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원 저

Response to Bee Venom Acupuncture and Polymorphism of Interleukin 4 Receptor and Interleukin 10 in Rheumatoid Arthritis

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Objectives : We investigated to find the relationship between single-nucleotide polymorphism (SNP) of IL4R, IL-10 and bee venom therapy efficacy in patients with RA treated with bee venom for 8 weeks.

Methods : Korean RA patients (n=114) and healthy subjects (n=109) were included in this prospective study. Korean bee venom was dissolved in saline (diluted 1:3000) and administrated into acupuncture points. Bee venom therapy was applied twice a week and continued for 8 weeks. The clinical response was evaluated using various assessments before and after treatment. Disease severity was measured by determining the number of tender joints and swollen joints. Laboratory studies included ESR, CRP, and rheumatoid factor. Genotyping for IL-4R and IL-10 polymorphism was done by pyrosequencing analysis.

Results : 1. In IL4R genotypes, there was significant difference between RA patients and controls group.

2. In IL4R genotypes, there was significant difference among Good, Mild and Bad responders to in RA patients, but in the frequency of alleles and carriers, there were no significant difference.

3. There was no significant difference between RA patients and controls group in IL-10 gene genotypes.

4. In IL-10 genotypes, there was no significant difference among Good, Mild and Bad responders to in RA patients.

5. There was no significant difference in the improvement of ESR, CRP and KHAQ scores after bee venom therapy in RA patients among the IL4R or IL-10 genotypes.

Conclusions : In IL-4R genotypes, there was significant difference between RA patients and control group, and among Good, Mild and Bad responders in RA patients. However, in IL-10 genotypes, there was no significant difference between RA patients and controls group and among Good, Mild and Bad responders in RA patients.

Key Words: Single-nucleotide polymorphism (SNP), IL4R (interleukin 4 receptor), IL-10 (interleukin 10), Rheumatoid Arthritis

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I. Introduction

Scientific research on bee venom acupuncture(BVA) for the treatment of arthritic and other inflammatory diseases began in the late 20th century[1,2]. In modern Oriental Medicine BVA is being widely used for herbal-acupuncture to relieve pain and to cure inflammatory disease such as rheumatoid arthritis(RA), osteoarthritis and gout. The chemistry and pharmacology of bee venom(BV) was already verified[3,4]. and now the anti-nociceptive and anti-inflammatory effect of BV is being demonstrated[5].

RA is one of systemic inflammatory disease characterized by chronic and progressive joint destruction. There are pro- inflammatory cytokines, such as tumour necrosis factor(TNF) and interleukin 1(IL-1) and anti-inflammatory cytokines, such as interleukin 10(IL-10) and interleukin 4(IL-4) in RA[6-9].

So far many kinds of anti-rheumatic drugs have been developed, but they have many side-effects. So as a complementary medicine, BVA has been utilized to treat inflammation of RA[10]. But, although BVA has anti-inflammatory effect, all RA patients doesn' t respond to the BVA. And some patients afflict themselves with side-effects such as edema and itching.

It would be very useful to predict which patients will have good improvement or not with BVA. But there is few data so that we predict the response to BVA treatment.

In the present study, we investigated the relationship between single-nucleotide polymorphism(SNP) of IL4R, IL-10 and BVA efficacy in patients with RA treated with BVA for 8 weeks.

II. Patients and methods

1. Controls

The control group consisted of apparently healthy Koreans. Controls were selected from healthy subjects who underwent the health examinations from May 2002 to April 2003. They were age and sex matched with the patients.

2. Patients

RA patients requiring BVA were included in this study. All of them were enrolled in the department of Acupuncture & Moxibustion at Oriental Medical hospital of Kyung Hee medical center in Seoul from May 2002 to April 2003. All patients fulfilled the American College of Rheumatology 1987 revised criteria for RA, and all had evidence of active disease despite conventional anti-rheumatic drugs treatment for at least 3 months. None of the patients had a history of chronic infectious disease, neoplasia, multiple sclerosis, or uncontrolled renal, hepatic, hematologic or cardiac disease.

3. BVA treatment

BV (Apis mellifera)[11] from Korea was dissolved in saline (diluted 1:3000) and intradermally administrated into acupuncture points. Selection of acupuncture points was based on the local points around the inflammed joints. A volume of 0.1ml of BV was injected into each acupoint and the total injected volume/patient did not exceed 1ml. BVA was applied twice a week and continued for 8 weeks. Previous conventional medication was permitted without drug change during BVA.

4. Evaluation of clinical response

The clinical response was evaluated using various assessments before and after BVA. Disease severity was measured by determining the number of tender joints and swollen joints. The number of tender joints was assessed by scoring pressure and joint motion, collapsing the various types of tenderness into a single tender-versus-nontender dichotomy for each joint, then counting the number of tender joints for each patient. The number of swollen joints was assessed by classifying 68 joints in each patient as either swollen or not swollen, then computing

the number of swollen joints for each patient. Morning stiffness was measured how long patient feel stiffness in and around the joints before maximal improvement. Patient' s assessment of RA-related pain was determined using a 100 mm visual analog scale(VAS), where 0 represented no pain and 100 represented severe pain. Health status was also assessed using a Korean language version of the HAQ disability index(KHAQ), which consists of 20 questions concerning activities of daily living and mobility that were aggregated to score a single index of disability. Responses to each question were scaled from 0 (no difficulty) to 3 (unable to do), then aggregated to produce a single index of disability that was also scored on a scale from 0 (no difficulty) to 3 (unable to do). Laboratory studies included erythrocyte sedimentation rate(ESR), C-reactive protein(CRP), and rheumatoid factor(RF).

5. Genotyping methodology

1) DNA samples

Blood samples were obtained from the antecubital vein without regarding to the time of the last meal. Human genomic DNA was extracted using the QIAGEN System (QIAmp DNA Blood Midi Kit, Hilden, Germany). DNA was stored at 4℃ until analyzed.

This study was approved by the ethics review committee of the Medical Research Institute, Kyung-Hee Medical Center. Informed consent was obtained from all subjects.

2) PCR amplification

A 110-bp fragment of the IL4R gene and 113-bp of the IL-10 gene were amplified using 25 ng of DNA, 5 pmol of each primer (IL4R sense 5' -GAAACCTGGGAGCAGATCCT-3' and IL4R antisense 5' -TCCACCGCATGTACAAACTC-3' , IL-10 sense 5' -GGGTAAAGGAGCCTGGAACAC-3' and IL-10 antisense 5' -GGGTGGGCTAAATATCCTCAAAGT-3'). The polymerase chain reaction (PCR) amplification was performed by using 0.5 unit Taq polymerase (HT Biotechnology Ltd., Cambridge, United Kingdom). The 25 ul of PCR reaction mixtures were 10 mM Tris-HCl, pH 9.0, 1.5 mM magnesium chloride, 50 mM potassium chloride, 0.1% Triton-X 100, 0.01 % [v/v] stabilizer, 0.25 mM of each deoxynucleotide triphosphate (dNTP), 0.1 M of each oligonucleotide primer. The PCR steps were denaturation of 5 minute at 95℃, 30 cycles of 30 seconds at 95℃, 45 seconds at 62℃, and 30 seconds at 72℃ with a Gene-Amp PCR System 9600 (Perkin-Elmer, Foster City, CA).

3) Sample preparation for pyrosequencing reactions

The antisense primer was biotinylated to allow the preparation of single-stranded DNA. The quality of PCR products was controlled by 2% of agarose gel electrophoresis. The immobilization of PCR products to streptavidin sepharose beads (Streptavidin Sepharose HP, Amersham Pharmacia Biotech, Uppsala, Sweden) was performed according to a standard protocol (Pyrosequencing AB, Uppsala, Sweden)[12]. The sequencing primers (IL4R seq 5' -CCCACCAGTGGCTAT-3' , IL-10 seq 5' -CTGGCTTCCTACAG-3') were designed so that the terminal residue hybridized to the base immediately adjacent to the A/G mutation. After immobilization of 20 ul of biotinylated PCR products onto streptavidin-coated sepharose beads by incubation at room temperature for 10 minutes, the immobilized PCR products were transferred to a Millipore 96-well filter plate (Millipore, Bedford, MA). Vacuum was used to remove the different solutions and reagents to obtain pure, single-stranded DNA while the beads remained in the wells[13].

The PCR strands were separated by incubating them with 50 ul of 0.2 M sodium hydroxide for 1 minute and washed twice with 150 ul of 10 mM Tris-acetate (pH 7.6). The beads with the immobilized template were resuspended in 55 ul of 4 M acetic acid containing 0.35 M sequencing primer IL4R seq and IL-10 seq. Then this 45 ul of suspension was transferred to a PSQ 96 plate (Pyrosequencing AB, Uppsala, Sweden)[14].

4) Pyrosequencing analysis

The PSQ 96 plate containing the samples was heated at 80℃ for 2 minutes using PSQ 96 Sample Prep Thermoplate (Pyrosequencing AB, Uppsala, Sweden) for sequencing primer annealing, and moved to room temperature for 5 minutes. Then the PSQ 96 Plate was placed into the process chamber of the PSQ 96 instrument (Pyrosequencing AB, Uppsala, Sweden). Enzymes, substrates, and nucleotides from the PSQ 96 SNP Reagent Kit (Pyrosequencing AB, Uppsala, Sweden) were dispensed from a reagent cassette into the wells, and a charge coupled device camera registered the light that was generated when a nucleotide is incorporated into a growing DNA strand[15-16]. From above process the polymorphism at position +1902 of the IL4R gene and -597 of the IL-10 gene was genotyped.

6. Statistical analysis.

To compare the distribution of the gender, genotypes and the frequency of alleles or carrier between RA patients and controls χ^2 tests was used. The odds ratios (OR) and 95% confidence intervals (CI) were used to quantify the association with RA. Statistical analysis was performed by using Statistical Package for SAS program (version 8.1.).

Before initiation of BVA treatment, the following parameters were estimated: age, rheumatoid factor, number of swollen and tender joints, duration of morning stiffness, pain intensity, KHAQ score, ESR, and serum CRP level. The results are expressed as the mean ± SD. Comparison of the parameters was performed by Student's 2-tailed t-test.

III. Results

A. IL4R polymorphism and BVA

1. Baseline characteristics of the patients and controls

The baseline characteristics of the patients are shown in Table 1. There was no significant difference between the patients and controls as for age and sex. The majority of patients were women and their mean ±SD age was 47.63 ± 9.43 years,

Before treatment RF of the patients was 124.48 ± 228.56 IU/ml. As clinical assessment swollen joints count was 4.94 ± 5.03, tender joints count was 9.61 ± 8.76, duration of morning stiffness was 132.50 ± 104.17 minutes. And pain intensity was 58.02 ± 20.47 by VAS, KHAQ score was 0.92 ± 0.57. As for biologic assessment ESR was 31.75 ± 16.67 mm/hr, serum CRP was 1.98 ± 8.05 mg/dl.

2. IL4R genotypes and BVA response

1) IL4R genotypes

The homozygote and heterozygote genotypes of IL4R gene are seen in Figure 1. The distribution of IL4R genotypes of patients were as follows: A/A, 72.81%; A/G, 26.32% and G/G, 0.88%. In contrast, genotypes of controls were A/A, 53.41%; A/G, 46.59% and G/G, 0.00%. And there was significant difference between patients and controls group (Table 2, $p<0.0001$).

Table 1. Baseline Characteristics of the IL4R Polymorphism Study Patients and Controls

	Patients (n=114)	Controls (n=88)	*p*
Demographics			
Age, years	47.63 ± 9.43	46.25 ± 15.61	0.4651
Women/Men(No.)	98 / 16	71 / 17	0.3139
Disease status			
RF(IU/ml)	124.48 ± 228.56		
Clinical assessment			
Swollen joints count	4.94 ± 5.03		
Tender joints count	9.61 ± 8.76		
Duration of morning stiffness(minutes)	132.50 ± 104.17		
Pain intensity (VAS; 0-100)	58.02 ± 20.47		
KHAQ score	0.92 ± 0.57		
Biologic assessment			
ESR(mm/hr)	31.75 ± 16.67		
Serum CRP(mg/dl)	1.98 ± 8.05		

Values are the mean ± SD. RF = rheumatoid factor; VAS = visual analog scale; KHAQ = Korean Health Assessment Questionnaire; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein.

Table 2. Correlation between Patients and Controls at Position +1902 of the IL4R Gene

	Patients n=114 (%)	Controls n=88 (%)	*p*	OR (95% CI)
Genotype				
AA	83 (72.81)	47 (53.41)	<0.0001	
AG	30 (26.32)	41 (46.59)		
GG	1 (0.88)	0 (0.00)		
Allele				
A	196 (85.96)	135 (76.70)	0.0165	1.86(1.12-3.10)
G	32 (14.04)	41 (23.30)		
Carrier				
A	113 (78.47)	88 (68.22)	0.0043	2.34(1.30-4.20)
G	31 (21.53)	41 (31.78)		

Table 3. Correlation between IL4R Gene Polymorphism and Response to BVA

	Good responders n=50 (%)	Mild responders n=49(%)	Bad responders n=15 (%)	*p*
Genotype				
AA	39 (78.00)	34 (69.39)	10 (66.67)	0.0080
AG	10 (20.00)	15 (30.61)	5 (33.33)	
GG	1 (2.00)	0 (0.00)	0 (0.00)	
Allele				
A	88 (88.00)	83 (84.69)	25 (83.33)	0.7237
G	12 (12.00)	15 (15.31)	5 (16.67)	
Carrier				
A	49 (81.67)	49 (76.56)	15 (75.00)	0.7249
G	11 (18.33)	15 (23.44)	5 (25.00)	

Data were evaluated Chi-square test. Good responders = patients whose level of ESR and CRP decreased after BVA. Mild responders = patients whose level of ESR or CRP decreased after BVA. Bad responders = patients whose level of ESR and CRP increased after BVA.

Table 4. Improvement in ESR, CRP and KHAQ with BVA and Correlation with Polymorphism at Position +1902 of the IL4R Gene

	A/A genotype (n=83)	A/G & G/G genotype (n=31)	*p*
Improvement in ESR	-0.72 ± 24.75	1.90 ± 8.79	0.405
Improvement in CRP	0.96 ± 9.57	-0.06 ± 1.10	0.341
Improvement in KHAQ	3.72 ± 7.25	1.54 ± 6.55	0.135

Values are the mean ± SD. P value was obtained by Student-t test.

The frequency of alleles of patients were as follows: A, 85.96%; G, 14.04%. In controls were A, 76.70%; G, 23.30%. And there was significant difference between patients and controls group (Table 2, p=0.0165, OR (95% CI) ; 1.86(1.12-3.10)).

The frequency of carriers of patients were as follows: A, 78.47%; G, 21.53%. In controls were A, 68.22%; G, 31.78%. And there was significant difference between patients and controls group (Table 2, p=0.0043, OR (95% CI) ; 2.34 (1.30-4.20)).

2) IL4R genotypes and BVA response

Then the patients were divided by their genotype group and response to BVA. Good responders were as patients who showed decrease of ESR and CRP, Mild responders were who showed decrease of ESR or CRP, and Bad responders were who showed increase of ESR and CRP after BVA. The distribution of IL4R genotypes of Good responders were as follows: A/A, 78.00%; A/G, 20.00% and G/G, 2.00%. Genotypes of Mild responders were A/A, 69.39%; A/G, 30.61% and G/G, 0.00%. Genotypes of Bad responders were A/A, 66.67%; A/G, 33.33% and G/G, 0.00%. And there was significant difference among the three groups (Table 3, p=0.0080).

The frequency of alleles of Good responders were as follows: A, 88.00%; G, 12.00%. In Mild responders were A, 84.69%; G, 15.31%. In Bad responders were A, 83.33%; G, 16.67%. And there was no significant difference among the three groups (Table 3, p=0.7237).

The frequency of carriers of Good responders were as follows: A, 81.67%; G, 18.33%. In Mid responders were A, 76.56%; G, 23.44%. In Bad responders were A, 75.00%; G, 25.00%. And there was no significant among the three groups (Table 3, p=0.7249).

3) IL4R genotypes and improvement scores

The improvement of ESR (before treatment - 4 weeks after) were as follows: A/A, -0.72 ± 24.75; A/G & G/G, 1.90 ± 8.79 mm/hr. And there was no significant difference between the genotypes (Table 4, p=0.405).

The improvement of CRP (before treatment - 4 weeks after) were as follows: A/A, 0.96 ± 9.57; A/G & G/G, -0.06 ± 1.10 mg/dl. And there was no significant

Table 5. Baseline Characteristics of the IL-10 Polymorphism Study Patients and Controls

	Patients (n=80)	Controls (n=109)	*p*
Demographics			
Age, years	47.06 ± 8.60	45.64 ± 15.18	0.4160
Women/Men(no)	72 / 8	87 / 22	0.0584
Disease status			
RF(IU/ml)	106.51 ± 177.40		
Clinical assessment			
Swollen joints count	5.11 ± 4.79		
Tender joints count	9.42 ± 8.37		
Duration of morning stiffness(minutes)	137.31 ± 108.15		
Pain intensity (VAS 0-100)	59.81 ± 21.84		
KHAQ score	0.98 ± 0.58		
Biologic assessment			
ESR(mm/hr)	30.71 ± 15.54		
Serum CRP(mg/dl)	1.21 ± 2.45		

Values are the mean ± SD. RF = rheumatoid factor; VAS = visual analog scale; KHAQ = Korean Health Assessment Questionnaire; ESR = erythrocyte sedimentation rate; CRP = C-reactive protein.

Table 7. Correlation between IL-10 Gene Polymorphism and Response to BVA

	Good responders n=33 (%)	Mild responders n=34 (%)	Bad responders n=13 (%)	*p*
Genotype				
AA	25 (75.76)	29 (85.29)	10 (76.92)	0.5934
AC	8 (24.24)	5 (14.71)	3 (23.08)	
Allele				
A	58 (87.88)	63 (92.65)	23 (88.46)	0.6288
C	8 (12.12)	5 (7.35)	3 (11.54)	
Carrier				
A	33 (80.49)	34 (87.18)	13 (81.25)	0.7031
C	8 (19.51)	5 (12.82)	3 (18.75)	

Data were evaluated Chi-square test.
Good responders = patients whose level of ESR and CRP decreased after BVA.
Mild responders = patients whose level of ESR or CRP decreased after BVA.
Bad responders = patients whose level of ESR and CRP increased after BVA.

difference between the genotypes (Table 4, p=0.341).

The improvement of KHAQ scores (before treatment - 4 weeks after) were as follows: A/A, 3.72 ± 7.25; A/G & G/G, 1.54 ± 6.55. And there was no significant difference between the genotypes (Table 4, p=0.135).

B. IL-10 polymorphism and BVA

Table 6. Correlation between Patients and Controls at Position -597 of the IL-10 Gene

	Patients	Controls n=80 (%)	*p* n=109 (%)	OR (95% CI)
Genotype				
AA	64 (80.00)	95 (87.16)	0.1835	1.70 (0.77-3.72)
AC	16 (20.00)	14 (12.84)		
Allele				
A	144 (90.00)	204 (93.58)	0.2035	1.62 (0.77-3.42)
C	16 (10.00)	14 (6.42)		
Carrier				
A	80 (83.33)	109 (88.62)	0.1835	0.59 (0.27-1.30)
C	16 (16.67)	14 (11.38)		

Table 8. Improvement in ESR, CRP and KHAQ with BVA and Correlation with Polymorphism at Position -597 of the IL-10 Gene

	A/A genotype (n=64)	A/C genotype (n=16)	*p*
Improvement in ESR	-2.39 ± 27.75	0.93 ± 3.85	0.3582
Improvement in CRP	-0.08 ± 3.50	-0.27 ± 1.40	0.7484
Improvement in KHAQ	3.62 ± 6.79	0.18 ± 6.80	0.0831

Values are the mean ±SD. P value was obtained by Student' s 2-tailed t-test.

1. Baseline characteristics of the patients and controls

The baseline characteristics of the patients are shown in Table 5. There was no significant difference between the patients and controls as for age and sex. The majority of patients were women and their mean ±SD age was 47.06 ± 8.60 years.

Before treatment RF of the patients was 106.51 ± 177.40 IU/ml. As clinical assessment swollen joints count was 5.11 ± 4.79, tender joints count was 9.42 ± 8.37, duration of morning stiffness was 137.31 ± 108.15 minutes. And pain intensity was 59.81 ± 21.84 by VAS, KHAQ score was 0.98 ± 0.58. As for biologic assessment ESR was 30.71 ± 15.54 mm/hr, serum CRP was 1.21 ± 2.45 mg/dl.

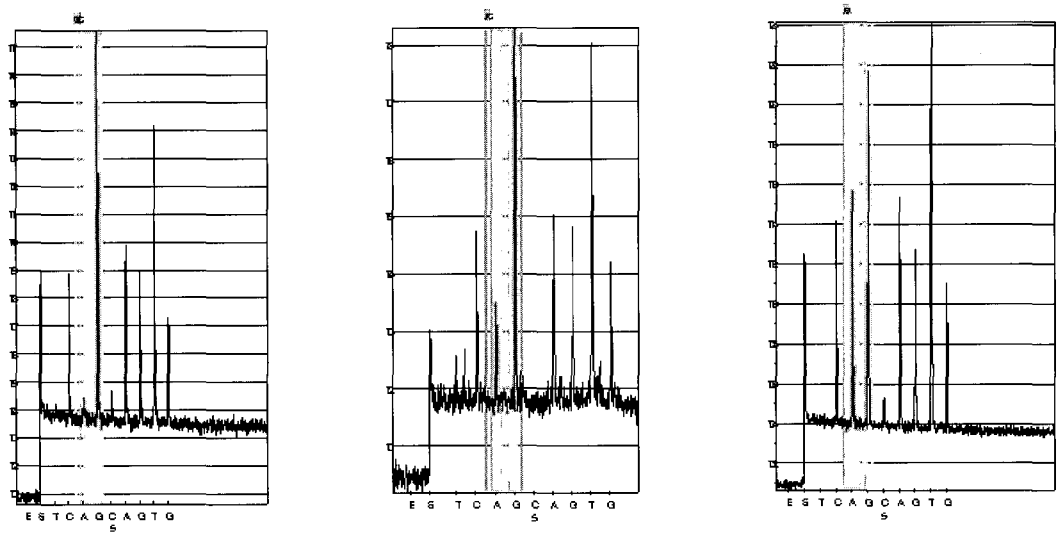

Figure 1. Pyrosequencing result of G/G, A/G and A/A genotype at position +1902 of the IL4R gene. Axes are nucleotide dispensation event (x-axis) versus observed signal intensity (y-axis).

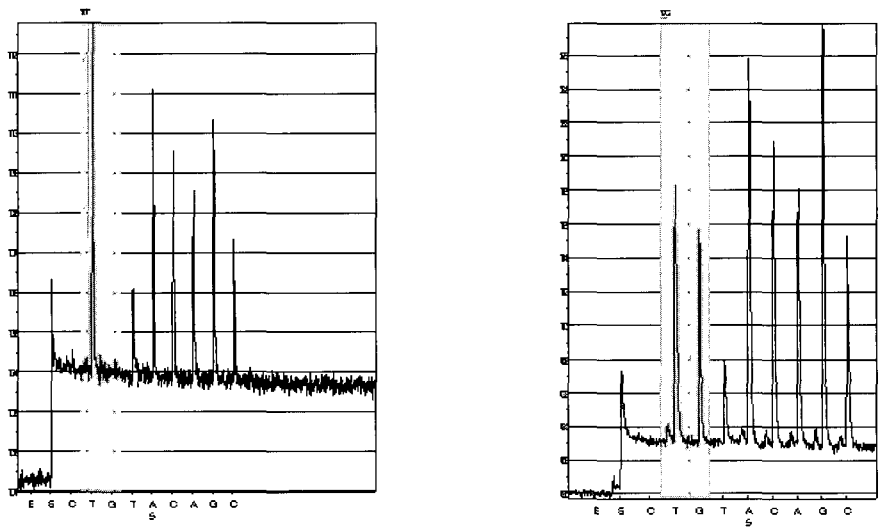

Figure 2. Pyrosequencing result of A/A and A/C genotype at position -597 of the IL-10 gene. Axes are nucleotide dispensation event (x-axis) versus observed signal intensity (y-axis).

2. IL-10 genotypes and BVA response

1) IL-10 genotypes

The homozygote and heterozygote genotypes of IL-10 gene are seen in Figure 2. The distribution of IL-10 genotypes of patients were as follows: A/A, 80.00%, A/C, 20.00%. In contrast, genotypes of controls were A/A, 87.16%, A/C, 12.84%. There was no significant difference between patients and controls group (Table 6, p=0.1835, OR (95% CI) ; 1.70 (0.77-3.72)).

The frequency of alleles of patients were as follows: A, 90.00%, C, 10.00%. In controls were A, 93.58%, C, 6.42%. And there was significant difference between

patients and controls group (Table 6, p=0.2035, OR (95% CI) ; 1.62 (0.77-3.42)).

The frequency of carriers of patients were as follows: A, 83.33%, C, 16.67%. In controls were A, 88.62%, C, 11.38%. And there was significant difference between patients and controls group (Table 6, p=0.1835, OR (95% CI) ; 0.59 (0.27-1.30)).

2) IL-10 genotypes and BVA response

Then the patients were divided by their genotype group and response to BVA. Good responders were as patients who showed decrease of ESR and CRP, Mild responders were who showed decrease of ESR or CRP, and Bad responders were who showed increase of ESR and CRP after BVA. The distribution of IL-10 genotypes of Good responders were as follows: A/A, 75.76%; A/C, 24.24%. Genotypes of Mild responders were A/A, 85.29%; A/C, 14.71%. Genotypes of Bad responders were A/A, 76.92%; A/C, 23.08%. And there was no significant difference among the three groups (Table 7, p=0.5934).

The frequency of alleles of Good responders were as follows: A, 87.88%; C, 12.12%. In Mild responders were A, 92.65%; C, 7.35%. In Bad responders were A, 88.46%; C, 11.54%. And there was no significant difference among the three groups (Table 7, p=0.6288).

The frequency of carriers of Good responders were as follows: A, 80.49%; C, 19.51%. In Mild responders were A, 87.18%; C, 12.82%. In Bad responders were A, 81.25%; C, 18.75%. And there was no significant difference among the three groups (Table 7, p=0.7031).

3) IL-10 genotypes and improvement scores

The improvement of ESR (before treatment - 4 weeks after) were as follows: A/A, -2.39 ± 27.75; A/C, 0.93 ± 3.85 mm/hr. And there was no significant difference between the genotypes (Table 8, p=0.3582).

The improvement of CRP (before treatment - 4 weeks after) were as follows: A/A, -0.08 ± 3.50; A/C -0.27 ± 1.40 mg/dl. And there was no significant difference between the genotypes (Table 8, p=0.7484).

The improvement of KHAQ scores (before treatment - 4 weeks after) were as follows: A/A, 3.62 ± 6.79; A/C 0.18 ± 6.80. And there was no significant difference between the genotypes (Table 8, p=0.0831).

IV. Discussion

There are several types of DNA sequence variation, including insertions and deletions, differences in the copy number of repeated sequences, and single base pair differences. The latter are the most frequent. They are termed SNP when the variant sequence type has a frequency of at least 1% in the population. Genetic factors appear to contribute to virtually every human disease, conferring susceptibility or resistance, affecting the severity or progression of disease, and interacting with environmental influences. Much of current biomedical research, in both the public and private sectors, is based upon the expectation that understanding the genetic contribution to disease will revolutionize diagnosis, treatment, and prevention. In trying to understand disease processes, information about genetic variation is critical for understanding how genes function or malfunction, and for understanding how genetic and functional variation are related.

BVA has been used to treat inflammatory diseases such as RA in experimental animals[17]. In experimental animals, the induction of arthritis is successfully suppressed by BV treatment[18] . Recently it was revealed that injection of BV into the Zusanli(ST36) acupoint produces anti-arthritic effect than into a non-acupoint in a rodent model of RA[18].

RA is an autoimmune inflammatory disease characterized by cartilage destruction. In Oriental Medicine, RA belongs to symptom such as Bi(痺), Poung Bi(風痺), Youk Jeol Poung(歷節風),etc[19]. Pro-inflammatory cytokines, such as TNFα and IL-1, play a significant pathogenic role and the disease can be ameliorated by treatments specifically targeting TNFα or

IL-1[20,21]. Anti-inflammatory cytokines can also be found in the affected joints and it has been postulated that chronic synovitis may reflect an imbalance in pro- and anti-inflammatory cytokine production in RA[22]. Consequently, the potential beneficial effects of anti-inflammatory cytokines such as IL-10[23] and IL-4[24] in RA are of great interest. In RA synovium relative absence of IL4 provided an explanation for uncontrolled pro-inflammatory cytokine production leading to joint destruction[25]. Conversely, treatment with IL-4 was able to reduce joint inflammation and destruction in vitro and in animal models[26].

IL-4 is a potent anti-inflammatory cytokine, which reduces the production of pro-inflammatory cytokines and destructive enzymes by monocytes as well as by RA synovium samples. The IL-4 gene has been located on the long arm of chromosome 5, together with genes for other Th2 cytokines, such as IL-5 and IL-13. For many cytokines and their receptors, genetic variants have been described[27]. The IL-4R is composed of multiple chains, including a specific chain and a rc chain, which is common to several cytokine receptors. In the IL-4Rα -chain gene, an A→G transition at nucleotide 1902, causing a change from glutamine to arginine at codon 576, has been described and the presence of this rare allele has been associated with familial hyper-IgE syndrome and atopy[28].

In our present study there was significant difference between patients and controls group in IL-4R genotypes (Table 2, p<0.0001). (RA patients : A/A, 72.81%; A/G, 26.32% and G/G, 0.88%. In controls, A/A, 53.41%; A/G, 46.59% and G/G, 0.00%.) And there was significant difference between patients and controls group in frequency of alleles (Table 2, p=0.0165, OR (95% CI) ; 1.86(1.12-3.10)). (RA patients : A, 85.96%; G, 14.04%. In controls, A, 76.70%; G, 23.30%.). Also there was significant difference between patients and controls group in frequency of carriers (Table 2, p=0.0043, OR (95% CI) ; 2.34 (1.30-4.20)). (RA patients : A, 78.47%; G, 21.53%. In controls, A, 68.22%; G, 31.78%).

The IL-4R chain gene polymorphism was strongly associated with IgE secretion and atopy in familial studies[28]. Such association was found in Korean RA patients. The present results indicate the possible contribution of IL-4 gene polymorphism to RA.

The immunomodulatory cytokine IL-10 is produced by a variety of cell types, including monocytes and B lymphocytes[29]. It is a potent up-regulator of B-cell production and differentiation[30]. but has anti-inflammatory capabilities that can directly down-regulate TNFα, IL-1, IL-8 and interferon- production[29,31]. The IL-10 gene maps to the junction of (1q31-q32)[32] and exhibits substantial polymorphism in the promoter region which appears to correlate with variation in transcription[33,34]. Eight SNPs have also been identified in the promoter region of this gene[35,36]. three of which have been studied in some detail: -1082(G to A), -819(C to T) and -592(C to A)[33,35,37].

In our present study there was no significant difference between patients and controls group in IL-10 genotypes (Table 6, p=0.1835, OR (95% CI) ; 1.70 (0.77-3.72)). (RA patients : A/A, 80.00%, A/C, 20.00%. In controls, A/A, 87.16%, A/C, 12.84%). And there was no significant difference between patients and controls group in frequency of alleles (Table 6, p=0.2035, OR (95% CI) ; 1.62 (0.77-3.42)). (RA patients : A, 90.00%, C, 10.00%. In controls, A, 93.58%, C, 6.42%). Also there was no significant difference between patients and controls group in frequency of carriers (Table 6, p=0.1835, OR (95% CI) ; 0.59 (0.27-1.30)). (RA patients ; A, 83.33% and C, 16.67%. In controls, A, 88.62%, C, 11.38%)

The IL-10 gene polymorphism was not associated in Korean RA patients. The present results indicate that IL-10 gene polymorphism would not be the possible contribution to RA.

Response to therapies can also be affected by genetic differences. Information about DNA sequence variation will thus have a wide range of application in the analysis of disease and in the development of diagnostic, therapeutic,

and preventative strategies. In this study, we followed up patients with RA who were being treated with BVA, and tested whether the patients clinically respond to BVA or not. The patients were divided by their genotype group and response to BVA. Good responders were as patients who showed decrease of ESR and CRP, Mild responders were who showed decrease of ESR or CRP, and Bad responders were who showed increase of ESR and CRP after BVA.

In our present study the distribution of IL4R genotypes of Good responders were as follows: A/A, 78.00%; A/G, 20.00% and G/G, 2.00%. Genotypes of Mild responders were A/A, 69.39%; A/G, 30.61% and G/G, 0.00%. Genotypes of Bad responders were A/A, 66.67%; A/G, 33.33% and G/G, 0.00%. And there was significant difference among the three groups (Table 3, p=0.0080). The frequency of alleles of Good responders were as follows: A, 88.00%; G, 12.00%. In Mild responders were A, 84.69%; G, 15.31%. In Bad responders were A, 83.33%; G, 16.67%. And there was no significant difference among the three groups (Table 3, p=0.7237). The frequency of carriers of Good responders were as follows: A, 81.67%; G, 18.33%. In Mid responders were A, 76.56%; G, 23.44%. In Bad responders were A, 75.00%; G, 25.00%. And there was no significant among the three groups (Table 3, p=0.7249).

Also the distribution of IL-10 genotypes of Good responders were as follows: A/A, 75.76%; A/C, 24.24%. Genotypes of Mild responders were A/A, 85.29%; A/C, 14.71%. Genotypes of Bad responders were A/A, 76.92%; A/C, 23.08%. And there was no significant difference among the three groups (Table 7, p=0.5934). The frequency of alleles of responders were as follows: A, 87.88%; C, 12.12%. In Mild responders were A, 92.65%; C, 7.35%. In Bad responders were A, 88.46%; C, 11.54%. And there was no significant difference among the three groups (Table 7, p=0.6288). The frequency of carriers of Good responders were as follows: A, 80.49%; C, 19.51%. In Mild responders were A, 87.18%; C, 12.82%. In Bad responders were A, 81.25%; C, 18.75%. And there was no significant difference among the three groups (Table 7, p=0.7031)

Predicting which RA patients are likely to respond well to the treatment and undergo a good improvement course based on genotype data would be a major clinical point. It would ensure that patients at highest risk of a severe outcome could be targeted with early aggressive therapies. With a better understanding of interactions between genotype and treatment response it would be possible to decide the treatments most likely to be efficacious for specific patients. Advances in genotyping technology have facilitated the investigation of genetic factors in both disease susceptibility and severity[38].

In this study, we followed up patients with RA who were being treated with BVA, and we tested if the clinical response to BVA could be predicted by analysis of the polymorphism of IL4R or IL-10 gene or not. Until now, there are a few similar studies to relate RA and recent developed anti-rheumatic drugs. For example the patients with a certain combination of alleles(-308 TNF1/TNF1 and -1087 IL-10 G/G) was associated with good responsiveness to etanercept[39]. But there is no report of a gene polymorphism predicting response to BVA in Korean RA patients.

In our present study in case of IL-4 the improvement of ESR (before treatment - 4 weeks after) were as follows: A/A, -0.72 ± 24.75; A/G & G/G, 1.90 ± 8.79 mm/hr. And there was no significant difference between the genotypes (Table 4, p=0.405). The improvement of CRP (before treatment - 4 weeks after) were as follows: A/A, 0.96 ± 9.57; A/G & G/G, -0.06 ± 1.10 mg/dl. And there was no significant difference between the genotypes (Table 4, p=0.341). The improvement of KHAQ scores (before treatment - 4 weeks after) were as follows: A/A, 3.72 ± 7.25; A/G & G/G, 1.54 ± 6.55. And there was no significant difference between the genotypes (Table 4, p=0.135).

In case of IL-10 the improvement of ESR (before treatment - 4 weeks after) were as follows: A/A, -2.39 ± 27.75; A/C, 0.93 ± 3.85 mm/hr. And there was no significant difference among the genotypes (Table 8, p=0.3582). The improvement of CRP (before treatment - 4 weeks after) were as follows: A/A, -0.08 ± 3.50; A/C -0.27 ± 1.40 mg/dl. And there was no significant difference among the genotypes (Table 8, p=0.7484). The improvement of KHAQ scores (before treatment - 4 weeks after) were as follows: A/A, 3.62 ± 6.79; A/C 0.18 ± 6.80. And there was no significant difference among the genotypes (Table 8, p=0.0831).

Genetic and environmental factors are both important in the development of RA. So far it is very difficult to quantify the predictive value of genetic markers and apply the results from genetic studies to clinic patients. Establishing high quality of prospective inception cohorts, a more systemic approach to suitable outcome measures, and estimating the effects of treatment, will be important to the identification of good predictive genetic markers.

This is one of association study between polymorphism of IL-4R, IL-10 and BVA response. Further cytokine gene studies including different positions of IL4R or IL-10 can be a useful tool for predicting efficacy and side-effect of BVA.

The findings of this study need to be confirmed in larger patients and further studies. Additional examinations of the relationship between interleukin and lifestyles with regard to RA risk factor will be helpful in the prevention of RA through the promotion of more suitable lifestyles.

V. Conclusion

We investigated the relationship between SNP of IL4R, IL-10 and BVA efficacy in patients with RA treated with BVA for 8 weeks. The results are as follows.

1. IL4R genotypes, there was significant difference between RA patients and controls group.
2. In IL4R genotypes, there was significant difference among BVA Good, Mild and Bad responders to in RA patients, but in the frequency of alleles and carriers, there were no significant difference.
3. There was no significant difference between RA patients and controls group in IL-10 gene genotypes.
4. In IL-10 genotypes, there was no significant difference among BVA Good, Mild and Bad responders to in RA patients.
5. There was no significant difference in the improvement of ESR, CRP and KHAQ scores after BVA in RA patients among the IL4R or IL-10 genotypes.

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