Lupus (2013) 22, 721–726

http://lup.sagepub.com

CONCISE REPORT

Interleukin-10 promoter polymorphisms and expression in Thai children with juvenile systemic lupus erythematosus

P Rianthavorn[1], C Chokedeemeeboon[2], T Deekajorndech[1] and K Suphapeetiporn[2]
[1]Division of Nephrology, Department of Pediatrics; and [2]Center of Excellence for Medical Genetics, Faculty of Medicine, Chulalongkorn University, Thailand

Interleukin (IL)-10 expression is regulated by its promoter and correlated with the activity of adult-onset lupus (systemic lupus erythematosus (SLE)). As the pathogenesis of adult-onset SLE may differ from SLE with the age at onset <18 years old (juvenile SLE or JSLE), we evaluated polymorphisms at positions −1082A/G, −819T/C and −592A/C of the *IL-10* promoter and serum IL-10 levels in 71 patients with JSLE. Disease activity was determined by the SLE disease activity index (SLEDAI). Active SLE was defined by SLEDAI ≥6 and inactive SLE was defined by SLEDAI equal to zero. The mean age was 14.5 ± 2.8 years. Nephritis occurred in 57 patients. In JSLE patients, −592 CC and −819 CC were identified with a higher frequency than in controls with the odds ratio (OR) of 2.75 (95% confidence interval (CI) 1.11–6.81, $p = 0.04$). GCC increased the susceptibility to nephritis in patients with JSLE (OR 2.16, 95% CI 1.07–4.35, $p = 0.03$). Serum IL-10 levels were significantly higher in 20 JSLE patients with active disease than in 27 patients with inactive disease and in 15 healthy children ($p < 0.001$). In conclusion, IL-10 expression was upregulated in active JSLE. The −819 CC and −592 CC genotypes increased the susceptibility to JSLE and GCC increased the susceptibility to nephritis. *Lupus* (2013) **22,** 721–726.

Key words: Interleukin-10; juvenile systemic lupus erythematosus; single-nucleotide polymorphisms

Introduction

Systemic lupus erythematosus (SLE) is a chronic inflammatory disorder characterized by the production of autoantibodies and immune complex deposition in various tissues. The etiology of SLE is partially elucidated although recent studies have shown that the interaction between polygenic and environmental factors could be an important mechanism. Multiple genes are proven to be a predisposing factor to SLE thus they are commonly named SLE susceptibility genes.[1]

IL-10, a gene located within the major SLE susceptibility locus on chromosome 1,[2] has gained much interest as an SLE susceptibility gene. IL-10, the gene product, plays a substantial role in the pathogenesis of SLE as the cytokine facilitates antibody production by stimulating the proliferation and differentiation but decreasing the apoptosis of B-lymphocytes. Additionally, IL-10 upregulation was observed in patients with SLE[3] and in some of their unaffected relatives.[4] Moreover, SLE activity was correlated with serum IL-10 levels.[5]

The IL-10 expression is under the regulation of its promoter gene at the transcriptional level as evidenced in previous studies that variants of the *IL-10* promoter are associated with either a decrease or an increase of IL-10 production, which in turn may reduce or magnify the susceptibility to SLE.[6–9] Many studies have confirmed that a number of single-nucleotide polymorphisms (SNPs) of the *IL-10* promoter, for example SNPs at the positions −1082, −819 and −592, are associated with the increase of the susceptibility and severity of SLE.[6,7,9]

The age of SLE onset can influence the severity, clinical manifestations and outcomes of the disease. Patients with juvenile SLE (JSLE), defined by the age of less than 18 years at SLE onset,[10] experience major organ involvement and positive

Correspondence to: Pornpimol Rianthavorn, Division of Nephrology, Department of Pediatrics, Faculty of Medicine, Chulalongkorn University, 1873 King Rama 4 Road, Pathumwan, Bangkok 10330, Thailand.
Email: pornpimol.r@chula.ac.th
Received 1 October 2012; accepted 19 March 2013

© The Author(s), 2013. Reprints and permissions: http://www.sagepub.co.uk/journalsPermissions.nav
10.1177/0961203313486192

autoantibodies more frequently than patients with adult-onset SLE do.[11,12] The discrepancy in the natural history between patients with JSLE and adult-onset SLE suggests that the pathogenesis of SLE in these two settings may be different.

Although the data on the genetic variations of *IL-10* promoter gene and IL-10 expression in adult-onset SLE are abundant, there have been no studies on the role of IL-10 and its promoter gene in JSLE. In order to better understand the role of IL-10 on the pathogenesis of JSLE, we studied the expression of IL-10 and SNPs at the positions −1082, −819 and −592 of the *IL-10* promoter gene in patients with JSLE.

Patients and methods

Patients

Patients aged less than 18 years old with the diagnosis of SLE based on the 1997 American College of Rheumatology (ACR) revised criteria[13] were recruited. Ethylenediaminetetraacetic acid (EDTA) blood was collected for genotyping and sera were obtained for IL-10 measurement. Additional blood and urine evaluation were performed in each patient as indicated by the clinical status. Demographic and clinical data were obtained by medical record review. Disease activity was evaluated using the SLE Disease Activity Index (SLEDAI).[14] Active SLE was defined as SLEDAI ≥6 and inactive SLE was defined as SLEDAI equal to zero.[15] Renal biopsy-proven nephritis was interpreted according to the International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification criteria.[16] The study was approved by the Institutional Review Board Committee of the Faculty of Medicine, Chulalongkorn University, and complied with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects. Informed consent was obtained at the time of enrollment, and patient anonymity was preserved.

DNA extraction

Genomic DNA was extracted from peripheral blood leukocytes using a genomic DNA isolation reagent kit (QIAamp DNA Blood Mini Kit, Valencia, CA, USA) according to the manufacturer's instructions. The DNA samples were then stored at 4°C until further use.

Il-10 genotyping

IL-10 promoter SNPs at the positions −1082 G/A, −819 C/T and −592 C/A were amplified by polymerase chain reaction (PCR) and detected by restriction fragment length polymorphism (RFLP) technique.

For the position −592 C/A, the PCR reaction was performed using a 20 μl reaction mixture containing 100 ng of sample DNA, 1X PCR buffer (Fermentas Life Sciences, Glen Burnie, MD, USA), 1.5 mM MgCl2, 0.2 μM forward primer (5′GGTGAGCACTACCTGACTAGC3′), 0.2 μM reverse primers (5′CCTAGGTCACAGTGACGT GG3′) and 0.5 U *Taq* polymerase (Fermentas Life Sciences, Glen Burnie, MD, USA) as follows: initial denaturation at 94°C for three minutes, 35 cycles of 94°C for 30 seconds, 56°C for 30 seconds, 72°C for 30 seconds and final extension at 72°C for five minutes. RFLP was performed using a 10 μl reaction mixture digested with 5 U of *Rsa*I (New England Biolabs, Ipswich, MA, USA) at 37°C for 16 hours followed by electrophoresis on 1.5% agarose gel (Vivantis Inc, Oceanside, CA, USA) stained with ethidium bromide. If the C allele is present, the PCR product will be uncleaved with 412 bp. If the A allele is present, the PCR product will be cleaved into two fragments with 176 and 236 bp.

For the position −819 C/T, the PCR reaction was performed using a 20 μl reaction mixture containing 100 ng of sample DNA, 1X PCR buffer (Fermentas, Life Sciences, USA), 1.5 mM MgCl2, 0.2 μM forward primer (5′AGTAAGGGACCTCCTATCCA GCC3′), 0.2 μM reverse primers (5′CTCAAAGTT CCCAAGCAGCC3′) and 0.5 U *Taq* polymerase (Fermentas, Life Sciences, USA) as follows: initial denaturation at 94°C for three minutes, 35 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 35 seconds and final extension at 72°C for five minutes. RFLP was performed using a 10 μl of reaction mixture digested with 5 U of *Msl*I (New England Biolabs, Ipswich, MA, USA) at 37°C for 16 hours followed by electrophoresis on 2.5% agarose gel (SeaKem® LE Agarose gel Cambrex Bio Science Rockland Inc, Rockland, ME, USA) stained with ethidium bromide. If the T allele is present, the PCR product will be uncleaved with 520 bp. If the C allele is present, the PCR product will be cleaved into two fragments with 279 and 241 bp.

For the position −1082 C/A, the PCR reaction was performed in a 30 μl reaction mixture containing 50 ng of sample DNA, 1X Immobuffer (Bioline USA Inc, Taunton, MA), 2.5 mM MgCl2, 0.1 μM forward primer (5′CACACAAATCCAAGACAA CACTACTAAGGCTTCTTTCTGA3′), 0.1 μM

reverse primers (5′ATAGTGAGCAAACTGA GGCACAGAG3′), 3 μL of DMSO and 0.5 U IMMOLASE™ DNA Polymerase (Bioline USA Inc, Taunton, MA, USA) as follows: initial denaturation at 94°C for 10 minutes, 40 cycles of 94°C for 30 seconds, 60°C for 60 seconds, 72°C for 10 seconds and final extension at 72°C for one minute. RFLP was performed using a 20 μl of reaction mixture digested with 5 U of *Acu*I (New England Biolabs, Ipswich, MA, USA) at 37°C for 16 hours followed by electrophoresis on 2.5% (SeaKem® LE Agarose gel Cambrex Bio Science Rockland Inc, Rockland, ME, USA) stained with ethidium bromide. If the G allele is present, the PCR product will be uncleaved with 331 bp. If the A allele is present, the PCR product will be cleaved into two fragments with 289 and 42 bp.

Each polymorphic site was checked for Hardy-Weinberg equilibrium and linkage disequilibrium as previously described.[17] Subsequently, haplotypes of the *IL-10* promoter were reconstructed by using PHASE calculation software.[18] Frequencies of genotypic and haplotypic variants of the *IL-10* promoter in patients with JSLE were compared with those of 160 unrelated healthy Thai people previously as reported.[17]

Measurement of serum IL-10 levels by enzyme-linked immunosorbent assay (ELISA)

The levels of serum IL-10 from patients with JSLE and 15 healthy children were measured in duplicates using a commercially available ELISA kit (Quantikine® Human IL-10 Immunoassay; R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. Optical density (OD) values were read at 450 nm using a BiotrakII Plate Reader (Biosciences, Amersham, Sweden).

Statistical analysis

Genotype frequencies were tested for Hardy-Weinberg equilibrium by Pearson's χ^2 goodness of fit test. Estimated haplotype frequencies from genotypic data were analyzed using PHASE calculation software.[18] Descriptive data are reported using mean and standard deviation. Allele and haplotype frequencies were compared between groups using the chi square tests or Fisher's exact test, where appropriate. The strength of association was assessed by calculating odd ratios (ORs) and the exact limits for the 95% confidential interval (95% CI). Serum IL-10 levels were compared by using non-parametric testing (Mann-Whitney U test or Kruskal-Wallis one-way analysis of variance). The data were analyzed using IBM Statistical Package for the Social Sciences version 19 software (IBM Corporation, Armonk, NY, USA), and *p* values of less than 0.05 were considered to be statistically significant.

Results

Seventy-one patients (63 females, eight males) with JSLE were enrolled in the study. The average age of the patients was 14.5 ± 2.8 (range 7–18) years. Nephritis occurred in 57 patients (80%) and was classified as ISN/RPS class 1, 2, 3, 4 and 5 in 10.5%, 15.8%, 17.5%, 43.9% and 12.3%, respectively. Autoimmune antibodies including antinuclear antibodies (ANA), anti-double-stranded DNA (anti-dsDNA), anti-cardiolipin antibodies (aCL), anti-Sm, anti-RNP, anti-Ro and anti-La antibodies were found positive in 99%, 85%, 44%, 32%, 13%, 13% and 4% of the patients, respectively. The mean SLEDAI was 5 ± 6.8 (range 0–29). Twenty patients had active SLE (SLEDAI ≥6) while 27 patients had inactive SLE (SLEDAI equal to zero), and 24 patients had intermediary SLEDAI of 1 to 5.

The frequencies of the three SNPs at the positions −1082, −819 and −592 of the *IL-10* promoter in patients with JSLE, JSLE with nephritis and controls are shown in Table 1. The −1082 GG genotype was found in one JSLE patient who had nephritis but not in any controls. The frequencies of the −819 CC and −592 CC

Table 1 Genotypes of single-nucleotide polymorphisms for *IL-10* promoter at −1082, −819 and −592 in JSLE, JSLE with nephritis and controls[17]

Allele (%)	*Control* (n = *160*)	*JSLE* (n = *71*)	*JSLE with nephritis* (n = *57*)
−1082			
GG	0	1.4	1.8
GA	13.1	19.7	22.8
AA	86.9	78.9	75.4
−819			
CC	6.3	15.4[a]	14.0
CT	43.7	43.7	45.6
TT	50.0	40.8	40.4
−592			
CC	6.3	15.4[a]	14.0
AC	43.7	43.7	45.6
AA	50.6	40.8	40.4

IL-10: interleukin-10; JSLE: juvenile systemic lupus erythematosus; [a]odds ratio 2.75 vs. control ($p = 0.04$, 95% confidence interval (CI) 1.11–6.81).

genotypes were significantly higher in patients with JSLE than in controls. The OR of developing JSLE in patients with −819 CC and −592 CC genotypes when compared to controls was 2.75 ($p = 0.04$, 95% CI 1.11–6.81).

There are five SNP haplotypes described in the Thai population. Their frequencies in patients with JSLE, JSLE with nephritis and controls are shown in Table 2. ATA was the most common haplotype observed in both patients with JSLE and in controls. However, the frequency of the ATA haplotype was significantly lower in controls than in patients with JSLE. The OR of having JSLE in patients with the ATA haplotype when compared to controls was 0.64 ($p = 0.03$, 95% CI 0.42–0.97). The frequency of the GCC haplotype was significantly higher in JSLE with nephritis than in controls. The OR of having JSLE with nephritis when compared to controls in patients with the GCC haplotype was 2.16 ($p = 0.03$, 95% CI 1.07–4.35).

Table 2 Haplotype of *IL-10* promoter in JSLE, JSLE with nephritis and controls[17]

Haplotype (%)	*Control* (n = 160)	*JSLE* (n = 71)	*JSLE with nephritis* (n = 57)
ATA	71.6	61.3[a]	62.3
ACC	21.2	26.0	22.7
GCC	6.6	11.3	13.2[b]
ATC	0.3	0.7	0.9
ACA	0.3	0.7	0.9

IL-10: interleukin-10; JSLE: juvenile systemic lupus erythematosus; [a]odds ratio 0.64 vs. controls ($p = 0.03$, 95% confidence interval (CI) 0.42–0.97); [b]odds ratio 2.16 vs. controls ($p = 0.03$, 95% CI 1.07–4.35).

The frequencies of ATC and ACA, two rare haplotypes reported in Thai people, did not differ between patients with JSLE and controls.

The mean of serum IL-10 levels in patients with JSLE was 12.75 ± 16.94 pg/ml. Serum IL-10 levels did not significantly differ in JSLE patients with nephritis when compared to JSLE patients without nephritis (13.96 ± 18.32 vs. 7.86 ± 8.22 pg/ml). Serum IL-10 levels of patients with active SLE were compared to those of patients with inactive SLE and controls. Serum IL-10 levels were significantly higher in patients with active SLE than in patients with inactive SLE or in controls (Figure 1). There was no correlation observed between serum IL-10 levels and genotypic variants of the *IL-10* promoter in patients with JSLE (Table 3).

Discussion

IL-10 plays a central role in the pathogenesis of SLE as this cytokine increases humoral immune response, which in turn induces autoantibody production. IL-10 also inhibits cell-mediated immune response by decreasing the functions of T-lymphocytes and antigen-presenting cells thereby reducing the clearance of nuclear antigens of apoptotic cells. Formation and multi-organ deposition of immune complexes as a result of circulating self-antigens and autoantibody overproduction lead to extensive tissue damage in SLE.

As the production of IL-10 is regulated at the transcriptional level by the promoter gene, genotypic variations in this region could explain the

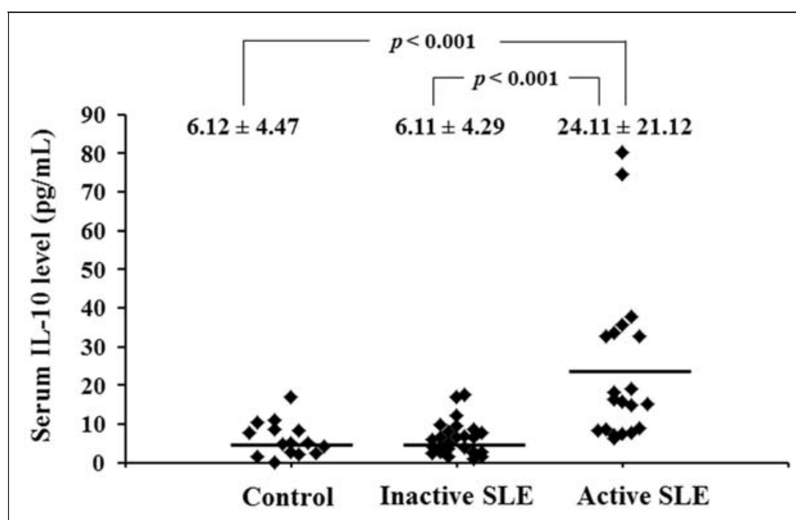

Figure 1 Serum interleukin (IL)-10 levels in controls, juvenile systemic lupus erythematosus (JSLE) patients with inactive disease and JSLE patients with active disease.

interindividual differences in the IL-10 production, which in turn influence SLE susceptibility.[6–9] The role of three SNPs at the positions −1082, −819 and −592 of the *IL-10* promoter upstream of the transcriptional initiation site has been widely investigated and many of these studies have confirmed the association between these SNPs and SLE susceptibility in patients with adult-onset SLE.[6,7,9] As the pathogenesis of adult-onset SLE may be different from that of JSLE, we investigated the association of these SNPs in patients with JSLE.

We observed that the frequencies of the −592 CC and −819 CC genotypes were significantly higher in patients with JSLE than in controls. The −592 allele is located within the negative regulatory region of the *IL-10* promoter. In peripheral blood mononuclear cells harboring −592 C, upregulation of IL-10 expression was evidenced because of the lack of the negative regulatory effect of the allele.[19] Thus, the increased susceptibility to JSLE could be the result of IL-10 upregulation in patients carrying −592 CC. The association between −592 CC and patients with adult-onset SLE was reported in Chinese but not found in Caucasians.[20,21] The conflicting findings between different studied populations suggest that ethnicity and environmental factors can modify the regulatory effect of −592 CC on IL-10 expression. The function of the −819 C allele in regards to IL-10 production is not completely validated. Increase of the −819 CC frequency in patients with JSLE may not entirely reflect the association of −819 CC and SLE susceptibility but could be the result of complete linkage disequilibrium between the −819 and −592 alleles.[17]

We did not observe the association between the −1082 GG genotype and JSLE susceptibility although the −1082 G allele is proven to be the most important positive regulatory factor for the constitutive and inducible expression of IL-10.[22,23] Moreover, the association of −1082 G with increased SLE susceptibility was reported in Caucasians with adult-onset SLE.[24] The significance of −1082 GG to JSLE susceptibility may be diminished by the rarity of −1082 G in Asians[25] as −1082 GG was found in one patient with JSLE and not in any controls.

The majority of the haplotypes identified in patients with JSLE and controls are ATA, GCC and ACC. We found an association between GCC and nephritis in patients with JSLE similar to the finding previously described in those with adult-onset SLE.[7] On the contrary, ATA was found with a lower prevalence in patients with JSLE than in controls, suggesting that ATA was associated with a decrease of susceptibility to JSLE. Genetic susceptibility to JSLE and nephritis could be attributed to the innate capability to produce IL-10. ATA is detected in low IL-10 secretors, which in turn decreases susceptibility to JSLE whereas GCC is proven to associate with high IL-10 production and may increase the prevalence of nephritis in patients with JSLE.[22,23]

The role of IL-10 in JSLE was confirmed by the finding that JSLE patients with active disease had higher serum IL-10 levels than JSLE patients with inactive disease and controls did. Although several studies have demonstrated that haplotypes of the *IL-10* promoter influence IL-10 expression, serum IL-10 levels were not correlated with variants of the promoter gene in patients with JSLE. There was no difference of serum IL-10 levels between −592 CC and the other genotypes or between GCC and ATA haplotypes.

The overall serum IL-10 levels are the combination of constitutive or innate and inducible IL-10 production. Constitutive IL-10 production in the general population has been proven to associate with its promoter.[22] However, inducible IL-10 levels are variable depending on ethnicity, patient's age, stimulating agents and cell types.[26] Moreover, tumor necrosis factor and interferon can regulate the IL-10 expression and fluctuation of these

Table 3 Serum IL-10 levels in relation to genotypic variants of the *IL-10* promoter gene in patients with JSLE

Genotypes	*JSLE (n = 71)*	*IL-10 level (pg/ml) Mean ± SD*
−1082		
GG	1	32.67
GA	14	8.33 ± 9.33
AA	56	13.50 ± 18.22
−819		
CC	11	12.33 ± 12.67
CT	31	10.25 ± 13.68
TT	29	15.59 ± 21.07
−592		
CC	11	12.33 ± 1267
AC	30	10.78 ± 13.79
AA	30	14.88 ± 20.93
GCC/GCC	1	32.67
ATA/ATC	2	12.92 ± 7.66
ATA/ACA	3	6.72 ± 8.00
GCC/ACC	5	11.16 ± 15.46
ACC/ACC	5	9.13 ± 7.38
GCC/ATA	9	6.59 ± 4.14
ACC/ATA	19	12.54 ± 16.82
ATA/ATA	27	15.79 ± 21.80

IL-10: interleukin-10; JSLE: juvenile systemic lupus erythematosus.

cytokine levels during the course of SLE can alter IL-10 activity.[27,28] Thus, serum IL-10 levels from JSLE patients with the same *IL-10* promoter could be different as in vivo IL-10 expression is not only under the regulation of its promoter gene but also depends on the levels of other cytokines.

In conclusion, a complex dysregulation of IL-10 observed in JSLE is a result of the interaction between multiple environmental and genetic factors. Genetic variants of the *IL-10* promoter are a predisposing factor to JSLE susceptibility and nephritis although it is unlikely that a single genetic variant of the *IL-10* promoter can explain the entire pathogenesis of JSLE. As a starting point, this study has shown that IL-10 is involved in the pathogenesis of JSLE and IL-10 levels are correlated with disease activity.

Funding

This work was supported by the Ratchadapiseksompoch Fund, Faculty of Medicine, Chulalongkorn University (grant number RA 40/52(2)).

Conflict of interest statement

The authors have no conflicts of interest to declare.

References

1 Sestak A, O'Neil KM. Familial lupus and antiphospholipid syndrome. *Lupus* 2007; 16: 556–563.
2 Johanneson B, Lima G, von Salomé J, Alarcón-Segovia D, Alarcón-Riquelme ME. A major susceptibility locus for systemic lupus erythemathosus maps to chromosome 1q31. *Am J Hum Genet* 2002; 71: 1060–1071.
3 Llorente L, Richaud-Patin Y, Wijdenes J, *et al*. Spontaneous production of interleukin-10 by B lymphocytes and monocytes in systemic lupus erythematosus. *Eur Cytokine Netw* 1993; 4: 421–427.
4 Llorente L, Richaud-Patin Y, Couderc J, *et al*. Dysregulation of interleukin-10 production in relatives of patients with systemic lupus erythematosus. *Arthritis Rheum* 1997; 40: 1429–1435.
5 Houssiau FA, Lefebvre C, Vanden Berghe M, Lambert M, Devogelaer JP, Renauld JC. Serum interleukin 10 titers in systemic lupus erythematosus reflect disease activity. *Lupus* 1995; 4: 393–395.
6 Lin YJ, Wan L, Huang CM, *et al*. IL-10 and TNF-alpha promoter polymorphisms in susceptibility to systemic lupus erythematosus in Taiwan. *Clin Exp Rheumatol* 2010; 28: 318–324.
7 Lazarus M, Hajeer AH, Turner D, *et al*. Genetic variation in the interleukin 10 gene promoter and systemic lupus erythematosus. *J Rheumatol* 1997; 24: 2314–2317.
8 Mok CC, Lanchbury JS, Chan DW, Lau CS. Interleukin-10 promoter polymorphisms in Southern Chinese patients with systemic lupus erythematosus. *Arthritis Rheum* 1998; 41: 1090–1095.
9 Rosado S, Rua-Figueroa I, Vargas JA, *et al*. Interleukin-10 promoter polymorphisms in patients with systemic lupus erythematosus from the Canary Islands. *Int J Immunogenet* 2008; 35: 235–242.
10 Ruperto N, Bazso A, Ravelli A, *et al*. The Paediatric Rheumatology International Trials Organization (PRINTO). *Lupus* 2007; 16: 670–676.
11 Brunner HI, Gladman DD, Ibañez D, Urowitz MD, Silverman ED. Difference in disease features between childhood-onset and adult-onset systemic lupus erythematosus. *Arthritis Rheum* 2008; 58: 556–562.
12 Hoffman IE, Lauwerys BR, De Keyser F, *et al*. Juvenile-onset systemic lupus erythematosus: Different clinical and serological pattern than adult-onset systemic lupus erythematosus. *Ann Rheum Dis* 2009; 68: 412–415.
13 Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997; 40: 1725.
14 Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. *Arthritis Rheum* 1992; 35: 630–640.
15 Abrahamowicz M, Fortin PR, du Berger R, Nayak V, Neville C, Liang MH. The relationship between disease activity and expert physician's decision to start major treatment in active systemic lupus erythematosus: A decision aid for development of entry criteria for clinical trials. *J Rheumatol* 1998; 25: 277–284.
16 Weening JJ, D'Agati VD, Schwartz MM, *et al*. The classification of glomerulonephritis in systemic lupus erythematosus revisited. *J Am Soc Nephrol* 2004; 15: 241–250.
17 Netsawang J, Tangwattanachuleeporn M, Hirankarn N, Wongpiyabovorn J. The distribution of IL-10 promoter polymorphism in Thais. *J Med Assoc Thai* 2004; 87(Suppl 2): S117–S122.
18 Stephens M, Donnelly P. A comparison of bayesian methods for haplotype reconstruction from population genotype data. *Am J Hum Genet* 2003; 73: 1162–1169.
19 Rosenwasser LJ, Borish L. Genetics of atopy and asthma: The rationale behind promoter-based candidate gene studies (IL-4 and IL-10). *Am J Respir Crit Care Med* 1997; 156: S152–S155.
20 Chong WP, Ip WK, Wong WH, Lau CS, Chan TM, Lau YL. Association of interleukin-10 promoter polymorphisms with systemic lupus erythematosus. *Genes Immun* 2004; 5: 484–492.
21 Crawley E, Woo P, Isenberg DA. Single nucleotide polymorphic haplotypes of the interleukin-10 5' flanking region are not associated with renal disease or serology in Caucasian patients with systemic lupus erythematosus. *Arthritis Rheum* 1999; 42: 2017–2018.
22 Suárez A, Castro P, Alonso R, Mozo L, Gutiérrez C. Interindividual variations in constitutive interleukin-10 messenger RNA and protein levels and their association with genetic polymorphisms. *Transplantation* 2003; 75: 711–717.
23 Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV. An investigation of polymorphism in the interleukin-10 gene promoter. *Eur J Immunogenet* 1997; 24: 1–8.
24 Nath SK, Harley JB, Lee YH. Polymorphisms of complement receptor 1 and interleukin-10 genes and systemic lupus erythematosus: A meta-analysis. *Hum Genet* 2005; 118: 225–234.
25 Hoffmann SC, Stanley EM, Cox ED, *et al*. Ethnicity greatly influences cytokine gene polymorphism distribution. *Am J Transplant* 2002; 2: 560–567.
26 Temple SE, Lim E, Cheong KY, *et al*. Alleles carried at positions −819 and −592 of the IL10 promoter affect transcription following stimulation of peripheral blood cells with *Streptococcus pneumoniae*. *Immunogenetics* 2003; 55: 629–632.
27 Platzer C, Meisel C, Vogt K, Platzer M, Volk HD. Up-regulation of monocytic IL-10 by tumor necrosis factor-alpha and cAMP elevating drugs. *Int Immunol* 1995; 7: 517–523.
28 Darnell Jr JE, Kerr IM, Stark GR. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science* 1994; 264: 1415–1421.