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BRIEF REPORT

Association of interferon-gamma gene polymorphism (+874A) with arthritis manifestation in SLE

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Abstract Systemic lupus erythematosus (SLE) is a complex autoimmune disease in which genetic factors strongly influence susceptibility. Cytokines such as the interferon-gamma (*IFNG*) gene play a key role in controlling the immunity and inflammation, and therefore their polymorphisms may affect these genes' expression levels among individuals. We investigated the frequency of *IFNG* gene intron (+874) polymorphism, previously reported to be associated with IFNG production, in SLE patients compared to a control group. This population-based case–control study includes 154 SLE patients and 154 healthy control subjects with similar ethnic backgrounds. The genotyping was determined by polymerase chain reaction sequence-specific primer method and using the Chi-squared test for analyzing the association between this single-nucleotide polymorphism and SLE. The allele frequencies of the *IFNG* (+874) gene polymorphism were not significantly different between SLE patients and control subjects (72.7 vs 77%). However, there was a significant association between A dominance model of inheritance with arthritis (odds ratio=7.64, 95% confidence interval=1.56–41.64, P=0.006, P_c=0.03). The result suggested that the +874 intron polymorphism of *IFNG* can be used as the marker for SLE susceptibility with arthritis in the Thai population.

Keywords Interferon-gamma · Polymorphisms · SLE · Thai population

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Introduction

Interferon-gamma (IFNG) is a signature cytokine of the Th1 subset of helper T cells. Several studies have indicated that alterable expression of the IFNG level has significant effects to the susceptibility of various autoimmune diseases [1]. There is a report showing significant increasing IFNG messenger ribonucleic acid expression in peripheral blood mononuclear cells of patients with SLE [2]. Increased IFNG plasma level as well as ex vivo production was observed in SLE patients compare to controls [3–5]. These findings support that the enhanced production of IFNG by mononuclear cells may trigger inflammatory responses. In addition, treatment with IFNG accelerated disease in the (NZBXNZW) F1 lupus model [6, 7]. A significant reduc-

Table 1 Genotype and allele frequencies for the +874 at the first intron of the *IFNG* gene in healthy controls and SLE patients

	Healthy controls (n=154)	SLE patients (n=154)	OR (95%CI)
Genotype frequencies			
A/A	92 (59.7%)	79 (51.3%)	0.71 (0.44–1.14)
A/T	53 (34.4%)	66 (42.8%)	1.43 (0.88–2.33)
T/T	9 (5.8%)	9 (5.8%)	1.00 (0.35–2.84)
Allele frequencies			
A	237 (77%)	224 (72.7%)	0.80 (0.55–1.17)
T	71 (23%)	84 (27.3%)	1.25 (0.86–1.83)

tion in SLE progression was observed in the (NZBXNZW) F1 mice treating anti-IFNG antibodies [8]. Some investigators observed an imbalance of Th1/Th2 cells in lupus nephritis using intracellular cytokine detection, indicating that Th1 cells was predominated in SLE patients with proteinuria [9] similar to the result from the mice model [10]. In contrary, decreasing number of Th1-like cells and IFNG production as well as the dysfunction of $CD8^+$ cells were also reported with nephritis in SLE [11–14]. The effect of IFNG on development of SLE-like syndrome in another animal model, MRL-lpr mice, was demonstrated to have dichotomic actions. The early prophylactic treatment exhibits favorable effects, and the late institution of treatment accelerates SLE development [15]. All evidences above indicated the complex role of IFNG in the regulation of SLE.

The *IFNG* gene is located on chromosome 12q24.1. In 1999, one study discovered a variable-length CA repeat in the first intron of this gene and allele 2; 12 CA repeat was associated with IFNG level production [16]. The next study of these researchers described +874 A/T single-nucleotide polymorphism (SNP) in the first intron of *IFNG*. The T allele of this SNP is associated with the high IFNG production [17]. One previous study reported that gene frequencies of eight different alleles for CA repeat in the first intron of the *IFNG* gene were not significantly different between SLE patients and controls in Eastern seaboard populations (Baltimore and Philadelphia). However, allele 2 appeared to be protective for arthritis while allele 6 was associated with more severe lupus. This data suggested that genetic variation in the *IFNG* gene might influence the disease course rather than being a disease-causing gene [18]. So far, there is no report of the association between +874 A/T SNP and SLE susceptibility. The aim of this study was to investigate the association of this SNP with SLE susceptibility and particularly with specific clinical manifestations that are influenced by this cytokine such as arthritis and nephritis.

Table 2 Clinical manifestation of patients with SLE in this study (n=144)

Clinical manifestation	Number of patients with SLE (%)
Malar rash	92 (63.9)
Discoid rash	48 (33.3)
Photosensitivity	63 (43.8)
Arthritis	110 (76.4)
Proteinuria	94 (65.3)

Materials and methods

Subjects

During August 2002 and September 2004, 154 Thai patients who fulfilled the criteria for diagnosis of SLE of the American College of Rheumatology 1982 (149 women and 5 men; mean age±SD=36±12.6 years) were enrolled from the lupus clinic at the King Chulalongkorn Memorial Hospital (tertiary referral center). Clinical and serological data were recorded as either absent or present, based on the data from the cumulative database obtained by chart review. One hundred and fifty-four volunteer-unrelated Thai healthy donors (117 women and 37 men; mean age±SD= 23±12.3 years) were recruited from the same ethnic background and examined after obtaining informed consent. The ethics committee of the faculty of Medicine, Chulalongkorn University, reviewed and approved this study.

DNA extraction

Deoxyribonucleic acid (DNA) was isolated from buffy coat collected with ethylenediaminetetraacetic acid as anticoag-

Table 3 Genotype and allele frequencies for the +874 at the first intron of the *IFNG* gene in SLE patients with and without arthritis

	SLE patients with arthritis (n=110)	SLE patients without arthritis (n=34)
genotype frequencies		
A/A	61 (55.5%)[a]	13 (38.2%)
A/T	46 (41.8%)[a]	15 (44.1%)
T/T	3 (2.7%)	6 (17.6%)
Allele frequencies		
A	168 (76.4%)[b]	41 (60.3%)
T	52 (23.6%)	27 (39.7%)

[a] AA and A/T genotype: OR=7.64, 95%CI=1.56–41.64, P=0.006, P_c=0.03
[b] OR=2.13, 95%CI=1.15–3.94, P=0.01, P_c=0.05

ulant, using a salting-out method as previously described [19].

Genotyping methodology and DNA sequencing

Polymorphisms at +874 within intron 1 of the *IFNG* gene were identified using the polymerase chain reaction (PCR) sequence-specific primer method. All DNA were amplified with the use of the *IFNG* gene-specific primers described by Pravica et al. [17]. The positive results of the *IFNG* gene and the human growth hormone gene showed a band of 261- and 428-bp fragments, respectively. Additionally, the PCR products were analyzed to confirm the results of *IFNG* genotyping by DNA sequencing. Specific primers for sequencing were described previously [20].

Statistical analysis

Hardy–Weinberg equilibrium (HWE) was determined by Pearson's χ^2 goodness-of-fit test. Allele and genotype frequencies were compared between groups using the Chi-square (χ^2) test or Fisher's exact probability test, when appropriate. A P value of less than 0.05 was considered significant. In the case of multiple comparisons, corrected P value (P_c) for a number of comparisons (Bonferroni correction) was applied. Odds ratios (OR) with 95% confidence interval (CI) were calculated using the statistical program Epi Info version 6 [21]. Furthermore, the mode of inheritance analysis was also included.

Result

Genotype and allele frequencies for the +874 at the first intron of the *IFNG* gene in healthy controls and SLE patients were summarized in Table 1. Genotype frequencies of healthy Thai individuals were in the HWE. There were no significant differences in allele frequency of +874 A/T polymorphism at the first intron of the *IFNG* gene between patients with SLE and the healthy controls. The clinical expression of SLE is tremendously varied among individuals. We analyzed the association between five common clinical manifestations in SLE patients and polymorphism of the *IFNG* gene. In this study, we obtained clinical data of 144 patients that are shown in Table 2. There was a significant association between the +874A allele with arthritis (OR=2.13, 95%CI=1.15–3.94, P=0.01, P_c=0.05). The effect of the +874A allele was similar to the autosomal dominance mode of inheritance in which the presence of one A allele (AA or AT) conferred the significant OR as high as 7.64 (95%CI=1.56–41.64, P=0.006, P_c=0.03; Table 3).

Discussion

The result from this study did not show a positive association between the +874 A/T polymorphism in the first intron of the *IFNG* gene and susceptibility to SLE in the Thai population. Because SLE is a complex disease, several genes may contribute to its pathogenesis. It is possible that the effect from the *IFNG* gene is very small in SLE susceptibility, and a larger study cohort is required to approach positive association. However, when we analyzed the polymorphism of the *IFNG* gene with clinical manifestations, we found significant association of the +874A allele with arthritis. One previous study indicated that individuals homozygous for allele 2; 12 CA repeat associated with a high level of IFNG production [16]. This allele 2 was later reported to be protective for arthritis in SLE [18]. Because there was an absolute correlation between the presence of +874T allele and the presence of high-producing allele 2, therefore, our result confirms previous observation regarding the protective role of IFNG to arthritis. It is interesting to note that this SNP coincides with the putative nuclear factor-kB-binding site that might have a functional consequence for the *IFNG* gene [17]. This observation strengthens prior suggestion that genetic variation in IFNG expression might influence the disease course rather than being a disease-causing gene.

In addition, the positive association was found between the amino acid polymorphism (Val14Met) within the IFNG receptor 1 gene and SLE [22, 23]. The genetic contribution that determined IFNG function might come from the receptor gene or the combination of both cytokine and receptor genes. These data help support that genetic variation in the *IFNG* gene might influence the disease course of SLE.

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