

Tumor necrosis factor- α and interleukin-4 gene polymorphisms in Chinese patients with gout

M.-L. Chen¹, F.-J. Tsai^{1,4}, C.-H. Tsai^{1,3}, C.-M. Huang^{2,4}

¹Department of Medical Genetics, Division of Immunology and Rheumatology, ²Department of Internal Medicine, and ³Graduate Institute of Bioinformatics, Asia University, China Medical University Hospital, Taichung, Taiwan; ⁴College of Chinese Medicine, China Medical University, Taichung, Taiwan.

Abstract

Objective

The purpose of this study was to examine whether polymorphisms of interleukin-4 (IL-4) (promoter-590 and intron 3) and tumor necrosis factor- α (TNF- α) promoter-308 genes are markers of susceptibility to or clinical manifestations of gout in Taiwanese patients.

Methods

The study included 196 Taiwanese patients with gout and 103 unrelated healthy control subjects living in central Taiwan. Polymorphisms of the IL-4 (promoter-590 and intron 3) and TNF- α (promoter-308) genes were typed from genomic DNA. Allelic frequencies and carriage rates were then compared between gout patients and control subjects. The correlation between allelic frequencies, carriage rates and clinical manifestations of gout were evaluated.

Results

No significant differences were observed in the allelic frequencies and carriage rates of the IL-4 (promoter-590 and intron 3) and TNF- α gene polymorphisms between patients with gout and healthy control subjects. Furthermore, the IL-4 (promoter-590 and intron 3) and TNF- α genotypes were not found to be associated with the clinical and laboratory profiles in gout patients. However, there was a significant difference in the TNF- α polymorphism genotype between patients with and without hypertriglyceridemia ($P=0.001$, $\chi^2=11.47$, $OR=10.3$, $95\%CI=3.57-29.7$).

Conclusions

The results of our study suggest that polymorphisms of the IL-4 (promoter-590 and intron 3) and TNF- α promoter-308 genes are not related to gout in Chinese patients in Taiwan.

Key words

IL-4, TNF- α , gout, gene polymorphism.

Man-Ling Chen, MD; Fuu-Jen Tsai, MD;
Chang-Hai Tsai, MD; Chung-Ming Huang,
MD.

This study was supported by a grant
(DMR-90-107) from China Medical
University Hospital of Taiwan.

Please address correspondence to: Chung-
Ming Huang, MD, Division of Immunology
and Rheumatology, Department of Internal
Medicine, China Medical University
Hospital, No. 2 Yuh Der Road, Taichung,
Taiwan.

E-mail: hcm.jeffrey@msa.hinet.net

Received on July 18, 2006; accepted in
revised form on December 15, 2006.

© Copyright CLINICAL AND
EXPERIMENTAL RHEUMATOLOGY 2007.

Introduction

Gout is one of the oldest known forms of arthritis (joint inflammation) and holds a unique place in the history of medicine. The most specific clinical manifestation is the appearance of monosodium urate (MSU) crystals in the joints that induce acute attacks of gouty arthritis. However, we are unable to explain why these crystals are present in the synovial fluid of asymptomatic uninfamed joints (1). MSU crystals in the joint are most probably macrophage-like synovial cells that induce the release of vasoactive prostaglandins, proteases, and proinflammatory cytokines including interleukin 1 (IL-1), TNF- α , IL-6, and IL-8, which in turn initiate a vigorous inflammatory response (2).

Cytokine is the general term for a large group of molecules involved in signaling between cells during immune responses. Among the cytokines, IL-4 has anti-inflammatory properties, but its production is decreased or absent in rheumatoid synovial tissue (3). The IL-4 gene has been mapped to the q arm (q23-31) of chromosome 5 in a cluster of cytokine genes (IL-3, IL-5, IL-9, IL-13, IL-15, GM-CSF, and interferon regulatory factor). Different polymorphisms of the IL-4 gene have been described, and at least two of them may influence protein production. One of these is a C to T change upstream of all previously described control elements of IL-4 at position -590 from the first ATG codon (4). The other is located in the third intron, and is composed of a variable number of tandem repeats (VNTR) of a 70-bp sequence (5).

IL-4 is a key cytokine that induces the activation and differentiation of B cells as well as development of the Th2 subset of lymphocytes. Th2 cytokines such as IL-4, IL-6, and IL-10 primarily support antibody production and many studies have confirmed that rheumatoid arthritis synovial fluids (RA SF) contain either no (< 15 pg/ml) or very low amounts (< 25 pg/ml) of IL-4 (3).

The gene encoding TNF- α is located in the major histocompatibility complex (MHC) class III region of chromosome 6 and is highly polymorphic. Previous studies have demonstrated consider-

able genetic diversity at the TNF locus (6). The importance of this locus in inflammatory disorders has been confirmed. The association of various alleles of TNF- α with several autoimmune disease phenotypes confirms the importance of this locus in inflammatory disorders (7). In particular, the -308 A allele has been shown to be associated with high promoter activity (8). However, no previous study has demonstrated the relationship between the IL-4 gene and TNF- α gene polymorphisms and gouty arthritis. Therefore, we examined two polymorphisms of the IL-4 gene (promoter-590 and intron 3) and one of the TNF- α gene (promoter-308) to determine whether they might serve as markers of susceptibility to and/or the severity of gout by comparing allelic frequencies in 196 patients with gout and 103 healthy individuals.

Patients and methods

Gout patients and healthy controls

We enrolled 196 patients with gout (14 women, 182 men; average age 49.5 yrs, range 18-84 yrs) according to the 1977 revised American College of Rheumatology (ACR) criteria (9). In addition, 103 sex- and age-matched unrelated, healthy individuals living in central Taiwan served as control subjects. Informed written consent was obtained from both groups participating in this study.

Definition of variables

Hyperuricemia was defined as ≥ 7.0 mg/dl serum uric acid for males and ≥ 6.0 mg/dl for females. Three consecutive blood pressure readings were taken from the right arm at least 5 min apart with the subject seated. Diastolic blood pressure was measured at the fifth phase. Definite hypertension was defined if the average of three readings was $\geq 160/95$ mmHg. Hypertriglyceridemia was defined as serum triglyceride ≥ 200 mg/dl, and hypercholesterolemia was defined as serum cholesterol ≥ 200 mg/dl.

DNA extraction

Genomic DNA was prepared from peripheral blood by a genomic DNA isolation reagent kit (Genomarker, Taiwan).

Competing interests: none declared.

Polymorphism analysis

IL-4 intron 3 polymorphism. PCR was performed in a total reaction volume of 25 μ l with 2.5–10 pmole of each primer containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.0 mM MgCl₂, 0.2 mM of each deoxyribonucleotide triphosphate, and 1 unit of Amplitaq DNA polymerase (Perkin Elmer, Foster City, CA, USA). The cycling conditions were as follows: one cycle at 94°C for 5 minutes; 35 cycles at 94°C for 20 seconds, 58°C for 20 seconds, 72°C for 20 seconds, and one final extension cycle at 72°C for 10 minutes. The cycles were performed in a Perkin-Elmer 2400 thermal cycler. Primers were upstream 5'-AGGCTGAAAGGGGAAAGC-3' and downstream 5'-CTGTTCACCTCAACTGCTCC-3'. The IL-4 intron 3 polymorphism PCR products, including 70-bp VNTR, were directly analyzed by electrophoresis on 3% agarose gel and each allele was recognized according to its size. The RP1 and RP2 alleles were 183-bp and 253-bp, respectively.

IL-4 polymorphism at position -590. PCR and cycling conditions were performed as previously described. Primers for this polymorphism were upstream 5'-ACTAGGCCTCACCTGATACG-3' and downstream 5'-GTTGTAATGCAGTCCTCCTG-3'. The PCR products were studied after *Bsm* FI restriction enzyme digestion (New England Biolabs, Beverly, MA, USA). Two alleles exist at the -590 position. *Bsm* FI causes cleavage of a restriction site at the -590 position when the *C allele is present, giving rise to fragments 60 and 192bp in length, or 252bp when the *T allele is present. The digested fragments were analyzed by electrophoresis on 3% agarose gels.

TNF- α polymorphism at position -308. The primer for the TNF α promoter -308 polymorphism was designed as upstream 5'-AGGCAA TAGGTTTTGAGGGCCAT-3' and downstream 5'-ACACTCCCCATCCTCCCGGCT-3' according to the procedure described by Galbraith *et al.* (10). The cycling conditions for promoter -308 were set as follows: one cycle at 94°C for 5 min, 35 cycles at 94°C for 30 sec, 60°C for 40

sec, and 72°C for 40 sec, and one final cycle of extension at 72°C for 7 min. The PCR product (117-bp) was mixed with 2 units of *Nco* I (New England Biolabs, Beverly, USA) and the reaction buffer according to the manufacturer's instructions. The restriction site was designed to be located at the frequently seen allele of promoter -308 (A) to form a digestible site. Two fragments of 97-bp and 20-bp were present if the product was digestible. The reaction was incubated for 3 hours at 37°C. Then, 10 μ l of the product was loaded onto 3% agarose gel containing ethidium bromide for electrophoresis. The polymorphism was subdivided into AA homozygote (digestible), GG homozygote (undigestible) and A/G heterozygote.

Statistical analysis

Allelic frequency was expressed as a percentage of the total number of alleles. Results from control subjects and gout patients were compared by the χ^2 test (2x2 contingency tables) for statistical significance. *P* values < 0.05 were considered statistically significant. Odds ratios (OR) were calculated from allelic frequencies with 95% confidence intervals (95% CI).

Results

The distribution of the TNF- α genotypes, allelic frequencies and carriage rates among the control subjects and the patients with gouty arthritis is shown in Table I. The distribution of the

G/G homozygote in the control group was 86.4%, and the distribution of the G/A homozygote was 13.6%. In gout patients, the distribution of G/G was 83.7%, and that of G/A was 16.3%. Using the chi-square test, we compared the distribution of the TNF- α genotypes, allelic frequencies and carriage rates by the χ^2 test and found no significant differences between the patients with gout and the healthy control subjects (*p* = 0.53, 0.55, and 0.59, respectively). Similarly, we detected no significant differences in genotype, allelic frequency or carriage rate, including those of the IL-4 promoter and IL-4 intron 3 regions, between the patients with gout and the healthy control subjects (Table II). The related clinical symptoms and laboratory findings, such as hypertension, tophi, alcohol drinking, hyperuricemia, hyperlipidemia, diabetic mellitus (DM), and gout family history are shown in Table III. Except for hypertriglyceridemia, we did not find any relationship between the TNF- α promoter genotype and the clinical symptoms and findings in patients with gout. The A/G genotype was significantly more common than the G/G in gout patients with hypertriglyceridemia (χ^2 = 11.47, *p* = 0.001), (OR = 10.3, 95% confidence interval 3.57–29.7). There was no significant association between the IL-4 intron 3 gene polymorphism and clinical symptoms and findings. We observed increased frequencies of tophi and hypercholesterolemia among patients with the C

Table I. Distribution of TNF- α genotypes, allelic frequencies and carriage rates among gout patients and healthy control subjects.

	Gout patients (%) n = 196	Controls (%) n = 103	<i>P</i>
Genotype			
TNF-alpha			
G/G	164 (83.7)	89 (86.4)	0.53
G/A	32 (16.3)	14 (13.6)	
A/A	0	0	
Allelic frequency			
TNF-alpha			
Allele G	360 (91.8)	192 (93.2)	0.55
Allele A	32 (8.2)	14 (6.8)	
Allelic carriage			
TNF-alpha			
Allele G	196 (86.0)	103 (88.0)	0.59
Allele A	32 (14.0)	14 (12.0)	

Table II. Distribution of IL-4 genotypes, allelic frequencies and carriage rates among gout patients and healthy control subjects.

	Gout patients (%) n = 196	Controls (%) n = 103	<i>P</i>
<i>Genotype</i>			
IL-4 promoter			
-590*C/-590*C	10 (5.1)	6 (5.8)	0.97
-590*C/-590*T	65 (33.2)	34 (33.0)	
-590*T/-590*T	121 (61.7)	63 (61.2)	
<i>IL-4 intron3</i>			
RP1/RP1	126 (64.3)	66 (64.1)	0.95
RP1/RP2	61 (31.1)	33 (32.0)	
RP2/RP2	9 (4.6)	4 (3.9)	
<i>Allelic frequency</i>			
IL-4 promoter			
Allele C	85 (21.7)	46 (22.3)	0.86
Allele T	307 (78.3)	160 (77.7)	
<i>IL-4 intron3</i>			
RP1	313 (79.8)	165 (80.1)	0.94
RP2	79 (20.2)	41 (19.9)	
<i>Allelic carriage rate</i>			
IL-4 promoter			
Allele C	75 (28.7)	40 (29.2)	0.92
Allele T	186 (71.3)	97 (65.7)	
<i>IL-4 intron 3</i>			
RP1	187 (72.8)	99 (72.8)	0.99
RP2	70 (27.2)	37 (27.2)	

Table III. Relationship between TNF- α genotypes and clinical symptoms and findings in patients with gout.

TNF- α	G/G (%)	A/G (%)	P
Hypertension	47 (28.9)	8 (25.0)	0.66
Tophi	32 (19.6)	10 (31.3)	0.16
Alcohol drinking	40 (24.5)	8 (25.0)	0.96
Hyperuricemia	127 (78.4)	22 (68.8)	0.25
Hypertriglyceridemia	33 (28.2)	8 (80.0)	0.001*
Hypercholesterolemia	62 (57.4)	8 (40.0)	0.15
Diabetic mellitus	19 (12.3)	5 (17.9)	0.42
Family history of gout	58 (37.7)	13 (46.4)	0.38

*Statistical analysis represents the results of hypertriglyceridemia compared with the subgroup with hypertriglyceridemia. $P = 0.001$, χ^2 test = 11.47, OR = 10.3, 95%CI = 3.57~29.7.

allele compared with patients with the T allele in the IL-4 promoter; however, the differences were not statistically significant ($p = 0.07$ and 0.09 , respectively). Similarly, we did not find any relationship between the IL-4 promoter polymorphism and other parameters of clinical symptoms of gout in Chinese patients in Taiwan (Table IV).

Discussion

Gout is not a single disease but a term used to describe a group of metabolic disorders characterized by deposition in the tissue of MSU monohydrate crystals from hyperuricaemic body

fluids. Acute gouty paroxysm is triggered by precipitation of MSU in the joints or neighboring tissues (1). MSU crystals in the joint are most probably macrophage-like synovial cells that induce the release of vasoactive prostaglandins, proteases, and proinflammatory cytokines including IL-1, TNF- α , IL-6, and IL-8, which in turn initiate vigorous inflammatory responses (2). TNF is a pluripotent cytokine that has been implicated in the pathogenesis of several chronic inflammatory diseases with an autoimmune component. TNF- α is a proinflammatory cytokine that regulates the acute-phase response,

vascular adhesion, bone marrow progenitor differentiation, and apoptosis (11). Mice transgenic for the overexpression or inappropriate expression of TNF- α develop systemic or organ-specific inflammation. An increase in the spontaneous release of TNF- α from the monocytes of gout patients has been reported by several researchers (12).

IL-4 is a cytokine with anti-inflammatory properties and is a specific and major defining product of Th2 cells. IL-4 has been shown to inhibit IL-1 β , IL-6, and TNF α as well as IgM and IgG production by human synovial tissue cultures, and IL-1 β , IL-6, and IL-8 by freshly isolated adherent synovial cells (13). The self-limitation of acute gouty inflammation has been known for a long time. Although many mechanisms can partly explain the spontaneous resolution in acute gout, the etiology of the disorder is still unknown. IL-4 may play an important role in the anti-inflammation effect of acute gout. Nucleotide variations in the human genome include single nucleotide polymorphisms (SNP), variable number tandem repeat (VNTR) polymorphisms, microsatellite polymorphisms and nucleotide deletions and insertions. The activity of individual genes may be regulated by certain nucleotide variations in the coding or non-coding region, which might influence gene transcription or alter the function of the gene product. Variations in the genome contribute to the genetic basis of human traits or disease. Both genetic and environmental factors contribute to the severity of susceptibility to gout. Gout and genetic polymorphisms of the genes responsible for immunity were not traditionally perceived as being causally related, but their relationship is being increasingly investigated. Some studies of the TNF- α gene polymorphism in rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) patients have shown conflicting results between patients and controls. Additionally, our previous studies demonstrated that IL-4 promoter and IL-4 intron 3 gene polymorphisms cannot serve as candidate gene markers for screening RA and SLE patients (14, 15). Gouty arthritis, like RA and

Table IV. Relationship between IL-4 intron 3 and promoter genotypes and clinical symptoms and findings in patients with gout.

IL-4 intron 3	Hypertension	Tophi	Alcohol drinking	Hyperuricemia	Hypertriglyceridemia	Hypercholesterolemia	Family history	DM
RP1/RP1 (%)	34 (27.2)	22 (17.6)	34 (27.2)	98 (78.4)	27 (33.3)	45 (54.2)	48 (41.4)	17 (14.7)
RP1/RP2 (%)	17 (27.9)	16 (26.2)	10 (16.4)	45 (75.0)	10 (25.0)	20 (51.3)	18 (31.6)	6 (10.5)
RP2/RP2 (%)	4 (44.4)	4 (44.4)	4 (44.4)	6 (66.7)	4 (66.7)	5 (83.3)	5 (55.6)	1 (11.1)
P value	0.57	0.12	0.10	0.10	0.13	1.30	0.27	0.74
Promoter	Hypertension	Tophi	Alcohol drinking	Hyperuricemia	Hypertriglyceridemia	Hypercholesterolemia	Family history	DM
C/C (%)	5 (50.0)	5 (50.0)	4 (40.0)	7 (70.0)	4 (57.1)	6 (85.7)	5 (50)	1 (10)
C/T (%)	19 (29.2)	14 (21.5)	12 (18.2)	49 (76.6)	14 (32.6)	19 (45.2)	23 (37.1)	6 (9.8)
T/T (%)	31 (25.8)	23 (19.2)	32 (26.7)	93 (77.5)	23 (23.0)	45 (57.0)	43 (39.1)	17 (15.5)
P value	0.29	0.07	0.24	0.87	0.35	0.09	0.74	0.56

SLE, is an inflammatory arthritis. In the present study we chose to screen two polymorphisms in the IL-4 gene (promoter and intron 3), and one in the TNF- α (-308) gene. No statistically significant differences in the IL-4 (promoter and intron 3) and TNF- α genotypes or allelic frequencies between gout patients and normal individuals were found. However, there was a significant difference in TNF- α -308 polymorphism between gout patients with and without hypertriglyceridemia. This suggests that TNF- α (promoter-308) is correlated with the hyperglyceridemia in gout, but not with gout itself.

Hyperuricemia is the most important direct risk factor for gout; other risk factors include male gender, age, hypertension, DM, renal insufficiency, alcohol abuse, family history, and coronary artery disease (16). Gout is also associated with hypertriglyceridemia. The association between gout and coronary artery disease is somewhat controversial. Gout may be associated with atherosclerosis only because of the high prevalence of obesity and hypertension (17) in patients with gout. In our study, the IL-4 (promoter and intron 3) and TNF- α genotypes and clinical signs, including hyperuricemia, tophus, hypertension, DM, alcohol drinking, family history and cholesterol, did not differ significantly between gout patients and controls. However, hypertriglyceridemia was statistically correlated to TNF- α (-308) gene polymorphism ($p = 0.001$). Inflammation, which is thought to be mediated by cytokines, is associ-

ated with hypertriglyceridemia. In fact, TNF- α was shown to increase serum triglyceride levels primarily by stimulating hepatic lipid synthesis and secretion (18). The association between TNF- α (-308) gene polymorphism and gout needs further confirmation.

In conclusion, the results from our present study suggest that the TNF- α promoter -308 polymorphism is correlated with the hyperglyceridemia in gout but not with gout itself.

References

- MASSEUD D, ROTT K, LIU-BRYAN R, AGUDELO C: Overview of hyperuricaemia and gout. *Curr Pharm Des* 2005; 11: 4117-24.
- DIGIOVINE FS: Interleukin 1 (IL-1) as a mediator of crystal arthritis. Stimulation of T cell and synovial fibroblast mitogenesis by urate crystal induced IL-1. *J Immunol* 1987; 275: 745-9.
- MIOSSEC P, NAVILIAT M, DUPUY D *et al.*: Low levels of interleukin-4 and high levels of transforming growth factor β in rheumatoid synovitis. *Arthritis Rheum* 1990; 33: 1180-7.
- LE BEAU MM, LEMONS RS, ESPINOSA R *et al.*: Interleukin-4 and interleukin-5 map to human chromosome 5 in a region encoding growth factors and receptors and are deleted in myeloid leukemias with a del (5q). *Blood* 1989; 73: 647-50.
- MOUT R, WILLEMZE R, LANDEGENT JE: Repeat polymorphisms in the interleukin-4 gene. *Nucleic Acid Res* 1991; 19: 3763.
- UDALOVA IA, NEDOSPASOV SA, WEBB GC, CHAPLIN DD, TURETSKAYA RL: Highly informative typing of the human TNF locus using six adjacent polymorphic markers. *Genomics* 1993; 16: 180-6.
- MONOS DS, KAMOUN M, UDALOVA JA *et al.*: Genetic polymorphism of the human tumor necrosis factor region in insulin-dependent diabetes mellitus: linkage disequilibrium of TNF microsatellite alleles with HLA haplotypes. *Hum Immunol* 1995; 44: 70-9.
- WILSON AG, SYMONS JA, MCDOWELL TL, MCDEVITT HO, DUFF GW: Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. *Proc Natl Acad Sci USA* 1997; 94: 3195-9.
- WALLACE SL, ROBINSON H, MASI AT: Preliminary criteria for the classification of the acute arthritis of primary gout. *Arthritis Rheum* 1977; 20: 895-900.
- GALBRAITH GMP, PANDEY JP: Tumor necrosis factor alpha (TNF α) gene polymorphism in alopecia areata. *Hum Genet* 1995; 96: 433-6.
- BEUTLER B, CERAMI A: The biology of cachectin/TNF- α primary mediator of the host response. *Annu Rev Immunol* 1989; 7: 625-9.
- MATSUKAWA A, MIYAZAKI S, MAEDA T: Production and regulation of monocytes chemoattractant protein-1 in lipopolysaccharide- or monosodium urate crystal-induced arthritis in rabbits: roles of tumor necrosis factor α , interleukin-1, and interleukin-8. *Lab Invest* 1998; 78: 973-85.
- MOSMANN TR, CHERWINSKI H, BOND MW, GIEDLIN MA, COFFMAN RL: Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 1986; 136: 2348-57.
- WU MC, HUANG CM, CHEN HY, TSAI FJ: Polymorphisms of the interleukin-4 gene in Chinese patients with Systemic lupus erythematosus in Taiwan. *Lupus* 2003; 12: 21-5.
- HUANG CM, WU MC, WU JY, TSAI FJ: No association of IL-4 gene polymorphisms in Chinese patients with RA in Taiwan. *Clin Exp Rheumatol* 2002; 20: 871-2.
- ROUBENOFF R, KLAG MJ, MEAD LA *et al.*: Incidence and risk factors for gout in white men. *JAMA* 1991; 266: 3004-7.
- ABBOTT RD, BTAND FN, KANNEL WB, CASTELLI WP: Gout and coronary artery disease: the Framingham Study. *J Clin Epidemiol* 1988; 41: 237-42.
- FEINGOLD KR, SOUED M, ADI S *et al.*: Effect of interleukin-1 on lipid metabolism in the rat: similarities to and differences from tumor necrosis factor. *Arterioscler Thromb* 1991; 11: 495-500.