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원 저

# Response to Bee Venom Acupuncture and Polymorphism of Matrix Metalloproteinase-1 Gene in Korean Patients with Rheumatoid Arthritis

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## 국문초록

# 한국인 류마티스 관절염 환자의 봉독약침 치료반응과 Matrix Metalloproteinase-1의 유전자 다형성 연구

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***목적*** : 류마티스 관절염 환자의 골 파괴에 중요한 역할을 하는 것으로 알려진 Matrix Metalloproteinase-1 (MMP-1) 유전자의 단일 염기 다형성을 분석하고, 나아가 봉독약침 치료에 대한 반응과의 연관성을 조사하기 위하여 본 연구를 시행하였다.

***방법*** : 미국류마티스학회의 류마티스 관절염 기준에 해당하는 122명의 한국인 류마티스 관절염 환자와 건강한 92명의 대조군을 대상으로 pyrosequencing 방법을 이용하여 MMP-1 유전자의 -519 위치의 다형성을 비교 분석하였으며, 류마티스 관절염 환자군을 다시 유전자 유형에 따라 동통 관절수, 종창 관절수, 조기 강직, 통증 강도, 삶의 질 평가도구인 HAQ, 환자 및 의사의 전반적 질병상태 평가, ESR, CRP 등의 항목을 치료 전후 평가하여 비교 분석하였다.

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*결과* :

1. 류마티스 환자군과 건강한 대조군간에 MMP-1 유전자의 단일 염기 다형성의 유전자형의 분포와 대립유전자 발현 빈도에 통계적으로 유의한 차이가 나타났으며, 이는 MMP-1 유전자 다형성이 한국인 류마티스 관절염 환자의 질병 감수성과 관련이 있음을 추정할 수 있다.

2. 각 유전자형 그룹간 치료전 질병의 중증도 평가에서 임상 평가와 혈액의 급성 염증 반응물질 평가에서 통계적으로 유의한 차이는 없었다.

3. 급성 염증 반응의 지표인 ESR과 CRP level의 봉독약침 치료 전후 변화는 MMP-1의 유전자 다형성과 유의한 연관이 없었다.

4. 각 유전자형 그룹간의 치료 전후 질병 호전도 비교에서, AA 유전자형이 종창 관절수 평가에서 더나은 호전을 보였으며, 다른 모든 평가에서는 통계적으로 유의한 차이가 없었으며, 향후 관련 유전자와의 연관성 연구가 필요하다고 사료된다.

***Key Words***: **bee venom acupuncture, matrix metalloproteinase-1, single nucleotide polymorphism, rheumatoid arthritis**

# I. Introduction

Bee venom has been applied to rheumatoid arthritis(RA) patients in order to relieve pain and cure inflammation[1]. In our previous studies, we reported that bee venom acupuncture (BVA) improved tender joint count, swollen joint count and morning stiffness[2], and that it also improved rehabilitation and health-related quality of life in RA patients[3]. The experiment group, in comparison to the control group, showed significant decreases in tender joint count, swollen joint count, morning stiffness, health assessment questionnaire, pain, ESR and CRP level in a randomized controlled double blind study[4]. However, despite of significant anti-inflammatory effect, not all RA patients respond well to BVA. Furthermore, some patients complained of side-effects such as itching and edema. It would be extremely useful to be able to predict which patients will have better improvement with BVA and which patients will not.

RA is recognized as a multigenic disorder, with genetic poymorphisms contributing to both the susceptibility and the severity of the disease[5]. Recently, single nucleotide polymorphism at position -519 in the promoter region of the matrix metalloproteinase-1(MMP-1) gene is reported[6]. MMPs are cytokine-modulated enzymes that play an important role in the pathogenesis of RA by inducing bone resorption and cartilage destruction. Especially, MMP-1 is detected in RA synovial fluid[7] and serum samples at higher levels than in healthy controls[8]. But there is no report on the association between clinical manifestations including response to BVA and -519 position polymorphism of the MMP-1 gene. This study was performed to investigate the association between MMP-1

-519 genotypes and response to BVA in Korean patients with RA.

## Ⅱ. Subjects and Methods

### 1. Subjects

122 Korean RA patients were included in this prospective study. All of them were enrolled at the Department of Acupuncture & Moxibustion, Oriental hospital of Kyung Hee University medical center in Seoul from May 2002 to April 2003. All patients fulfilled the American College of Rheumatology 1987 revised criteria for RA[9], and all had evidence of active disease despite conventional DMARDs 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.

92 healthy subjects were randomly selected as a control group from adult volunteers who cleared yearly health examinations. They had the same ethnic background as the patients and were selected for age and gender.

### 2. Treatment protocol

Korean bee venom[10] was dissolved in saline (diluted 1 : 3000) and administrated into acupuncture points. Selection of acupuncture points was based on the local points around the inflammed joints. A volume of 0.1ml of bee venom was injected into each acupoint and the total injected volume/patient did not exceed 1 ml. BVA was applied twice a week and continued for 8 weeks. Previous conventional medication was permitted without drug change during BVA treatment.

### 3. 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 66 joints in each patient as either swollen or not swollen, then computing the number of swollen joints for each patient.

Morning stiffness was measured for duration of time that patients feel stiffness in and around the joints before maximal improvement.

The patient and physician global assessments were consisted of the patient's rating of how he/she is doing, considering all of the ways that RA affects him/her, and the physician's rating of how the patient is doing, considering all of the ways that RA affects the patient. The patient and physician global assessments were based on a 5-point scale, in which 1=very good(asymptomatic and no limitation of normal activities), 2=good(mild symptoms and no limitation of normal activities), 3=fair(moderate symptoms and limitation of some normal acti-

vities), 4=poor(severe symptoms and inability to carry out most normal activities), and 5= very poor(very severe symptoms that are intolerable and inability to carry out all normal activities)[11].

Patient's assessment of RA-related pain was determined using a 100mm visual analog scale, 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[12], 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 ESR, CRP, and rheumatoid factor.

## 4. Genotyping for MMP-1 polymorphism

### 1) DNA samples

Human genomic DNA was extracted from peripheral blood samples using the QIAGEN System(QIAmp DNA Blood Midi Kit, Hilden, Germany). DNA was stored at 4℃ until analyzed.

### 2) PCR amplification

After primers of MMP-1 gene were designed (MMP-1 sense 5'-TGGCTCTGAGTAAA GAT TAAGGGAAG-3' and MMP-1 antisense 5'-G TGGCTCTTCGGGGTTCTCT-3'), a 103 bp fragment of the MMP-1 gene was amplified using 25ng of DNA. Polymerase chain reaction(PCR) amplification was performed by using 0.5 unit Taq polymerase(HT Biotechnology Ltd., Cambridge, United Kingdom) 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 agarose gel electrophoresis. The immobilization of PCR products to streptavidin separose beads (Streptavidin Sepharose HP, Amersham Pharmacia Biotech, Uppsala, Sweden) was performed according to the manufacturer's directions.

The sequencing primer was 5'-TGTTAAG CTGCCTGG-3' and it was designed so that the terminal residue hybridized to the base immediately adjacent to the A/G mutation. After immobilization of 20$\mu$L of biotinylated PCR products on 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. The PCR strands were separated by incubating them with 50$\mu$L of 0.2M sodium hydroxide for 1 minute and washed twice with 150$\mu$L of 10mM Tris-

acetate(pH 7.6). The beads with the immobilized template were resuspended in 55 $\mu$L of 4M acetic acid containing 0.35 $\mu$M sequencing primer. Then 45 $\mu$L of this suspension was transferred to a PSQ 96 plate(Pyrosequencing AB, Uppsala, Sweden)[13)~15)].

### 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. After this process, the A/G polymorphism at position −519 of the MMP−1 gene was genotyped<Fig. 1, 2, 3>.

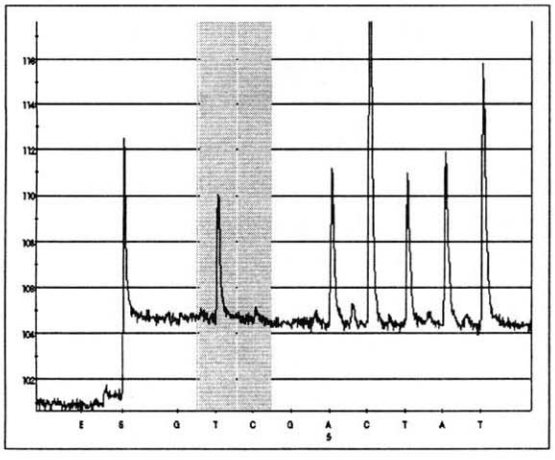

Fig. 1. Pyrosequencing result of AA homozygote genotype at position −519 of the MMP−1 gene

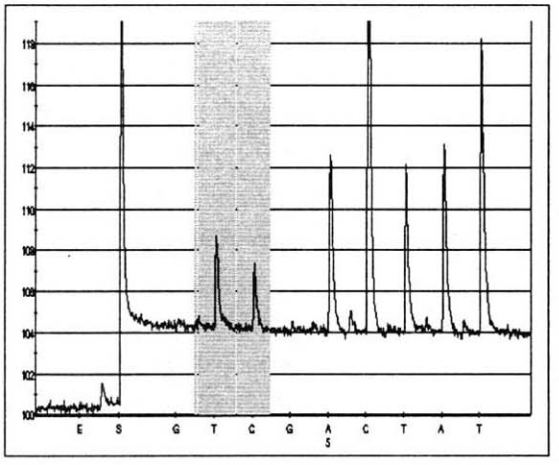

Fig. 2. Pyrosequencing result of AG heterozygote genotype at position −519 of the MMP−1 gene

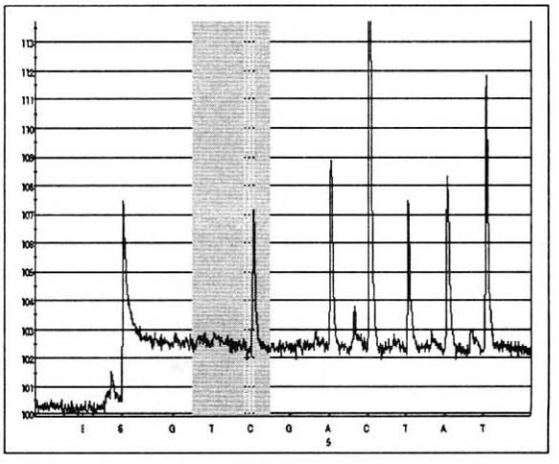

Fig. 3. Pyrosequencing result of GG homozygote genotype at position −519 of the MMP−1 gene

### 5. Statistical analysis

Statistical analyses were performed using SPSS 11.0. The results were considered statistically significant at $p<0.05$. Data were compared between groups by the independent t-test. Qualitative data were analyzed by the Chi-square test. Allele frequencies were estimated by the gene-counting method. Odds ratios(OR), and 95% confidence intervals(CI) were calculated as estimates of the relative risks.

## Ⅲ. Results

### 1. Baseline characteristics of patients

The baseline characteristics of the 122 patients are shown in <Table 1>. The majority of patients(86.9%) were women. Their mean±SD age was 47.7±9.4 years, and their mean±SD disease duration was 84.3±69.5 months. 54.9% of the patients were rheumatoid factor positive. In the 92 healthy control group, 81.5% were women and their mean±SD age was 45.5±15.1 years. There were no significant differences in gender, and age between RA patients and healthy control groups.

Table 1. Baseline characteristics of RA patients and healthy controls

| | RA patients (n=122) | Healthy controls (n=92) | p |
|---|---|---|---|
| Demographics | | | |
| Age, years | 47.7±9.4 | 45.5±15.1 | NS |
| Women, no.(%) | 106(86.9%) | 75(81.5%) | NS |
| Disease status | | | |
| Disease duration, months | 84.3±69.5 | | |
| RF seropositive, no.(%) | 67(54.9%) | | |
| Clinical assessment | | | |
| Tender joints count | 9.6±8.7 | | |
| Swollen joints count | 4.9±4.9 | | |
| Morning stiffness, minutes | 131.7±104.6 | | |
| Pain intensity(VAS 0-100) | 58.5±20.8 | | |
| HAQ score | 0.94±0.58 | | |
| Patient global assessment | 3.3±0.6 | | |
| Physician global assessment | 3.3±0.6 | | |
| Laboratory assessment | | | |
| ESR, mm/hour | 31.2±16.5 | | |
| Serum CRP level, mg/dl | 1.08±1.30 | | |

Values are the mean±SD. RF : rheumatoid factor ; VAS : visual analog scale ; HAQ : health assessment questionnaire ; ESR : erythrocyte sedimentation rate ; CRP : C-reactive protein ; NS : not significant. Data were evaluated by independent t-test.

### 2. Distribution of genotypes, alleles, and carriers of MMP-1 gene polymorphism

-519 position of MMP-1 gene was investigated in 122 RA patients and 92 healthy controls. There were 97 patients with genotype AA, 24 with genotype AG, and 1 with genotype GG, compared to 86 with AA, 6 AG, and 0 GG in the healthy control group. The observed genotype frequencies of the RA patients($p=0.935$) and the control group($p=0.949$) did not show significant difference predicted by the Hardy-Weinberg equation. The frequency of AA genotype in patients was significantly lower than that in healthy control group($p=0.004$, OR=0.27), while while the frequency of AG genotype in patients was higher than that in healthy

control group(p=0.006, OR=3.51). Allelic frequencies and carriage rates between A and G also showed significant differences(p=0.004). Therefore, compared to healthy controls, RA patients showed higher G allele frequency(p=0.004, OR=3.54) and G carriage rate(p=0.004, OR= 3.69)<Table 2>.

### 3. MMP-1 genotype and clinical manifestations of RA

Patients were divided into genotype group AA and genotype group AG & GG. The mean values of all the assessments at the baseline did not show any significant differences between the genotype groups<Table 3>.

Table 2. Distribution of genotypes, alleles, and carriers of MMP-1 gene polymorphism in RA patients and healthy controls

| | Patients n=122 | Controls n=92 | p | OR | 95% CI |
|---|---|---|---|---|---|
| Genotype | | | | | |
| AA | 97 | 86 | 0.004 | 0.27 | 0.11~0.69 |
| AG | 24 | 6 | 0.006 | 3.51 | 1.37~8.99 |
| GG | 1 | 0 | NS | | |
| Allele | | | | | |
| A | 218 | 178 | 0.004 | 0.27 | 0.11~0.69 |
| G | 26 | 6 | 0.004 | 3.54 | 1.43~8.79 |
| Carrier | | | | | |
| A | 121 | 92 | NS | | |
| G | 25 | 6 | 0.004 | 3.69 | 1.45~9.43 |

Data were evaluated by Chi-square test. NS : not significant ; OR : Odds ratio ; CI : confidence interval.

Table 3. MMP-1 genotype and clinical manifestations at the baseline

| | AA genotype (n=97) | AG & GG genotypes (n=25) | p |
|---|---|---|---|
| Demographics | | | |
| Age, years | 47.7±9.2 | 47.6±10.3 | NS |
| Women, no.(%) | 84 (86.6%) | 22 (88.0%) | NS |
| Disease status | | | |
| Disease duration, months | 81.6±67.5 | 94.9±77.4 | NS |
| RF seropositive, no.(%) | 66 (68.0%) | 12 (48.0%) | NS |
| Clinical assessment | | | |
| Tender joints count | 10.0±9.2 | 8.2±6.2 | NS |
| Swollen joints count | 5.2±5.3 | 3.8±2.5 | NS |
| Morning stiffness, minutes | 133.7±107.3 | 124.0±95.0 | NS |
| Pain intensity(VAS 0~100) | 58.8±20.6 | 57.3±21.6 | NS |
| HAQ score | 0.97±0.60 | 0.83±0.47 | NS |
| Patient global assessment | 3.3±0.7 | 3.2±0.4 | NS |
| Physician global assessment | 3.3±0.7 | 3.2±0.4 | NS |
| Laboratory assessment | | | |
| ESR, mm/hour | 31.3±17.0 | 31.0±14.9 | NS |
| Serum CRP level, mg/dl | 1.14±1.32 | 0.84±1.22 | NS |

Values are the mean±SD. RF : rheumatoid factor ; VAS : visual analog scale ; HAQ : health assessment questionnaire ; ESR : erythrocyte sedimentation rate ; CRP : C-reactive protein ; NS : not significant. Data were evaluated by independent t-test.

### 4. MMP-1 genotype and ESR change after BVA

Patients were divided by their genotype and ESR change after BVA. 67 patients showed ESR decrease after BVA. The observed genotype frequencies of patients showing ESR decrease (p=0.940) and the others(increase or no change) (p=0.662) were not significantly different as predicted by the Hardy-Weinberg equation. There were no significant differences in the genotype, allele, and carrier between the two groups divided by ESR change<Table 4>.

### 5. MMP-1 genotype and CRP level after BVA

Patients were divided by their genotype and CRP level after BVA. 42 patients showed CRP level decrease after BVA. The observed genotype frequencies of patients showing CRP level decrease(p=0.841) and the others(increase or no change) (p=0.991) were not significantly different as predicted by the Hardy-Weinberg equation. There were no significant differences in the genotype, allele, and carrier between the two groups divided by CRP level change<Table 5>.

### 6. Comparison of improvement in RA assessment between MMP-1 genotype groups

In the comparison of improvement between genotype groups, all the other clinical and laboratory assessments showed no significant difference, except in the swollen joint count <Table 6>.

Table 4. Correlation between MMP-1 gene polymorphism and ESR change after BVA

| | Decreased n=67 | Increased or No change n=55 | p |
|---|---|---|---|
| Genotype | | | |
| AA | 54 | 43 | NS |
| AG | 12 | 12 | NS |
| GG | 1 | 0 | NS |
| Allele | | | |
| A | 120 | 98 | NS |
| G | 14 | 12 | NS |
| Carrier | | | |
| A | 66 | 55 | NS |
| G | 13 | 12 | NS |

NS : not significant. Data were evaluated by Chi-square test.

Table 5. Correlation between MMP-1 gene polymorphism and CRP level after BVA

| | Decreased n=42 | Increased or No change n=80 | p |
|---|---|---|---|
| Genotype | | | |
| AA | 35 | 62 | NS |
| AG | 7 | 17 | NS |
| GG | 0 | 1 | NS |
| Allele | | | |
| A | 77 | 141 | NS |
| G | 7 | 19 | NS |
| Carrier | | | |
| A | 42 | 79 | NS |
| G | 7 | 18 | NS |

NS : not significant. Data were evaluated by Chi-square test.

Table 6. Comparison of improvement in RA assessment between MMP-1 genotype groups

| | AA genotype (n=97) | AG & GG genotype (n=25) | p |
|---|---|---|---|
| Clinical assessment | | | |
| Tender joint count | −2.29±4.14 | −1.68±2.73 | NS |
| Swollen joint count | −1.58±2.77 | −0.84±1.07 | 0.019* |
| Morning stiffness | −41.30±60.59 | −21.80±40.41 | NS |
| Pain | −11.9±19.20 | −6.60±19.69 | NS |
| HAQ | −0.18±0.37 | −2.26±6.62 | NS |
| Patient global assessment | −0.77±0.78 | −0.84±0.47 | NS |
| Physician global assessment | −0.75±0.78 | −0.84±0.47 | NS |
| Laboratory assessment | | | |
| ESR | −1.54±8.79 | −1.40±6.80 | NS |
| CRP | 0.02±1.36 | 0.04±0.35 | NS |

Values are the mean±SD. HAQ : health assessment questionnaire ; ESR : erythrocyte sedimentation rate ; CRP : C-reactive protein ; NS : not significant. Data were evaluated by independent t-test.

## Ⅳ. Discussion

In the pathogenesis of RA, some cytokines play a major role being able to induce bone resorption and cartilage destruction[16]. And this activation causes to release MMPs, which can degrade a range of extracelluar matrix proteins and have been implicated in connective tissue destruction and remodelling associated with various diseases[17]. It is suggested that seeping of MMP proteins from diseased tissues into the blood circulation[18] may result to increase plasma/serum levels of certain MMPs in patients with rheumatoid diseases such as rheumatoid arthritis[19] and systemic lupus erythematosus[20]. The expression of most metalloproteinases is regulated at the transcription level by growth factors, hormones, and cytokines.

Out of many kinds of human MMPs, collagenase(MMP-1) and stromelysin 1(MMP-3) are thought to be probably involved in the pathogenesis of RA[21]. MMP-1 is the interstitial collagenase expressed most widely among tissues and therefore plays a prominent role in degrading collagens Ⅰ, Ⅱ, Ⅲ, Ⅶ, and Ⅹ, the most abundant compounds of connective tissue. MMP-1 level is elevated in patients with RA, both in plasma[8] and in synovial fluid where it correlates with the degree of synovial inflammation[22]. It is commonly observed in both synovium and cartilage, especially prominent at cartilage erosion sites in rheumatoid lesions[23].

There are many reports about the association between polymorphism in the MMP-1 gene

and various diseases. A polymorphism at position-1607 in the promoter of the MMP-1 gene has been identified and demonstrated that the 2G allele binds substantially more recombinant Ets-1 transcription factor and has significantly higher transcriptional activities and a more aggressive matrix degradation than the 1G allele in normal fibroblasts and in melanoma cells[24], and the MMP-1 gene may be a susceptibility locus for reduced BMD at the distal radius in postmenopausal women[25]. Also, higher circulating MMP-1 levels are associated with rapidly progressive erosive RA[26].

In order to control rheumatoid arthritis(RA), many kinds of disease modified anti-rheumatic drugs(DMARDs) have been widely used. But, sometimes withdrawal of the drugs is required due to side-effects such as gastrointestinal effects[27] and bone marrow toxicity[28]. As an alternative medicine, bee venom was used in the treatment of RA. Yet the mechanisms of bee venom components to activate the therapeutic effect is unclear, which requires further investigation. Kwon et al. reported that bee venom administration into the Zusanli acupoint produced a significant anti-nociceptive effect on arthritis-induced inflammatory pain symptoms[29]. In the present study, we selected the specific acupoints around the inflamed joints in the same way as clinical practice at hospital.

A newly identified single nucleotide polymorphism(SNP) at position -519 in the promoter region of the MMP-1 gene is reported. This polymorphism consists in a guanine to adenine substitution[6]. However, there is no report on the relationship between clinical manifestations including -519 position polymorphism of the MMP-1 gene and response to BVA in RA patients.

In the present study, we followed up 122 patients with RA who were being treated with BVA in order to investigate the association between individual response to BVA and MMP-1 gene polymorphism in Korean patients with RA. -519 position of MMP-1 gene was investigated in 122 RA patients and 92 healthy controls. There were 97 patients with genotype AA, 24 with genotype AG, and 1 with genotype GG, compared to 86 with AA, 6 AG, and 0 GG in the healthy control group. The frequency of AA genotype was significantly lower than that in healthy control group($p=0.004$, OR=0.27), while the frequency of AG genotype in patients group was higher than that in healthy control group($p=0.006$, OR=3.51). Allelic frequencies and carriage rates between A and G also showed significant difference($p=0.004$). Therefore, RA patients showed higher G allele frequency($p=0.004$, OR=3.54) and G carriage rate($p=0.004$, OR=3.69). This result suggests that MMP-1 gene polymorphism could be involved in the susceptibility of RA patients. There are several previous reports that MMP-1 was detected in RA synovial fluid[7] and serum samples at higher levels than in healthy controls[8]. However, generally ethnic origin should be carefully considered while studying the association between genetic factors and disease susceptibility of genetic polymorphism among populations of different genetic background. For example, 100 Caucasian sub-

jects of Czech nationality showed AA 37%, AG 38%, and GG 25% at −519 position MMP−1 gene polymorphism[6]. This suggests MMP−1 gene polymorphism also has significant difference between ethnic population.

Patients were divided into genotype group AA and genotype group AG & GG. The mean values of all the assessments at the baseline showed no significant difference between the genotype groups. It is still unclear that the polymorphism MMP−1 gene is involved in clinical manifestations including disease severity of RA patients, because there are many controversial reports on this issue. Constantin et al. reported that there was no association between polymorphism in the MMP−1 gene promotor and susceptibility to, and severity of, RA[30]. On the other hand, Massarotti et al. reported that MMP−1 promotor gene polymorphism was significantly associated with erosive RA, although he also agreed that MMP−1 gene did not contribute to the RA susceptibility[31]. In addition, several investigations reported that the serum level of MMP−1 correlated with the number of new erosions that developed in RA patients over 18 months of follow−up[32], and that the decrease in the serum level of MMP−1 could partly explain the delay in radiological progression in patients with RA during etanercept treatment[33]. A recent study reported that a high level of synovial expression of MMP−1 mRNA in early RA distinguished patients with more rapidly progressive erosive disease[34].

Patients were divided by their genotype and ESR change after BVA. 67 patients showed ESR decrease after BVA. There were no significant differences in the genotype, allele, and carrier between the two groups divided by ESR change. Patients were also divided by their genotype and CRP level after BVA. 42 patients showed CRP level decrease after BVA. There were no significant differences in the genotype, allele, and carrier between the two groups divided by CRP level change. This suggests that MMP−1 polymorphism are not associated with acute inflammatory reactants such as ESR and CRP, which requires further investigation. Furthermore, in comparison of improvement between genotype groups, no significant difference was found in all the clinical and laboratory assessments, except in the swollen joint count. This result suggests that general response to BVA is not associated with polymorphism of −519 position in the MMP−1 gene in this study.

This first study of gene polymorphism and BVA response suggests that the search for the association between gene polymorphism and response to medical therapies including BVA remains an important issue to optimize the effect. It is necessary to continue studies including related cytokines and different positions of the MMP−1 gene in future.

## V. Conclusions

We investigated the association of a polymorphism of the MMP−1 gene with BVA response in 122 Korean patients with RA. The results

are as follows :

1. There were significant differences between genotypes, alleles, and carriers in RA patients and healthy controls. Compared to controls, patients showed higher allele frequency and carriage rate of G allele.

2. The mean values of all the assessments at the baseline showed no significant difference between the genotype groups.

3. MMP-1 polymorphism is not significantly associated with the change of ESR and CRP level after BVA.

4. In the comparison of improvement between different genotype groups, the AA genotype responded better than other genotypes in the swollen joint count, and the other assessments had no significant difference.

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