alpha 2 type IX collagen (COL9A2) gene Trp2 polymorphism, which is located at 1p32.2–33, has been extensively analyzed in the Japanese osteoarthritis patients [10]. However, it is unclear whether the Trp2 polymorphism is a marker of susceptibility to, or severity of, RA. Therefore, we have performed

association analysis of the COL9A2 gene Trp2 polymorphism with RA in the Japanese population.

Materials and methods

Subjects for association study Seven hundred forty-nine unrelated Japanese patients (155 males and 594 females) with RA and 124 unrelated Japanese healthy controls (23 males and 101 females) were studied (Table 1) [11]. All RA patients satisfied the American College of Rheumatology revised criteria for the classification of RA [12], with a mean disease duration of 13.6 years (3 to 54). We obtained clinical information including age at onset, family history, and distribution of affected joints. Based on the extent of joint destruction evident on plain radiograms, the disease status of RA was classified into three subsets [least erosive subset (LES) n=418, more erosive subset (MES) n=275, or mutilating disease (MUD) n=56] using the criteria described by Ochi et al. [13]. Control samples were taken from a pool of healthy Japanese individuals with no medical history of RA. Genomic DNA was prepared from peripheral blood samples that were obtained from all participants after receiving written informed consent, as approved by the ethical committee of Tokushima University.

Polymerase chain reaction (PCR) Primers for DNA amplification and sequencing were designed manually, according to the genomic sequence flanking exon 19 of the COL9A2 gene [14]. The sequences were forward 5′-GATTCTAGACACCAAGAGCC-3′ and reverse 5′-GATTCTAACCTCATCAGCCAC-3′. The 20 μl of reaction mixture contained genomic DNA (10 ng), standard polymerase chain reaction (PCR) buffer, dNTPs (2 nM each), Ampli *Taq* Gold (0.1 μl), and the primer pair (10 μM each). PCRs were performed in a GeneAmp PCR system 9700 (Applied Biosystems, Foster City, CA) with an initial denaturation at 95°C for 10 min, followed by 35 cycles at 95°C for 30 s, 56°C for 10 s and 72°C for 30 s, and a final step at 72°C for 5 min.

Direct sequencing and allele identification of Trp2 Five microliters of PCR products was treated with 2 units of shrimp alkaline phosphatase and 10 units of exonuclease I (Amersham, Buckinghamshire, UK) at 37°C for 15 min, followed by incubation at 80°C for 15 min for enzyme inactivation. Sequencing reactions were performed using ABI Prism BigDye Terminator kit (Applied Biosystems) in a GeneAmp PCR system 9700 with an initial denaturation at 96°C for 5 min, followed by 25 cycles at 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. Products were purified using Sephadex G-50 Fine (Amersham) and MultiScreen-PCR filter plate (Millipore, Bedford, MA). Purified products were analyzed on an ABI Prism 3100 multicapillary sequencer (Applied Biosystems).

Table 1 Features of patients and controls

	Patients (n=749)	Controls (n=124)
Mean age	61.8 (16–92)	58.9 (29–97)
Female (%)	79.3	81.5
Race, Japanese (%)	100	100

Table 2 Distribution of the alpha 2 type IX collagen gene tryptophan polymorphism (Trp2) between patients and controls

	Total	Trp2(+)	Trp2(−)	P value	Odds ratio	95% CI
Patients	749	169 (22.5)	580 (77.5)	0.69[a]	1.10[b]	0.69–1.75
Controls	124	26 (21.0)	98 (79.0)			

[a]Chi-squared test, α=0.05
[b]Unadjusted odds ratio calculated for a 2×2 table for case and Trp2 allele (exposure), α=0.05
CI Confidence interval

Statistical analysis The patients and controls were divided into two groups: (1) positive for 1 or 2 Trp2 allele [Trp2(+) group] and (2) no Trp2 allele [Trp2(−) group]. Tests of Hardy–Weinberg equilibrium (HWE) were carried out among cases and controls, separately. Comparison of the frequency of the Trp2(+) subjects was performed using 2×2 contingency table by chi-squared test. Unadjusted odds ratios and 95% confidence interval were calculated for the presence of Trp2(+) allele.

Results

Allele frequencies of Trp2 polymorphism were in Hardy–Weinberg equilibrium in both RA patients and controls (p>0.05). Among patients, 155 out of 749 (20.7%) were heterozygote for Trp2 allele, and 14 (1.8%) were homozygote. Among controls, 25 out of 124 (20.2%) were heterozygote for Trp2 allele, and 1 (0.8%) was homozygote. Although the RA patients had higher number of Trp2(+) subjects with odds ratio of 1.10, there was no significant difference between patients and controls (Table 2).

Next, we analyzed RA patients by categorization (Table 3). Categorizations by gender and age at onset showed no significant differences. When the patients were categorized into family history positive and family history negative subgroups, odds ratio (family history positive) reads 1.36, which was not statistically significant. Categorization by disease status did not show significant differences, with slightly higher odds ratio of 1.28 in MUD.

Discussion

In the most extensive analysis to date on the role of COL9A2 gene Trp2 polymorphism in the susceptibility to, or severity of, RA, we found negative association. Previous studies of the COL9A2 gene Trp2 polymorphism have primarily focused on investigation of association with lumbar disc diseases or osteoarthritis [8–10]. As far as we

Table 3 Distribution of the alpha 2 type IX collagen gene tryptophan polymorphism (Trp2) according to selected characteristics of interest of the patients

Characteristics	Total	Trp2(+)	Trp2(−)	*P* value[a]	Odds ratio[b]	95% CI
Gender						
Female	594	136 (22.9)	458 (77.1)	0.67	1.10	0.71–1.69
Male	155	33 (21.3)	122 (78.7)			
Age at onset						
>40	574	132 (22.3)	442 (77.7)	0.66	1.10	0.73–1.66
< or = 40	173	37 (21.3)	136 (78.7)			
Family history						
Positive	132	36 (27.3)	96 (72.7)	0.15	1.36	0.89–2.09
Negative	617	133 (21.6)	484 (78.4)			
Disease status						
MUD	56	15 (26.8)	41 (76.3)	0.43	1.28	0.69–2.38
MES	275	55 (20.0)	220 (80.0)	0.20	0.79	0.55–1.14
LES	418	99 (23.7)	319 (76.3)	0.41	1.16	0.82–1.64

[a]Chi-squared test, α=0.05
[b]Unadjusted odds ratios calculated for a 2×2 table for each risk factor (case) and Trp2 allele (exposure), α=0.05
CI Confidence interval, *MUD* mutilating disease, *MES* more erosive subset, *LES* less erosive subset

know, this is the first report attempting to determine whether Trp2 polymorphism might usefully predict RA outcome.

A number of methodological issues need to be considered in interpreting these data. We classified RA patients into three disease subsets according to the number of joints with erosion and their distribution [13]. The LES patients had less than 20 joint erosions in the peripheral small joints. The MES patients had more than 20 joint erosions including the central large joints. The MUD had rapid joint involvement in almost all (more than 50 out of 68) joints. Disease status evaluated at the time of peripheral blood collection might be hazardous, especially in the patients with shorter disease duration, given the changing nature of RA as it evolves. Furthermore, usage of, or responsiveness against, disease-modifying antirheumatic drugs (DMARDs) may vary among patients, which might mask the disease status in the natural course. With these limitations, Trp2 polymorphism was not associated with the disease status in our study. It is not clear whether the polymorphism is related to disease activity or degrees of joint destruction.

The functional impact of the COL9A2 gene Trp2 polymorphism is not known to date. However, it is quite unusual to have a bulky tryptophan residue in the collagen triple helix. Regarding the matrix deposition of COL9A2 tryptophan-containing allelic variant of type IX collagen in the cartilage extracellular matrix [15], it is plausible to speculate that Trp2(+) individuals may have deficient collagen triple helix susceptible to degradation. The association of degenerative lumbar disc diseases and COL9A2 gene Trp2 polymorphism strongly suggests this possibility. Although the structure of type IX collagen is different between intervertebral disc and articular cartilage [16], Trp2(+) individuals may be susceptible to inflammatory joint destruction in some conditions.

RA is apparently a multifactorial disease, and the distribution of each factor may be weak. In conclusion, the negative correlation between COL9A2 gene Trp2 polymorphism and RA shown in the present study implies that this polymorphism may not function as a candidate gene marker for screening RA patients.

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