中 國 醫 藥 大 學
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碩士學位論文

紅斑性狼瘡病患併腎病變 Interleukin-10 基因多型性之關連性

The Association of Interleukin-10 Gene Polymorphism in Systemic Lupus Erythematosus Patients with Renal Disease

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論文題目

中　文：紅斑性狼瘡病患併腎病變 Interleukin-10 基因多型性之關連性

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本論文係 林博文 於中國醫藥大學醫學研究所完成之碩士論文，經考試委員審查及口試合格，特此證明。

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中文摘要

背景：紅斑性狼瘡是一種會影響全身各種器官的自體免疫疾病，流行病學上已經有強烈的證據指出基因是導致許多常見疾病發生的危險因素。之前的研究已經顯示在罹患紅斑性狼瘡的白人身上 interleukin-10 (IL-10) -1082G、IL-10-819C 和 IL-10-592C haplotype 與腎臟病的侵犯有關連性；但在中國人，另一組不同的 IL-10-1087*A /-824*T /-597*A haplotype 也和腎臟病的影響有相關。我们的研究將探討 IL-10 promoter 基因多型性是否為易導致狼瘡性腎病變的一個標記。

方法：我们納入 119 位有紅斑性狼瘡的中國人為研究對象，另一組居住在中台灣的 100 位無血緣關係的健康人當作控制組。在紅斑性狼瘡病患這組，不僅腎臟病變(定義為每日蛋白尿超過 1g)也包括其他臨床症狀和血清學上的數據都納入分析中。每組基因 DNA 是經由抽取受試者周邊血液進一步使用基因組 DNA 分離 kit 所取得的(Genomaker, Taiwan)。每一種基因的多型性是以 polymerase chain reaction (PCR)為基礎的限制酶方法來分析及進一步去探討。我们將基因多型性的變化分成可切除的 AA homozygote、不可切除的 CC homozygote 同型合子和 A/C heterozygote 異型合子。此外，IL-10 基因多型性和紅斑性狼瘡臨床表現的關係亦納入評估。

結果:我们在紅斑性狼瘡病患身上並未發現 IL-10 基因型和腎臟病變有相關性。同時針對紅斑性狼瘡其他系統的表現包括顴骨疹、光致敏性、中樞神經系統和抗核抗體(ANA)在基因型上皆無達到顯著的發現(all p＞0.05)。但更進一步在基因的 genotype 以及 allelic frequency 上正常控制組和紅斑性狼瘡病患組卻有統計學上的差異(分別 p 值為 0.007 和 0.003)。

結論:雖然我们不能夠在紅斑性狼瘡併腎病變病患與健康人之間發現 IL-10 基因型有顯著的差異性，然而罹患紅斑性狼瘡者其-627IL-10 基因型包括 genotype 與 allelic frequency 確實有明顯的關連性。這進一步顯示在紅斑性狼瘡病患身上，IL-10 基因多型性在未來的研究可當作一個基因的標記。

英文摘要

Background: Systemic lupus erythematosus (SLE) is a prototype of autoimmune diseases that affects practically every organ in the body. There are strong epidemiological evidences that genes contribute to the risk of developing many common diseases. The previous study had shown that interleukin-10 (IL-10)-1082G, IL-10-819C, and IL-10-592C haplotype were associated with renal involvement in white patients with SLE. In Chinese patients, a different IL-10-1087*A /-824*T /-597*A haplotype was also associated with renal involvement. Our study will investigate whether IL-10 promoter polymorphism is a marker of susceptibility of lupus nephropathy.

Methods: The study included 119 Chinese patients with SLE. One hundred unrelated, healthy individuals living in central Taiwan served as control subjects. In SLE patients, not only renal disease (defined as proteinuria >1g/day) but also other clinical and serological data were analyzed. The genomic DNA was prepared from peripheral blood by use of a genomic DNA isolation reagent kit (Genomaker, Taiwan). Each polymorphism was detected as a result of a polymerase chain reaction (PCR)-based restriction analysis. The polymorphism was divided into digestible (AA homozygote), indigestible (CC homozygote) and A/C heterozygote. The relationship between the IL-10 gene polymorphism and clinical manifestations of SLE was evaluated.

Results: We did not detect any association of IL-10 genotype with renal disease involvement in the SLE patients. There were also no significant finding

in other parameters, including malar rash, photosensitivity, central nervous system involvement and ANA (all $p > 0.05$). Furthermore, for the genotype and allelic frequency, there were statistically significant differences between the SLE patients and the normal control subjects ($p=0.007$, and 0.003, respectively).

Conclusion: We were unable to find significant differences in IL-10 gene polymorphism between SLE patients with renal disease and the healthy control subjects. However, there were significant relation of -627 IL-10 genotype and allelic frequencies with SLE. It implies that the IL-10 gene polymorphism can serve as a candidate gene marker for further study in patients with SLE.

序言

非常感謝我的指導教授黃秋錦副院長在繁忙的醫院行政及臨床工作之餘，指導與協助我能夠順利地完成這次研究工作。首先要說明的是這是一篇系列性的研究，謝謝學長鍾錫裕醫師及免疫風濕科黃春明主任啟發我進入紅斑性狼瘡的研究領域中，並不吝提供研究病患讓我能長期觀察這群受試者，再者是特別感激蔡輔仁教授在基因的基礎研究上幫忙完成檢體的實驗室分析，最後再度強調在紅斑性狼瘡併腎病患者基因型的分析仍有一些值得探索和努力的空間，再次由衷地感謝黃副院長在學生研究生涯中所提供的學習，讓我成長及獲益良多。

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第一章前言

第一節 研究背景

Systemic lupus erythematosus (SLE) is a prototype for systemic autoimmune disease with a wide spectrum of laboratory and clinical manifestations. It is known to involve multi-systems and the production of autoantibodies to intracellular antigens. The etiology is largely unknown although valuable knowledge has been accomplished through intensive research in this field in recent years. IL-10 is produced at a high level by the B lymphocytes and monocytes of patients with SLE. The high IL-10 production contributes to the abnormal production of immunoglobulins (Ig) and of auto-antibodies in SLE [1]. It may be a risk factor for susceptibility and severity of rheumatoid arthritis and SLE [2-4]. The spontaneous in vitro production of IgM, IgG, and IgA by peripheral blood mononuclear cells from SLE patients was weakly increased by recombinant IL (rIL)-6, but strongly by rIL-10. IL-10 is an important pleotropic cytokine with anti-inflammatory and stimulatory activities [5]. It exerts its anti-inflammatory effect by inhibiting the synthesis of proinflammatory cytokines such as IL-α, IL-1ß, IL-6, IL-8, IL-12, and TNF-α in activated macrophages [6]. IL-10 also enhances B cell proliferation and differentiation and survival [7-9]. Levels of IL-10 production are critical in immunity regulation. Thus, the expression of IL-10 has been implicated in a number of autoimmune disorders, including RA , Sjogren's syndrome, and SLE [4].

第一章　前言

第二節 研究目的

This study has examined the polymorphism of the gene IL-10, to determine whether it was a marker of susceptibility to SLE by focusing on single nucleotide polymorphisms (SNPs). We compared allelic and genotypic frequencies between 119 Chinese patients with SLE and 100 healthy individuals in Taiwan.

第二章 研究方法

第一節 研究材料

Subjects

One hundred nineteen patients with definite SLE according to the 1982 revised American College of Rheumatology criteria for SLE [10], and 100 unrelated, healthy individuals living in central Taiwan who served as control subjects were enrolled in this study. Informed consent was obtained from all patients involved. Clinical and serological data were available on these patients, including malar rash, Photosensitivity, antinuclear antibody (ANA), central nervous involvement, and renal disease (defined as proteinuria>1 g/day). The central nervous system involvement was evaluated by a neurologist.

第二章 研究方法

第二節 研究設計

Methods

The genomic DNA was prepared from peripheral blood by use of a genomic DNA isolation reagent kit (Genomaker, Taiwan).

Division of IL-10 gene polymorphism

The Polymerase chain reaction (PCR) for IL-10 gene polymorphism was carried out to a total volume of 25 μl, containing genomic DNA (2-6 pmole of each primer); 1X Taq polymerase buffer (1.5 mM $MgCl_2$); and 0.25 units of AmpliTaq DNA polymerase (Perkin Elmer, Foster City, CA, USA). The primer for the IL-10 promoter gene at position -627 was 5'-CCTAGGTCACAGTGACGTGG-3' and 5'-GGTGAGCACTACCTGACTAGC-3. The polymerase chain reaction(PCR) amplification was performed in a programmable thermal cycler Gene Amp PCR System 2400 (Perkin- Elmer, Foster City, Calif., USA). The cycling conditions for IL-10 polymorphism were set as follows: one cycle at 96°C for 5 min, 35 cycles at 96° C for 30 sec, 35 cycles at 60°C for 30 sec, and 35 cycles at 72°C for 45 sec, and one final cycle of extension at 72°C for 7 min. The PCR product of 412-bp was mixed with two units Rsa I (New England Biolabs, Beverly, USA) and two fragments of 236-bp and 176-bp were present when the product was able to be digested (AA homozygote). The reaction was incubated for 3 hours at 37°C. Then, 10 μl of the product was loaded onto a 3% agarose gel containing

ethidium bromide for electrophoresis. The polymorphism was divided into digestible (AA homozygote), indigestible (CC homozygote) and A/C heterozygote.

Carriage rate and allelic frequency

The carriage rate of an allele is the number of individuals carrying at least one copy of the allele relative to the total number of individuals. Allelic frequency was expressed as a percentage of the total number of alleles.

第二章 研究方法

第三節 統計方法

Results from the control subjects and the SLE patients were compared using the χ^2 test (3 x 2 and 2 x 2 contingency tables) for statistical significance. When the assumption of the chi-squared test was violated and 1 cell had an expected count of <1, or > 20% of the cells had an expected count of <5, Fisher's exact test was used. The distributions of the IL-10 gene polymorphisms in each group were evaluated. A P value less than 0.05 was considered statistically significant. The odds ratios (OR) were calculated from allelic frequency with a 95% confidence interval (95% CI) for the polymorphism of the IL-10 gene.

第三章 研究結果

第一節 描述性統計分析

3-1-1 紅斑性狼瘡患者與健康人兩組基因型資料比較

The frequencies of the genotype in the SLE and control groups are shown in Table 1. It shows that among the 119 SLE patients, 66 patients (55.5%) had IL-10 genotype AA, 48 patients (40.3%) had A/C, and 5 patients (4.2%) had CC. Among the 100 control volunteers, the IL-10 genotype AA was found in 40 (40.0%), A/C in 45 (45.0%), and CC in 15 (15.0%). There were significant differences in the distribution of the IL-10 gene polymorphism between the healthy control subjects and the SLE patients (p=0.007). In addition, there was a significant difference in the allelic frequency of the IL-10 between the SLE patients and healthy controls (p=0.003), giving an odds ratio of 1.210 for A allele (95% confidence interval 1.063-1.377). There was no significant association in the carriage rates of the IL-10 (p=0.077), giving an odds ratio of 1.164 for A allele (95% confidence interval 0.981-1.382, respectively).

3-1-2 紅斑性狼瘡患者與健康人臨床症狀及實驗數據差異性

Clinical manifestations and laboratory findings of the SLE patients are shown in Table 2. The associations of IL-10 with particular clinical features of SLE were examined in the 119 Chinese patients. We did not detect any association of IL-10 genotype with the antinuclear antibody (ANA),malar rash,

photosensitivity, discoid lupus, mucosal ulcer, arthritis, serositis, hematology, immunology, central nervous system involvement and renal disease involvement in the SLE patients (all $p > 0.05$).

第三章 研究結果

第二節 推論性統計分析

3-2-1 紅斑性狼瘡影響腎臟病變和IL-10基因型的探討

Relationship between IL-10 genotype and renal involvement in patients with SLE are shown in Table 3 & 4.

3-2-2 狼瘡性腎病變的表現與IL-10基因多型性的關連性

In comparison with different genotype , there are no significant difference (all $p > 0.05$) in microhematuria, serum Cr and hypertension association, despite patients with CC-genotype had less proteinuria manifestation than AA- & AC-genotype. In addition, only few patients accepted renal biopsy examination.

第四章 討論

第一節 結果討論

4-1-1 紅斑性狼瘡的臨床表現受基因所控制

Systemic lupus erythematosus (SLE) is a prototype of autoimmune diseases that affects practically every organ in the body. There are strong epidemiological evidences that genes contribute to the risk of developing many common diseases. However, the genetic background of patients with SLE is still mostly unknown. To date, genetic researches of multi-factorial diseases have been studied with difficulty, due to the vast uncertainty surrounding the presence of a polygenic trait. In the present study, we chose the IL-10 gene polymorphism to examine whether IL-10 gene polymorphism was a marker of susceptibility to SLE in Chinese patients in Taiwan.

4-1-2 有關IL-10基因型的差異影響紅斑性狼瘡各系統疾病表現的文獻報告

IL-10 is a 36 kDa homodimeric cytokine, which is mainly produced by macrophage, monocytes and lymphocytes. The IL-10 gene maps to the junction of 1q31-q32 [11]. IL-10 production appears to be controlled at the transcriptional level [12]. The IL-10 5’ flanking region, which controls transcription, is polymorphic, with 2 microsatellites between -4000 and -1100 and three single base pair substitutions that have been described in the IL-10

promoter at positions -1082(G/A), -819(C/T), and -592(C/A) [13]. Reports from Lazarus et al [14] found the IL-10-1082G, IL-10-819C, and IL-10-592C haplotype were associated with Ro auto-antibodies and renal involvement in white patients with SLE. In Chinese patients, a different il-10-1087*A/-824*T/-597*A haplotype was also associated with renal involvement but not Ro autoantibodies[15]. These studies found no association with disease susceptibility. In contrast, Gibson et al [16] found novel single nucleotide polymorphisms in the distal region of the IL-10 promoter significantly associated with SLE susceptibility in African Americans. The Lim et al. study had shown that -627*A allele is associated with severe asthma [17], which is detected with low levels of IL-10 [18]. Grove J. et al also showed that the -627*A IL-10 promoter gene is related to advanced alcoholic liver disease [19]. They proposed that the -627*A allele is associated with low IL-10 expression which will favor the inflammatory, immune mediated, and profibrotic mechanisms of an alcohol related liver injury. Guseva I et al found that polymorphism of genes FcgRIIIA and IL-10 is associated with predisposition to development of SLE in the Kazakh population. The analysis of combined genotypes of the studied genes suggests a synergic action of genes FcgRIIIA and IL-10-627 with the risk to develop SLE [20].

4-1-3 紅斑性狼瘡患者與健康人兩組IL-10基因型的變化

To our knowledge, an association between SLE and the gene polymorphism of -627 IL-10 including genotype, allelic frequencies and carriage rate has not been demonstrated before. In the present study, significant differences were observed in the allelic frequencies and genotype of IL-10 gene polymorphism between the patients with SLE and the healthy control subjects. We were unable to find significant differences in the carriage rate of IL-10 gene polymorphism between the normal controls and SLE patients.

第四章 討論

第二節 其他相關性討論

Even though we followed up these patients for 2-3 years, there are no significant difference between different IL-10 genotype in renal involvement, including microhematuria, proteinuria and renal function (serum Creatinine). We observed that patients with CC-genotype had less proteinuria manifestation than AA- & AC-genotype.

第四章 討論

第三節 研究限制

Due to only 5 pateints carried with CC-genotype, there are no significant difference statistically (P> 0.05). Patients with early lupus nephritis may have no obvious symptoms and signs, so only few patients had renal pathology reports.

第五章 結論與建議

Recently, single nucleotide polymorphisms (SNPs) assist in determining the exact prognosis of patients, which in turn leads to the selection of a specific therapy based on specific genetic variations [25-30]. Numerous linkage and association studies, as well as the analysis of murine models, have provided ample evidence for a genetic basis for SLE. Genetic susceptibility to SLE results from the combined actions of multiple alleles, each of them conferring a modest incremental risk. There is evidence that susceptibility alleles that are associated with a greater disease severity also are associated with lupus nephritis. There also is evidence for a set of kidney-specific genes that are likely to amplify or to sensitize to the autoimmune pathology [31].

This study provides a useful method for the further study of genes in patients with SLE.

Table1. Comparison of IL-10 genotype distributions, allele frequencies and carriage rates observed in SLE patients and healthy control subjects.

All the p values represent chi-square test results.

IL-10	SLE patients N=119 (%)	Controls n=100 (%)	P	OR (95% CI)
Genotype				
AA	66(55.5)	40 (40)	0.007	
A/C	48 (40.3)	45 (45)		
CC	5 (4.2)	15 (15)		
Allelic frequencies				
A	180(75.6)	125 (62.5)	0.003	1.210 (1.063~1.377)
C	58(24.4)	75 (37.5)		0.650 (0.488~0.865)
Carriage rate				
A	114(95.8)	85 (85.0)	0.077	1.164 (0.981~1.382)
C	53(44.5)	60 (60.0)		0.767 (0.571~1.030)

All the p values represent chi-square test results.

Table 2. Relationship between IL-10 genotype and clinical signs and findings in patients with SLE

	AA (n=66)	A/C (n=48)	CC (n= 5)	Total (n=119)	p-value
Malar rash	33 (50.0%)	30 (62.5%)	4 (80.0%)	67 (56.3%)	0.228
Photosensitivity	32 (48.5%)	29 (60.4%)	4 (80.0%)	65 (54.6%)	0.229
Renal involvement	27 (40.9%)	17 (35.4%)	2 (40.0%)	46 (38.7%)	0.836
CNS involvement	11 (16.7%)	4 (8.3%)	1 (20.0%)	16 (13.4%)	0.396
ANA(+)	64 (97.0%)	47 (98.0%)	5 (100%)	116 (97.5%)	0.449

Table 3 Relationship between IL-10 genotype and renal involvement in patients with SLE

	AA n(%)	A/C n(%)	CC n(%)	Total n(%)	P value
Hematuria	5(62.5)	2(25)	1(12.50)	8(100)	0.322
Hypertension	6(54.5)	3(27.3)	2(18.2)	11(100)	0.126
Serum Cr	1.39	3.39	0.82		0.495

Table 4 Relationship between IL-10 genotype and proteinuria in patients with SLE

		IL-10			Total
		AA	CA	CC	
Proteinuria	Positive	31	26	0	57
		54.4%	45.6%	0.0%	100.0%
	Negative	31	23	5	59
		52.5%	39.0%	8.5%	100.0%
Total		62	49	5	116
		53.4%	42.2%	4.3%	100.0%

- Pearson chi-square *P* 0.076
- Likelihood ratio *p* 0.029

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