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Original Article

Association of single nucleotide polymorphisms and haplotypes of interleukin-10 5'flanking region with systemic lupus erythematosus susceptibility in Han Chinese

Bin Wang[1,2], Jin-Sen Lu[1,2], Xiao-Ke Yang[1,2], Fei-Fei Yuan[1], Hong Wang[1], Tian-Tian Lv[1,2], Yin-Guang Fan[1,2], Dong-Qing Ye[1,2]

[1]Department of Epidemiology and Biostatistics, School of Public Health, Anhui Medical University, Hefei, Anhui, P. R. China; [2]The Key Laboratory of Major Autoimmune Diseases, Anhui Medical University, Hefei, Anhui, P. R. China

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Abstract: Objective: To investigate the association of three SNP at -1082, -819, -592 and haplotypes of IL-10 5'flanking region with systemic lupus erythematosus susceptibility in Han population. Methods: 579 Han Chinese SLE patients fulfilling the ACR criteria and 632 ethnically matched controls were genotyped for IL-10 -1082 G>A, -819 C>T and -592C>A by TaqMan assay. Haplotypes were reconstructed via a mathematical model and analyzed by SHEsis software. Results: IL-10-1082G/A was associated with the susceptibility to SLE (OR=2.858, 95% CI: 2.245-3.640, P<0.001; GG vs. AA, OR=5.623, 95% CI: 2.299-13.754, P<0.001; GA vs. AA, OR=3.147, 95% CI, 2.324-4.261, P<0.001; GG+GA vs. AA, OR=3.313, 95% CI, 2.472-4.440, P<2=22.936, Pχ0.001). No significant differences were observed between SLE patients and controls in IL-10 promoter at position -819 C/T and -592 C/A. Analysis of the haplotypes revealed the haplotype ATA (formed by -1082A, -819T and -592A) that appeared to be a significantly protective effect against Chinese SLE patients (OR=0.663, 95% CI: 0.560-0.785, <2=3.736, P=0.053). χ^2=3.752, P=0.053; GCC, OR=1.311, 95% CI: 0.996-1.727, χ0.001). The ACC and GCC appeared no significance between patients and controls (ACC, OR=0.824, 95% CI: 0.677-1.002). Conclusion: Our findings strongly suggested that -1082G/A polymorphism of IL-10 promoter was associated with SLE and ATA haplotype may play a protective role in SLE of Chinese Han population.

Keywords: Systemic lupus erythematosus, interleukin-10, single nucleotide polymorphism, haplotype, susceptibility

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease driven by inflammation and characterized by imbalance of immune-reactive substance and multiple organ lesion which threatened the health of global population [1]. Past epidemiological evidence has revealed that the prevalence varies according to different regions but it is universally recognized that females are more susceptible than males. Han population rank the 2rd among nations worldwide for the prevalence of SLE with a 1:9 male to female ratio [2]. The pathogenesis of SLE is still in completely deciphered. However, there are two typical forms of immune abnormality in SLE: (1) An activation of humoral-immunity characterized by B cell hyperactivity and autoantibody production; (2) An impaired cell mediated immunity which results from both T lymphocyte and NK cell abnormalities [3]. Above-mentioned pathological damage causes angionecrosis and eventually leads to completely multiple organ dysfunctions.

Various pro-and anti-inflammatory cytokines, including IFN-α, IFN-γ, TGF-β, BAFF, Interleukin (IL)-6, IL-10, IL-17, and IL-23, participate in the pathogenesis of SLE [4]. Among these cytokines, IL-10 is an essential pleiotropic-immuno-

regulatory cytokine which not only inhibits the activation and the production of Th1 cell but also enhances B-cell survival, differentiation, proliferation and antibody production, which play a crucial role in autoimmune pathologies [5]. Preliminary studies have shown a correlation between high IL-10 levels and SLE, suggesting that the elevated IL-10 may trigger immune imbalance for SLE [6]. Treatment with anti-IL-10 antibodies can delay the onset of lupus disease due to up-regulation of TNF-α production [7]. Accumulating data have revealed that both genetic and environmental factors were responsible for SLE occurrence and genetic polymorphisms within promoter and coding regions of cytokine genes could influence the expression profile and eventually affect disease susceptibility. Multiple genome-wide linkage analysis, sequencing, association and variant researches demonstrated that the genetic variants of IL-10 contribute to SLE prevalence and clinical outcome. The human IL-10 gene is located on chromosome1 (1q31-1q32). Three common single nucleotide polymorphisms (SNPs): A G to A substitution at position-1082 (rs1800896), a T to C at -819 (rs1800871) and a C to A at -592 (rs1800872) are from the transcriptional start site in the 5'flanking region of the IL-10 gene regulating the expression level of IL-10.

In the past decade, various haplotypes in the IL-10 promoter including GCC, ACC, ATA, ATC, ACA, GTA and GTC have been thoroughly studied [8]. From all the possible haplotypes resulting from the combination of the three SNPs, only three have been described in all populations including ACC, ATA and GCC. It has been reported that lower ATA haplotype was in association with neuropsychiatric lupus in Chinese and Dutch populations while a recent meta-analysis based on 12 well-designed case control study demonstrated the lack of association between the haplotype GCC/ATA polymorphism in the IL-10 promoter and SLE risk [3, 8]. Moreover, other studies revealed that non-G carrier associated with lower IL-10 expression indicating a protective effect [9, 10]. However, investigators presented the controversial effects of these haplotypes to susceptibility of SLE [11].

To the best of our knowledge, the studies on IL-10 promoter polymorphisms or haplotypes and the risk of SLE in Han Chinese are quite rare. Therefore, we perform the present study to investigate the association of 3 SNPsat-1082, -819, -592 and haplotypes of IL-10 5'flanking region with systemic lupus erythematosus susceptibility in Han population.

Materials and methods

Patients and healthy controls

579 local patients with SLE were recruited from First Affiliated Hospital of Anhui Medical University and Anhui Provincial Hospital (61 men and 518 women). The diagnosis of SLE fulfilled the American College of Rheumatology (ACR) 1997 revised diagnostic criteria [12]. The mean age of SLE patients was 37.64 years (range 11-77 years). A total of 632 geographical-matched healthy controls (215 men and 417 women) were recruited. The mean age of controls was 30.57 years (range 21-78 years). All subjects were Han nationality from the same area and all written consents were obtained after explanations of the benefits and risks to them. Demographic data and routine laboratory data were collected from hospital records or by standard questionnaires. Medical history was reviewed from the onset of disease and detailed clinical characteristics of all patients were recorded.

Genotyping

Genomic DNA was extracted from EDTA-anticoagulated venous blood using FlexiGeneRDNA kits (QIAGEN, Hilden, Germany) according to the manufacturer's protocol [13]. The DNA concentration was measured and adjusted to 50 ng/μl by using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, Maltham, Massachusetts, USA). Finally, amplified samples were diluted to 10 ng/μl. Genotyping was performed by TaqMan SNP with an Applied Biosystems Prism 7300 PCR System (Applied Biosystems, Foster City, CA, USA) [14]. SNPs rs1800896, rs1800871 and rs1800872 were genotyped with assay-on-demand probes and primers (C_1747360_10 for rs1800896, C_1747362_10 for rs1800871 and C_1747363_10 for rs1800872; Applied Biosystems, USA). 4.0 μl of DNA sample, 0.1 μl of SNP Genotyping Assay Mix (Applied Biosystems), 5.0 μl of TaqMan Universal PCR Master Mix (Applied Biosystems), and 0.9 μl of ddH_2O were

Table 1. Analysis of Hardy-Weinberg equilibrium of polymorphisms in IL-10 5'flanking region [actual n (theoretical n)]

SNP	Subjects	Genotype*			χ^2	*P*
		11	12	22		
-1082G/A	Case	27 (26.13)	192 (193.74)	360 (359.13)	0.047	0.829
	Control	7 (4.70)	95 (99.60)	530 (527.70)	1.348	0.246
-819C/T	Case	48 (55.65)	263 (247.70)	268 (275.65)	2.208	0.137
	Control	72 (68.46)	272 (279.465)	288 (284.46)	0.408	0.523
-592C/A	Case	48 (55.34)	262 (247.32)	269 (276.34)	2.039	0.153
	Control	71 (68.13)	273 (278.75)	288 (285.12)	0.269	0.604

*11, 12 and 22 refer to G/G, C/C and C/C of rs100896, G/A, C/T and C/A of rs1800871, and A/A, T/T and A/A of rs1800872 respectively.

combined and PCR-amplified (50°C for 2 min, 95°C for 10 min, followed by 45 cycles of 95°C for 15 s and 60°C for 1 min).

Statistical analysis

The Chi-square test was used to estimate the Hardy-Weinberg equilibrium (HWE) in both SLE patients and healthy controls. Odds ratios (ORs) and 95% confidence interval (CIs) were also calculated by non-conditional logistic regression analyses. In order to exclude the impact of age and gender, the adjusted ORs and 95% CIs were also calculated. These analyses were performed using the SPSS 17.0 software (SPSSInc, Chicago, USA). Haplotype analysis on the polymorphisms was conducted with the SHEsis software [15, 16] (http://analysis.bio-x.cn/myAnalysis.php). Probability level, 0.05 in two-tailed test was used as a criterion of significance.

Results

The Hardy-Weinberg equilibrium results by goodness-of-fit Chi-square tests

As seen in **Table 1**, no deviations from Hardy-Weinberg equilibrium (HWE) were observed in either the patients with SLE or the controls in each polymorphism (HWE for IL-10 -1082G/A: Control χ^2=1.348, *P*=0.246, case, χ^2=0.047, *P*=0.829; For IL-10 -819C/T: control, χ^2=0.408, *P*=0.523, case, χ^2=2.208, *P*=0.137; For IL-10 -592C/A: Control, χ^2=0.269, *P*=0.604, case, χ^2=2.039, *P*=0.153).

Association between IL-10 polymorphisms and risk of SLE

IL-10-1082G/A: The genotype frequencies of IL-10 promoter -1082 GG, GA, and AA were 4.66%, 33.16%, and 62.18% in SLE patients and 1.11%, 15.03%, and 83.86% in healthy controls. Both genotype GG and GA were associated with the susceptibility to SLE as compared with the GG genotype (GG vs. AA, OR=5.623, 95% CI: 2.299-13.754, *P*<0.001; GA vs. AA, OR=3.147, 95% CI, 2.324-4.261, *P*<0.001). We also found a statistical significance in the dominant model (GG+GA vs. AA, OR=3.313, 95% CI, 2.472-4.440, *P*<0.001).

The allele frequencies of G and A were 21.24% and 78.76% in SLE patients and 8.62% and 91.38% in healthy controls. SLE patients had a potently higher frequency of the G allele compared with control subjects in Han population (OR=2.858, 95% CI: 2.245-3.640, *P*<0.001).

The genotype and allele frequencies of IL-10-1082G/A were shown in **Table 2**.

IL-10-819C/T: The genotype frequencies of IL-10 promoter -819 CC, CT and TT were 8.29%, 45.42% and 46.29% in SLE patients and 11.39%, 43.04% and 45.57% in healthy controls. There were no significant differences in genotype frequencies between patients and controls (CC vs. TT, OR=0.758, 95% CI: 0.494-1.164, *P*=0.206; CT vs. TT, OR=1.057, 95% CI, 0.819-1.366, *P*=0.669; CC+CT vs. TT, OR=0.995, 95% CI, 0.779-1.270, *P*=0.965).

The allele frequencies of C and T were 31.00% and 69.00% in SLE patients and 32.91% and 67.09% in healthy controls and no significant difference was detected (OR=0.916, 95% CI: 0.772-1.087, *P*=0.314).

The genotype and allele frequencies of IL-10-819C/T were shown in **Table 2**.

Table 2. Comparison of the allele and genotype frequencies of three SNPs in interleukin-10 flanking region

Alleles and genotypes	SLE9 (N=579)	Controls (N=632)	Unadjustment		Adjustment with sex and age	
			OR (95% CI)	*P*	*OR (95% CI)*	*P*
-1082 (G/A)					-	-
A	912 (0.7876)	1155 (0.9138)	1.000 (Ref.)	-	-	-
G	246 (0.2124)	109 (0.8620)	2.858 (2.245-3.640)	1.769×10^{-18}	-	-
AA	360 (62.18)	530 (83.86)	1.000 (Ref.)	-	1.000 (Ref.)	-
GG	27 (4.66)	7 (1.11)	5.679 (2.447-13.180)	0.000053	5.623 (2.299-13.754)	0.000154
GA	192 (33.16)	95 (15.03)	2.975 (2.249-3.937)	2.2685×10^{-14}	3.147 (2.324-4.261)	1.2381×10^{-13}
GG+GA	219 (37.84)	102 (16.14)	3.161 (2.412-4.142)	7.3237×10^{-17}	3.313 (2.472-4.440)	1.0385×10^{-15}
-819C/T						
T	799 (69.00)	848 (67.09)	1.000 (Ref.)	-	-	-
C	359 (31.00)	416 (32.91)	0.916 (0.772-1.087)	0.314	-	-
TT	268 (46.29)	288 (45.57)	1.000 (Ref.)	-	1.000 (Ref.)	-
CT	263 (45.42)	272 (43.04)	1.039 (0.819-1.318)	0.752	1.057 (0.819-1.366)	0.669
CC	48 (8.29)	72 (11.39)	0.716 (0.480-1.070)	0.103	0.758 (0.494-1.164)	0.206
CC+CT	311 (53.71)	344 (54.43)	0.972 (0.775-1.218)	0.802	0.995 (0.779-1.270)	0.965
-592C/A						
A	800 (69.08)	849 (67.17)	1.000 (Ref.)	-	-	-
C	358 (30.92)	415 (32.83)	0.915 (0.771-1.086)	0.312	-	-
AA	269 (46.46)	288 (45.57)	1.000 (Ref.)	-	1.000 (Ref.)	-
CA	262 (45.25)	273 (43.20)	1.027 (0.810-1.303)	0.823	1.039 (0.805-1.342)	0.768
CC	48 (8.29)	71 (11.23)	0.724 (0.484-1.082)	0.115	0.791 (0.514-1.216)	0.285
CA+CC	310 (53.54)	344 (54.43)	1.036 (0.827-1.300)	0.756	1.013 (0.793-1.293)	0.920

Table 3. Haplotype analysis of IL-10 promoter gene polymorphisms in SLE patients and controls [n (%)]

Haplotype	SLE (fre)	Control (fre)	χ^2	*P*	OR (95% CI)
ATA	678 (58.6)	816 (64.6)	22.936	1.700×10^{-6}	0.663 (0.560-0.785)
ACC	233 (20.1)	281 (22.2)	3.752	0.053	0.824 (0.677-1.002)
GCC	125 (10.8)	101 (8.0)	3.736	0.053	1.311 (0.996-1.727)

All the haplotype with a frequency <0.03 were ignored in the analysis.

IL-10-592C/A: The genotype frequencies of IL-10 promoter -592CC, CA and AA were 8.29%, 45.25%, 46.46% in SLE patients and 11.23%, 43.20%, 45.57% in healthy controls. There were no significant differences in genotype frequencies between patients and controls (CC vs. AA, OR=0.791, 95% CI: 0.514-1.216, *P*=0.285; CA vs. AA, OR=1.039, 95% CI, 0.805-1.342, *P*=0.768; CC+CA vs. AA, OR=1.013, 95% CI, 0.793-1.293, *P*=0.920).

The allele frequencies of C and A were 30.92% and 69.08% in SLE patients and 32.83% and 67.17% in healthy controls and no significant difference was detected (OR=0.915, 95% CI: 0.771-1.086, *P*=0.312).

The genotype and allele frequencies of IL-10-592C/A were shown in **Table 2**.

Haplotype analysis

Three main haplotypes (ATA, ACC and GCC) were determined by using SHEsis (**Table 3**). The haplotype ATA formed by -1082A, -819T and -592A showed a protective effect against Chinese SLE patients (OR=0.663, 95% CI: 0.560-0.785, χ^2=22.936, *P*=1.700×10^{-6}). The ACC and GCC appeared no significance between patients and controls (ACC, OR=0.824, 95% CI: 0.677-1.002, χ^2=3.752, *P*=0.053; GCC, OR=1.311, 95% CI: 0.996-1.727, χ^2=3.736, *P*=0.053).

Discussion

Accumulating evidence had revealed that the pathogeneticagents of SLE are prone to genetic heterogeneity of diverse population. Since the initiation of immune response is mediated by regulation of more or less cytokines, investigators have linked the genetic variation in the upstream controlling region of various cytokine with the susceptibility and progression of several autoimmune disease such as SLE [9, 17]. IL-10, a 36 kD homodimer cytokine once defined as cytokine synthesis inhibitory factor (CSIF) can suppress the secretion of IL-1, tumor necrosis factor (TNF-α), IL-6, IL-8 and IL-12 from monocytes/macrophages as well as interferon-γ (IFN-γ) and IL-2 from T cells [18, 19]. Diverse expressive level of IL-10 has not only participated in pathogenesis of several autoimmune disorders, including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and Sjogren's syndrome but also the maintenance of long-term transplantation tolerance [20]. Moreover, serum IL-I0 have also been indicated as a prognostic indicator of non-Hodgkin's lymphoma [21]. It is reported that SLE patients have an elevation of Th2 cytokines including IL-4, IL-6, IL-10 and accompanied elevation of IL-10/IFN-gamma ratio [22].

Solid evidence have demonstrated that the IL-10 production is under strong genetic control and a number of polymorphisms located in the promoter region of IL-10 gene could affect the transcriptional regulation [23]. Previous studies have addressed 3 significant single pair substitutions in the IL-10 gene promoter at positions -592, -819, -1082 from the transcriptional start site in the 5'flanking region [24]. Besides, IL-10 genotypes at position -1082 G/A are located within a recognized Ets transcription factor binding site [25]. Therefore, uncover the relationship between the three polymorphisms and susceptibility of SLE is essential and prospective. In the present study, significant difference in the frequencies of three genotypes -1082 GG, GA, and AA in IL-10 promoter were observed between SLE patients and healthy controls. It is suggested that elevated expression level of IL-10 are associated with a G at position -1082 and patients with a G at position -1082 showed significantly higher IL-10 protein production under the treatment of Con A stimulation of peripheral blood leucocytes (PBL) in vitro [18]. Our results demonstrated that SLE patients had a significantly higher frequency of the G allele compared with control subjects indicating more G at position -1082 may lead to high level of IL-10 and subsequently increase the risk of SLE in Han Chinese population. However, there was no difference in the frequencies of genotypes at position -819 C/T and -592 C/A between SLE patients and controls.

Past investigations have demonstrated that the 3 dimorphisms exhibit strong linkage disequilibrium and present in 3 haplotypes: ACC, ATA, GCC. In the past 2 decades, investigators have demonstrated the contribution of IL-10 production made by IL-10 promoter haplotypes but still cannot reach a consensus. Some reports indicated that patients with ATA or ACC haplotypes have the higher IL-10 level whereas others exposed that individuals with ATA haplotypes produce potently less IL-10 than other haplotypes [26-28]. It has been reported that GCC haplotype of the IL-10 promoter associates with elevated susceptibility to SLE in Spanish population. In the present study, our findings indicate that individuals with ATA haplotype are less susceptible to SLE in Han population. This phenomenon may be associated with lower level of IL-10 expression and this finding was consistent with the previous study.

In summary, our findings strongly suggested that -1082G/A polymorphism of IL-10 promoter was associated with SLE and ATA haplotype may play a protective role in SLE of Chinese Han population. Besides, high frequency of G allele at position -1082 may elevate the susceptibility of SLE while high frequency of C allele at position -819 and -592 might present protective effect in male Han Chinese population. However, accumulating evidence has reported two CA-repeat microsatellites IL-10.R and IL-10.G are involved with the regulation of IL-10 and SLE susceptibility [29]. It is also reported that several SNPs in the distal region of IL-10 promoter including -3575T/A, -2849G/A, -2763C/A and the extended SNP haplotypes AAA-AGCC and AGA-AGCC all contributed to the diverse IL-10 production level in larger African-American population [30]. Many well recognized transcriptional binding sites located throughout the 4-kb promoter region. Apart from aforementioned -592 and -1082 SNP,

-3573 SNP situated within a putative Pit-1 binding site indicating a potential association between female sex hormones and SLE [31]. Therefore, further study was needed to investigate the relationship between other genetic variation including microsatellite, SNP in the distal region and extended haplotypes and the pathogenesis of SLE.

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Disclosure of conflict of interest

None.

Address correspondence to: Dong-Qing Ye, Department of Epidemiology and Biostatistics, School of Public Health, Anhui Medical University, 81 Meishan Road, Hefei 230032, Anhui, P. R. China. Fax: +86 551 5161171; E-mail: ydq@ahmu.edu.cn

References

[1] Chen GM, Feng CC, Ye QL, Tao JH, Li R, Peng H, Zhou M, Leng RX, Li J, Cen H, Fan YG, Pan HF, Ye DQ. Association of P2X7R gene polymorphisms with systemic lupus erythematosus in a Chinese population. Mutagenesis 2013; 28: 351-355.

[2] Tikly M, Navarra SV. Lupus in the developing world--is it any different? Best Pract Res Clin Rheumatol 2008; 22: 643-655.

[3] Schotte H, Willeke P, Becker H, Poggemeyer J, Gaubitz M, Schmidt H, Schlüter B. Association of extended interleukin-10 promoter haplotypes with disease susceptibility and manifestations in German patients with systemic lupus erythematosus. Lupus 2014; 23: 378-385.

[4] Su DL, Lu ZM, Shen MN, Li X, Sun LY. Roles of pro- and anti-inflammatory cytokines in the pathogenesis of SLE. J Biomed Biotechnol 2012; 2012: 347141.

[5] Zeid SA, Khalifa G, Nabil M. Nabil, IL10 in lupus nephritis: detection and relationship with disease activity. Electron Physician 2015; 7: 1680-1685.

[6] Park YB, Lee SK, Kim DS, Lee J, Lee CH, Song CH. Elevated interleukin-10 levels correlated with disease activity in systemic lupus erythematosus. Clin Exp Rheumatol 1998; 16: 283-288.

[7] Ishida H, Muchamuel T, Sakaguchi S, Andrade S, Menon S, Howard M. Continuous administration of anti-interleukin 10 antibodies delays onset of autoimmunity in NZB/W F1 mice. J Exp Med 1994; 179: 305-310.

[8] Wang B, Fan YG, Ye DQ. Lack of association between the haplotype GCC/ATA polymorphism in the IL-10 promoter and SLE risk: evidence from a meta-analysis. Z Rheumatol 2013; 72: 705-708.

[9] Mörmann M, Rieth H, Hua TD, Assohou C, Roupelieva M, Hu SL, Kremsner PG, Luty AJ, Kube D. Mosaics of gene variations in the Interleukin-10 gene promoter affect interleukin-10 production depending on the stimulation used. Genes Immun 2004; 5: 246-255.

[10] 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.

[11] Eskdale J, Gallagher G, Verweij CL, Keijsers V, Westendorp RG, Huizinga TW. Interleukin 10 secretion in relation to human IL-10 locus haplotypes. Proc Natl Acad Sci U S A 1998; 95: 9465-9470.

[12] Hochberg MC. Updating the American College of rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997; 40: 1725.

[13] Sagreiya H, Berube C, Wen A, Ramakrishnan R, Mir A, Hamilton A, Altman RB. Extending and evaluating a warfarin dosing algorithm that includes CYP4F2 and pooled rare variants of CYP2C9. Pharmacogenetics Genomics 2010; 20: 407-413.

[14] Cheng G, Wang H, Chen M, Li L, Gong Y, Liu Q. Lack of evidence to support the association of polymorphisms within the TNFSF4 gene and coronary heart disease in a Chinese Han population. Exp Ther Med 2011; 2: 275-280.

[15] Shi YY, He L. SHEsis, a powerful software platform for analysesof linkage disequilibrium, haplotype construction, and genetic associationat polymorphism loci. Cell Res 2005; 15: 97-98.

[16] Li Z, Zhang Z, He Z, Tang W, Li T, Zeng Z, He L and Shi Y. A partition-ligation-combination-subdivision EM algorithm forhaplotype inference with multiallelic markers: update of the SHEsis (http://analysis.bio-x.cn). Cell Res 2009; 19: 519-523.

[17] Mok CC, Lau CS. Pathogenesis of systemic lupus erythematosus. J Clin Pathol 2003; 56: 481-490.

[18] 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.

[19] Rood MJ, Keijsers V, van der Linden MW, Tong TQ, Borggreve SE, Verweij CL, Breedveld FC,

Huizinga TW. Neuropsychiatric systemic lupus erythematosus is associated with imbalance in interleukin 10 promoter haplotypes. Ann Rheum Dis 1999; 58: 85-9.

[20] Lal G, Kulkarni N, Nakayama Y, Singh AK, Sethi A, Burrell BE, Brinkman CC, Iwami D, Zhang T, Hehlgans T, Bromberg JS. IL-10 from marginal zone precursor B cells controls the differentiation of Th17, Tfh and Tfr cells in transplantation tolerance. Immunol Lett 2016; 170: 52-63.

[21] Blay JY, Burdin N, Rousset F, Lenoir G, Biron P, Philip T, Banchereau J, Favrot MC. Serum interleukin-10 in non-Hodgkin's lymphoma: a prognostic factor. Blood 1993; 82: 2169-2174.

[22] Hagiwara E, Gourley MF, Lee S, Klinman DK. Disease severity in patients with systemic lupus erythematosus correlates with an increased ratio of interleukin-10: interferon-gamma-secreting cells in the peripheral blood. Arthritis Rheum 1996; 39: 379-385.

[23] Eskdale J, Gallagher G, Verweij CL, Keijsers V, Westendorp RG, Huizinga TW. Interleukin 10 secretion in relation to human IL-10 locus haplotypes. Proc Natl Acad Sci U S A 1998; 95: 9465-9470.

[24] Sobkowiak A, Lianeri M, Wudarski M, Łacki JK, Jagodziński PP. Genetic variation in the interleukin-10 gene promoter in polish patients with systemic lupus erythematosus. Rheumatol Int 2009; 29: 921-925.

[25] Kube D, Platzer C, von Knethen A, Straub H, Bohlen H, Hafner M, Tesch H. Isolation of the human interleukin 10 promoter. Characterization of the promoter activity in Burkitt's lymphoma cell lines. Cytokine 1995; 7: 1-7.

[26] Huizinga TW, Keijsers V, Yanni G, Hall M, Ramage W, Lanchbury J, Pitzalis C, Drossaers-Bakker WK, Westendorp RG, Breedveld FC, Panayi G, Verweij CL. Are differences in interleukin 10 production associated with joint damage? Rheumatology 2000; 39: 1180-1188.

[27] Temple SE, Lim E, Cheong KY, Almeida CA, Price P, Ardlie KG, Waterer GW. 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.

[28] Crawley E, Kay R, Sillibourne J, Patel P, Hutchinson I, Woo P. Polymorphic haplotypes of the interleukin-10 5'flanking region determine variable interleukin-10 transcription and are associated with particular phenotypes of juvenile rheumatoid arthritis. Arthritis Rheum 1999; 42: 1101-1108.

[29] Eskdale J, Kube D, Gallagher G. A second polymorphic dinucleotide repeat in the 5'flanking region of the human IL10 gene. Immunogenetics 1996; 45: 82-83.

[30] Gibson AW, Edberg JC, Wu J, Westendorp RG, Huizinga TW, Kimberly RP. Novel single nucleotide polymorphisms in the distal IL-10 promoter affect IL-10 production and enhance the risk of systemic lupus erythematosus. J Immunol 2001; 166: 3915-3922.

[31] Ostensen M. Sex hormones and pregnancy in rheumatoid arthritis and systemic lupus erythematosus. Ann N Y Acad Sci 1999; 876: 131-143; discussion 144.