 adultos de edad avanzada (AOSD). Se investigó la asociación funcional de –607 (C/A) IL-18 promotor polimorfismos con enfermedad de curso en pacientes con AOSD.

**Métodos.** Se utilizó el reacción en cadena de la polimerasa específica de secuencia y el método de longitud del fragmento de restricción para analizar los genotipos de IL-18 promotor polimorfismos en 96 pacientes no relacionados con AOSD y 164 controles etnicamente-matching.

**Resultados.** Se observó una frecuencia significativamente más baja de polimorfismo de nucleotido único –607/AA en pacientes con AOSD comparado con controles sanos (18.8% vs 31.1%, respectivamente; p < 0.05). Los niveles mediano de IL-18 fueron significativamente más bajos en pacientes con AOSD con genotipo AA comparado con los pacientes con genotipo CA o CC genotipo (147.5 pg/ml vs 410.5 pg/ml o 262.4 pg/ml, respectivamente; ambos p < 0.05). Significativamente niveles de IL-18 fueron observados en pacientes con sistema monocíclico con un sistema monoclínico y un sistema políclico con una enfermedad crónica articulada. El genotipo AA fue más frecuentemente observado en pacientes con sistema monoclínico y un sistema monoclínico, que tenía el mejor pronóstico, que en aquellos con otras 2 enfermedades. En contraste, una frecuencia más baja del genotipo AA en la CA o el CC genotipo fue observado en pacientes con enfermedad crónica dismúltiple artritis (5.5% vs 25.0% o 19.2%, respectivamente).


**Palabras clave:** IL-18, promotor polimorfismo, enfermedad de adulto, AOSD.
in the promoter region of human IL-18 exon 1 have been identified. A recent report showed that only the SNP at position –607 in the IL-18 promoter is associated with the development of AOSD in a Korean population. The SNP at position –607 of the IL-18 promoter region has been predicted to be a nuclear factor-binding site for the cAMP-responsive element binding protein (CREBP), and the change from cytosine (C) to adenosine (A) nucleotide may affect promoter activity. The sequence upstream from human IL-18 exon 1 has clear promoter activity, which can be enhanced by stimulation with PMA/ionomycin.

Patients with AOSD may vary regarding clinical features and disease course. In contrast to the distribution of patterns of disease course reported by Cush, et al, Chinese patients with AOSD tend to be less susceptible to development of disabling arthritis. To our knowledge, the association of promoter polymorphisms of IL-18 exon 1 with disease course in patients with AOSD has not previously been reported.

We investigated whether promoter polymorphisms at position –607 of IL-18 exon 1 predispose to susceptibility of Chinese patients with AOSD. The functional consequences of the SNP –607 of IL-18 promoter were assessed by measuring circulating IL-18 levels in patients with AOSD during the active phase of disease. The association of IL-18 promoter polymorphisms with clinical manifestations and disease course was examined after adjustment for potential confounders in patients with AOSD who were followed for at least 2 years.

MATERIALS AND METHODS

Patients. Ninety-six consecutive unrelated Chinese patients (71 women, 25 men; mean age at disease onset, 37.1 ± 15.0 yrs) fulfilling the Yamaguchi criteria for AOSD were enrolled. Patients with infections, malignancies, or other rheumatic diseases were excluded. The clinical activity score (range 0–12) for each patient with AOSD was assessed according to the criteria described by Pouchot, et al. A prospective cohort design with collected outcome data was used in our study. Data were obtained on presenting manifestations and disease courses of all patients using standardized definitions and a standard extraction form. After diagnosis of AOSD and initial investigation of IL-18 levels, all patients were treated with non-steroidal antiinflammatory drugs or corticosteroids with or without disease-modifying antirheumatic drugs including hydroxychloroquine, sulfasalazine, methotrexate, and/or cyclosporin. According to the modified classification of the proposed disease course for AOSD, 96 patients, followed for at least 2 years, were classified into 3 patterns defined as follows: monocular systemic — only 1 episode of systemic manifestation, followed by complete remission within 1 year after disease onset; polycyclic systemic — more than 1 episode of systemic manifestation, followed by partial or complete remission after onset of the initial or subsequent attack; and chronic articular — persistent arthritis involving at least 1 joint area, with radiographic evidence of bone erosion or ankylosis, lasting longer than 6 months. One hundred sixty-four unrelated ethnically matched healthy volunteers (83 women and 81 men; mean age, 39.8 ± 12.4 yrs) living in Taiwan, without rheumatic disease, served as healthy controls. The Ethics Committee of Clinical Research, Taichung Veterans General Hospital, approved the study protocol and informed consent was obtained from each participant.

Determination of the –607* promoter polymorphisms of the IL-18 gene.

Genomic DNA was extracted and purified from whole blood of 96 patients with AOSD and 164 control subjects using the phenol-chloroform method. The –607 genotypes of the IL-18 promoter were detected by restriction digestion of the polymerase chain reaction (PCR) product with MseI. PCR was performed using a set of primers described by Takada, et al. The sense primer 5’-CTT TGC TAT CAT TCC AGG AA-3’ and antisense primer 5’-TAA CCT CAT TCA GGA CTT CC-3’ were designed to amplify a 301-bp DNA fragment covering the polymorphic site (GenBank accession number: rs1946518). Optimal PCR was performed in a thermocycler 9600 (Perkin-Elmer-Cetus, Norwalk, CT, USA) using a 30-cycle program (denaturation at 95°C for 5 min, followed by 95°C for 20 s, 60°C for 50 s, 72°C for 20 s, and a final extension at 72°C for 3 min). PCR products were digested with 5.0 U of MseI (New England Biolabs, Beverly, MA, USA) at 37°C overnight, and run on a 4% ethidium bromide NuSieve GTG (BioWhittaker Molecular Applications, East Rutherford, NJ, USA) agarose gel (0.5 µg/ml gel). MseI digested the 301-bp DNA segment from CC homozygous individuals into 199, 73, and 29-bp fragments; the DNA segment from AA homozygous individuals into 101/98, 73, and 29-bp fragments; and the DNA segment from CA heterozygous individuals into 199, 101/98, 73, and 29-bp fragments (Figure 1).

Determination of serum levels of IL-18. Serum levels of IL-18 were determined in 96 patients with active untreated AOSD using ELISA according to the manufacturer’s instructions (Bender MedSystems, Vienna, Austria). The overall intra- and interassay coefficients of variation were 2.9% and 12.5%, respectively.

Statistical analysis. Data were analyzed using SPSS 10.0 for Windows (SPSS, Chicago, IL, USA). The nonparametric Kruskal-Wallis test was used for between-group comparisons of serum IL-18 levels. Only when this test showed significant differences were the exact p values determined using the Mann-Whitney U-test. The correlation coefficient was obtained by the nonparametric Spearman’s rank correlation test. A multivariate logistic regression model was used to evaluate the simultaneous effects of genotypic variables on the occurrence of clinical manifestations in patients with AOSD. The differences in frequencies of alleles and genotypes of IL-18 promoter polymorphisms between patients with AOSD and healthy controls or among AOSD patients with different patterns of disease course were examined using the chi-squared method and Fisher’s exact test. p values less than 0.05 were considered significant.

Figure 1. MseI digested the 301-bp DNA segment from CC homozygous individuals into 199, 73, and 29-bp fragments; DNA segment from AA homozygous individuals into 101/98, 73, and 29-bp fragments; and DNA segment from CA heterozygous individuals into 199, 101/98, 73, and 29-bp fragments.
RESULTS
Frequencies of –607* promoter polymorphisms of IL-18 gene. The frequencies of genotypes and alleles at position –607 of IL-18 promoter polymorphisms are summarized in Table 1. A significantly lower frequency of AA genotype was found in patients with AOSD compared to healthy controls (18.8% vs 31.1%, respectively; p < 0.05). We observed an increased frequency of CA genotype in patients with AOSD compared with controls (54.2% vs 42.1%), although this difference did not reach statistical significance (p = 0.079). Among patients with AOSD, the odds ratios of CA and CC genotypes for comparison with AA genotype were 2.14 [95% confidence interval (CI) 1.12–4.08, p = 0.030] and 1.67 (95% CI 0.82–3.45, p = 0.223), respectively. No gene dosage effect was found with the presence of the C allele. No significant differences in the distribution of allelic frequencies at position –607 were observed between patients with AOSD and controls.

Association of –607* promoter polymorphisms of IL-18 gene with clinical manifestations. All patients with AOSD had a high fever (> 39°C). Typical Still’s rash was present in 82 patients (85.4%) and arthritis was seen in 72 (75.0%). Hepatic dysfunction (alanine aminotransferase, ALT ≥ 40 IU/l) was present in 30 patients (31.2%), and lymphadenopathy was seen in 29 (30.2%). Multivariate logistic regression analysis showed no significant association between IL-18 promoter polymorphisms and clinical manifestations of patients with AOSD (data not shown).

Association of IL-18 promoter polymorphism with serum IL-18 levels in patients with AOSD during the active phase. Serum IL-18 levels correlated positively with clinical activity scores (r = 0.248, p < 0.05) in patients with AOSD (Figure 2A). Serum IL-18 levels were significantly lower in AOSD patients with SNP –607/AA genotype [median = 147.5 pg/ml, interquartile range (IQR) 90.9–330.7] than those with SNP –607/CA genotype (median = 410.5 pg/ml, IQR 160.0–696.9; p < 0.05) or those with SNP –607/CC genotype (median = 262.4 pg/ml, IQR 164.4–607.0; p < 0.05; Figure 2B). No significant difference in serum IL-18 levels between patients with CA genotype and those with CC genotype was observed.

Association of IL-18 promoter polymorphisms and serum IL-18 levels with patterns of disease course. A followup analysis of 96 patients with AOSD was performed over a mean period of 70.9 ± 36.4 months (range 28 to 206 mo); 45 patients (46.9%) had a poly cyclic systemic course, 32 (33.3%) a monocy clic systemic course, and the remaining 19 (19.8%) a chronic articular course. As shown in Figure 3A, significantly lower levels of serum IL-18 were demonstrated in AOSD patients with a monocy clic systemic course (median 140.1 pg/ml, IQR 80.0–309.6) than in those with a poly cyclic systemic course (median 505.2 pg/ml, IQR 190.3–707.0; p < 0.01) or those with a chronic articular course (median 263.2 pg/ml, IQR 183.0–562.0; p < 0.05). As shown in Figure 3B, the AA genotype was more frequently observed in patients with monocy clic systemic course than in those with poly cyclic systemic course and in those with chronic articular course (66.7% vs 27.8% or 5.5%, respectively; both p < 0.05). A significantly higher proportion of AOSD patients with a monocy clic systemic course had AA genotype (12/18, 66.7%) compared with CA genotype (14/52, 26.9%), CC genotype (6/26, 23.1%), and CA+CC genotype (20/78, 25.6%); all p values < 0.01 by chi-squared test with Yates’ correction of contingency. Although there was no statistical significance (p = 0.112, Fisher’s exact test), the frequency of patients with AA genotype (1/18, 5.5%) was lower than of CA genotype (13/52, 25.0%), CC genotype (5/26, 19.2%), and CA+CC genotype (18/78, 23.1%) in patients with chronic disabling arthritis. There was no significant difference in the frequency of chronic disabling arthritis between patients with CC genotype and AA genotype, or between those with CC genotype and AA genotype.

DISCUSSION
Our study, which includes the largest number of patients...
with AOSD from a single center, was to investigate functional association of promoter polymorphisms at position –607 of IL-18 exon 1 with clinical manifestations and various patterns of disease course in Chinese patients. We found that the frequencies of the SNP –607/AA genotype were significantly lower in patients with AOSD compared to healthy controls, suggesting that the SNP –607/AA genotype of the IL-18 promoter might be a genetically protective factor against occurrence of AOSD in the Chinese population. In contrast, significantly higher frequencies of the CA genotype relative to the AA genotype were observed in our patients with AOSD. Although the studied diseases were different, our data were similar to the findings of previous studies, which showed that the SNP –607/AA genotype might confer a protective effect against development of rheumatoid arthritis and lupus nephritis in Chinese populations. A recent study showed that the A allele at position –607 in the IL-18 promoter region may be associated with the susceptibility to AOSD in a Japanese population. The A allele at position –607, one component of haplotype S01 of IL-18 gene reported by Sugiura, et al, was associated with the development of AOSD in a Japanese population. An explanation for this discrepancy between previous reports and our study might be the variation of genetic susceptibility between ethnic groups. Nevertheless, our results and recent reports supposed that the regulatory regions of IL-18 gene are related to the development of AOSD.

Previous studies have shown that promoter polymorphisms of IL-18 exon 1 were associated with variable levels of IL-18 mRNA expression in Swedish patients with multiple sclerosis, and that promoter activity at the upstream site of exon 1 could be upregulated by activation. Our results demonstrated that serum IL-18 levels were significantly lower in AOSD patients with the AA genotype than in those with the CA genotype or CC genotype (Figure 2B). Our data were consistent with recent results that showed lupus patients with AA genotype had significantly lower IL-18 levels than those with the CC and CA genotypes. Comparison of promoter polymorphisms of IL-18 gene with sequences in the TRANSFAC (Gene Transcription Factor) database suggest that the change from C to A at position –607 possibly disrupts a potential CREBP binding site and may reduce IL-18 production. However, interindividual differences in the capacity to produce IL-18 may be affected by differences in transcription rate, translation efficiency, or protein processing: this gene dosage effect was not expected. It is possible that unidentified genetic polymorphisms exist in IL-18 gene and affect the expression of IL-18 protein.

The disease course and prognosis of patients with AOSD may vary considerably. Although the prognosis of AOSD is generally considered to be relatively benign, patients with the chronic articular course exhibited greater disability and had a worse prognosis than those with the other 2 disease courses. In our study, significantly lower frequencies of the SNP –607/AA genotype were observed in those with chronic articular course compared with those with the SNP –607/CA genotype (Figure 3B). Concerning their functional associations, we found that the AA genotype was more frequently observed in patients with monocyclic systemic course, which had lower levels of serum IL-18, than in those with the other 2 disease courses. Based on these data, we speculate that patients with AOSD carrying the SNP –607/AA genotype might have a lower risk of progression to chronic disabling arthritis. However, we cannot exclude the possibility that other genetic factors are important in determining the disease outcome of AOSD, and additional investigation will be needed to clarify the prognostic importance of polymorphisms in other cytokines.

The SNP –607/AA genotype, which is associated with...
lower IL-18 levels, is also associated with a favorable prognosis in Chinese patients with AOSD. Because AOSD has diverse disease phenotypes, complex roles of multiple cytokines, and the possible polygenic etiology, investigation of a single gene polymorphism is probably insufficient to explain the effects of a particular gene on the susceptibility of this disease. Further investigation of genetic polymorphisms of various pathogenesis-associated molecules in different ethnic groups is needed.

REFERENCES