J Clin Immunol (2008) 28:440–444
DOI 10.1007/s10875-008-9212-5

IkBα Promoter Polymorphisms in Patients with Primary Sjögren's Syndrome

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Received: 5 March 2008 / Accepted: 19 May 2008 / Published online: 4 July 2008

Abstract
Introduction To investigate the association of *IkBα* promoter polymorphisms with the development of primary Sjögren's syndrome in Taiwan, 98 patients with primary Sjögren's syndrome and 110 unrelated healthy controls were enrolled in this study.
Materials and Methods The *IκBα* −881 A/G, *IκBα* −826 C/T, *IκBα* −550 A/T, *IκBα* −519 C/T, and *IκBα* −297 C/T polymorphisms were determined by the methods of polymerase chain reaction/restriction fragment length polymorphism.
Results This study demonstrated that the genotype frequencies of *IκBα* −826 C/T and *IκBα* −826 T/T, in comparison with that of *IκBα* −826 C/C, were significantly higher in the patients with primary Sjögren's syndrome than in the controls. The allele frequency of *IκBα* −881 G was significantly decreased in the patients with primary Sjögren's syndrome compared with that of the controls. In contrast, the allele frequency of *IκBα* −826 T was significantly higher in the patients with primary Sjögren's syndrome than in the controls. The similar findings could also be found in the allele carriage frequencies. The patients with primary Sjögren's syndrome had lower allele carriage frequencies of *IκBα* −881 G and *IκBα* −826 C, and a higher allele carriage frequency of *IκBα* −826 T. We also found that the estimated haplotype frequency of *IκBα* −881A-826T-550A-519C-297C was significantly increased in the patients with primary Sjögren's syndrome in comparison with that of the controls.
Discussion This study demonstrated that the *IkBα* −826T allele and *IkBα* −881A-826T-550A-519C-297C haplotype were associated with susceptibility to primary Sjögren's syndrome in Taiwan. However, these findings may not be disease-specific but may be related to inflammatory responses.

Keywords *IkBα* · NFkB inhibitor · polymorphisms · Sjögren's syndrome

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Introduction

Primary Sjögren's syndrome is a chronic inflammatory autoimmune disease with exocrinopathy including salivary and lacrimal gland involvement, which results in keratoconjunctivitis sicca and xerostomia. The exocrine glands of the skin, urogenital, respiratory, and gastrointestinal tract may also be involved. Moreover, extra-glandular involvement is common. The extra-glandular manifestations include synovitis, neuropathy, vasculitis, and presence of autoantibodies [1].

Many human leukocyte antigen (HLA) and non-HLA genes including HLA-DRB1, HLA-DRB3, HLA-DQA1, HLA-DQB1, and cytokine genes have been revealed to be associated with the pathogenesis of primary Sjögren's syndrome [2]. Other immune-related genes may also be associated with this disease [3].

Many pro-inflammatory cytokines are involved in the inflammatory process of primary Sjögren's syndrome [4]. NFκB is related to the transcription of these pro-inflammatory cytokines, immune response, and anti-apoptotic genes [5–8]. Therefore, NFκB plays an important role in inflammatory diseases and in the development of autoimmunity [7, 9]. Many studies showed that patients with primary Sjögren's syndrome had increasing salivary gland cell apoptosis and the resistance of apoptosis cell death in salivary infiltrating mononuclear cells [10]. The anti-apoptogenic effect of NFkB is well known. It is caused by the expression of anti-apoptogenic molecules including Bcl-xL, XIAP, IAP, and TRAFs induced by NF-kB. IkB is an inhibitor of NFkB, which binds with NFkB in the cytoplasm and influences the transcriptional activity of NFkB. Therefore, IkB may also play an important role in chronic inflammatory autoimmune diseases.

Several polymorphisms in the promoter region of *IκBα* including *IκBα* −881 A/G (rs 3138053), *IκBα* −826 C/T (rs 2233406), *IκBα* −550 A/T (rs 2233407), *IκBα* −519 C/T (rs 2233408), and *IκBα* −297 C/T (rs 2233409) have been reported [11]. Three NFkB-binding sites have been demonstrated in the promoter of *IκBα*, which are required for induction of gene expression [12]. A putative binding site for transcription factors ROR alpha 1 and ROR alpha 2 is in the position of *IκBα* −881 (TFsearch website). Another putative binding site for transcription factor C/EBP is in the position of *IκBα* −519 (TFsearch website). Therefore, the polymorphisms in the *IκBα* promoter may affect the binding of transcriptions and then influence the expression of *IκBα*. Mozzator-Chamay showed that the *IκBα* −881G/*IκBα* −826T haplotype might protect the development of scarring trachoma, an inflammatory disease, in Gambia [11]. The polymorphisms of the *IκBα* promoter may be related to the pathogenesis of inflammatory diseases. The purpose of the present study is to investigate the association of *IκBα* promoter polymorphisms with the development of primary Sjögren's syndrome.

Materials and Methods

Ninety-eight patients with primary Sjögren's syndrome (eight men and 90 women; mean ± SD=47.45±10.58 years) and 110 unrelated healthy controls (12 men and 98 women; mean ± SD=45.26±9.48 years) were enrolled in this study. All of the patients and controls are Taiwanese. The diagnosis of primary Sjögren's syndrome was according to the revised US–European criteria for the classification of Sjögren's syndrome [13]. The *IkBα* −881 A/G, *IkBα* −826 C/T, *IkBα* −550 A/T, *IkBα* −519 C/T, and *IkBα* −297 C/T polymorphisms were detected by the polymerase chain reaction (PCR)/restriction fragment length polymorphism (RFLP) method and confirmed by direct sequencing as demonstrated in our previous study [14].

To determine the *IkBα* −881 A/G and *IkBα* −826 C/T polymorphisms, a set of primers with the following sequences were used: 5′-GGTCCTTAAGGTCCAATCG-3′ and 5′-GTTGTGGATACCTTGCACTA-3′ (underlined, mismatched nucleotide). The amplification conditions consisted of initial denaturation at 95°C for 3 min, followed by five cycles of denaturation at 95°C for 1 min, annealing at 56°C for 1 min, and extension at 72°C for 1 min; and 35 cycles of denaturation at 95°C for 1 min, annealing at 54°C for 1 min, and extension at 72°C for 1 min; and then a final extension phase at 72°C for 7 min. The restriction enzymes, *Tsp*RI and *Bfa*I, were used to determine the *IκBα* −881 A/G and *IκBα* −826 C/T polymorphisms, respectively.

The primers 5′-GCTTTCACAACTTCTACCTG-3′ and 5′-AGAGTGGAAATGATGGCTG-3′ were used to determine the *IkBα* −519 C/T polymorphisms. PCR was performed under the following conditions: initial denaturation at 96°C for 3 min and five cycles of denaturation at 95°C for 1 min, annealing at 56°C for 1 min, and extension at 72°C for 1 min, and then 30 cycles of denaturation at 95°C for 1 min, annealing at 54°C for 1 min, and extension at 72°C for 1 min. A final extension phase was also performed at 72°C for 7 min. Then the PCR product was digested with *Mnl*I.

To determine the *IkBα* −550 A/T polymorphisms, a nested PCR was performed with the PCR product to determine *IkBα* −519 C/T polymorphisms and a set of new primers. The sequences of primers were 5′-TTGCTGCAAAGAGCCTGCT-3′ (underlined: mismatched nucleotide) and 5′-AGAGTG GAAATGATGGCTG-3′. The amplification conditions were as follows: initial denaturation at 96°C for 3 min, followed by five cycles of denaturation at 95°C for 1 min, annealing at 57°C for 1 min, and extension at 72°C for 1 min; and 30 cycles of denaturation at 95°C for 1 min, annealing at 54°C for 1 min, and extension at 72°C for 1 min; and then a final extension at 72°C for 7 min. Then the PCR product was subjected to digestion with *Sfc*I.

The polymorphisms of *IkBα* −297 C/T were also determined by the PCR/RFLP method. The primers 5′-GAAAG GACCGGCAGTTGG-3′ and 5′-GTACTTCCCTGCAG CCTG-3′ were used. The PCR was carried out under the following conditions: initial denaturation at 96°C for 3 min and five cycles of denaturation at 95°C for 1 min, annealing at 57°C for 1 min, and extension at 72°C for 1 min, followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 52°C for 1 min and extension at 72°C for 1 min, and then a final extension phase at 72°C for 7 min. The restriction enzyme *Hpy*8 I was used.

Table I The Genotype Frequencies of *IkBα* Promoter Polymorphisms in the Patients with Primary Sjögren's Syndrome and the Controls

IkBα genotype	Primary Sjögren's, n=98 (%)	Controls, n=110 (%)	OR (95% CI)	p value
IkBα −881 A/A	85 (86.7)	83 (75.5)	1	
A/G	13 (13.3)	25 (22.7)	0.5 (0.2–1.1)	NS
G/G	0 (0)	2 (1.8)	0.0 (0.0–4.1)	NS
IkBα −826 C/C	4 (4.1)	71 (64.5)	1	
C/T	30 (30.6)	33 (30.0)	16.1 (5.3–49.6)	<0.001
T/T	64 (65.3)	6 (5.5)	189.3 (51.1–701.3)	<0.001
IkBα −550 A/A	93 (94.9)	100 (90.9)	1	
A/T	4 (4.1)	9 (8.2)	0.5 (0.1–1.6)	NS
T/T	1 (1.0)	1 (0.9)	1.1 (0.1–17.4)	NS
IkBα −519 C/C	91 (92.9)	99 (90.0)	1	
T/C	6 (6.1)	10 (9.1)	0.7 (0.2–1.9)	NS
T/T	1 (1.0)	1 (0.9)	1.1 (0.1–17.6)	NS
IkBα −297 C/C	85 (86.7)	94 (85.5)	1	
C/T	12 (12.2)	14 (12.7)	0.9 (0.4–2.2)	NS
T/T	1 (1.0)	2 (1.8)	0.6 (0.0–6.2)	NS

NS: not significant

The chi-square test or the Fisher's exact test was used for statistical analysis. The p value was corrected by the multiplication of the number of comparisons (Pc). The OR was calculated by the Haldane modification method if the number of cases was 0. The estimated haplotype frequencies were determined by the EH program (Web Resources of Genetic Linkage Analysis).

Results

The distributions of the genotype in the controls were compatible with Hardy–Weinberg equilibrium. This study demonstrated that the genotype frequency of *IkBα* −826 C/T, in comparison with that of *IkBα* −826 C/C, was significantly higher in the patients with primary Sjögren's syndrome than in the controls (Table I). A similar finding could also be found in the genotype frequency of *IkBα* −826 T/T (primary Sjögren's vs controls, p<0.001). However, the differences of genotype frequencies of *IkBα* −881, *IkBα* −550, and *IkBα* −297 between the patients with primary Sjögren's syndrome and the controls were not significant.

The patients with primary Sjogren's syndrome had a significantly lower allele frequency of *IkBα* −881 G than the controls (Table II). Moreover, the allele frequency of *IkBα* −826 T was significantly increased in the patients with primary Sjögren's syndrome in comparison with that of the controls.

The allele carriage frequency of *IkBα* −881 G was significantly decreased in the patients with primary Sjögren's syndrome compared with that of the controls (Table III). This study also revealed that the allele carriage frequency of *IkBα* −826 T was significantly higher in the patients with primary Sjögren's syndrome than in the controls (p<0.001). In contrast, the allele carriage frequency of *IkBα* −826 C was significantly decreased in the patients with primary Sjögren's syndrome in comparison with that of the controls (p<0.001).

We also found that the estimated haplotype frequency of *IkBα* −881A-826T-550A-519C-297C was significantly higher in the patients with primary Sjögren's syndrome than in the

Table II The Allele Frequencies of *IkBα* Promoter Polymorphisms in the Patients with Primary Sjögren's Syndrome and the Controls

IkBα polymorphisms	Primary Sjögren's, 2n=196 (%)	Controls, 2n=220 (%)	OR (95% CI)	p value
IkBα −881 A	183 (93.4)	191 (86.8)		
G	13 (6.6)	29 (13.2)	0.5 (0.2–0.9)	0.02
IkBα −826 C	38 (19.4)	175 (79.5)		
T	158 (80.6)	45 (20.5)	16.2 (10.0–26.2)	<0.001
IkBα −550 A	190 (96.9)	208 (94.5)	1.7 (0.6–4.6)	NS
T	6 (3.1)	12 (5.5)		
IkBα −519 C	188 (95.9)	208 (94.5)	1.4 (0.5–3.4)	NS
T	8 (4.1)	12 (5.5)		
IkBα −297 C	182 (92.9)	202 (91.8)	1.2 (0.6–2.4)	NS
T	14 (7.1)	18 (8.2)		

NS: not significant

Table III The Allele Carriage Frequencies of *IkBα* Promoter Polymorphisms in the Patients with Primary Sjögren's Syndrome and the Controls

IkBα polymorphisms	Primary Sjögren's, n=98 (%)	Controls, n=110 (%)	OR (95% CI)	p value
IkBα −881 A	98 (100.0)	108 (98.2)	4.5 (0.5–44.4)	NS
G	13 (13.3)	27 (24.5)	0.5 (0.2–0.9)	0.04
IkBα −826 C	34 (34.7)	104 (94.5)	0.03 (0.01–0.08)	<0.001
T	94 (95.9)	39 (35.5)	42.8 (14.6–125.3)	<0.001
IkBα −550 A	97 (99.0)	109 (99.1)	0.9 (0.1–14.4)	NS
T	5 (5.1)	11 (10.0)	0.5 (0.2–1.6)	NS
IkBα −519 C	97 (99.0)	109 (99.1)	0.9 (0.1–14.4)	NS
T	7 (7.1)	11 (10.0)	0.7 (0.3–1.9)	NS
IkBα −297 C	97 (99.0)	108 (98.2)	1.8 (0.2–20.1)	NS
T	13 (13.3)	16 (14.5)	0.9 (0.4–2.0)	NS

NS: not significant

controls (Table IV). The p value was still significant even after correction (Pc<0.007).

Discussion

This study demonstrated that the *IkBα* −881A-826T-550A-519C-297C haplotype was related to susceptibility to primary Sjögren's syndrome in Taiwan.

IκB inhibits the transcription function of NFκB. Different IκB molecules preferentially inhibit distinct NFκB/Rel protein dimmers [15]. The central portion of the IκB molecules contains several ankyrin repeats. Ankyrin repeats bind to the Rel homology domain of NFκB/Rel, which causes NFκB to remain in the cytoplasm by masking the nuclear localization sequence of NFκB. Nuclear import of IκBα is also found [16, 17]. When IκBα is expressed in the nucleus, it can inhibit the interaction of NFκB with DNA and promote the export of NFκB from the nucleus to the cytoplasm [17, 18]. The C-terminal domain of IκB may block DNA binding by NFκB, dissociate DNA-bound NFκB dimmers, and insure a nuclear export of NFκB [18, 19].

Cytokines play an important role in the pathogenesis of primary Sjögren's syndrome by triggering and perpetuating the immune responses. IL-18 is involved in local glandular inflammation in primary Sjögren's syndrome. The B-cell activation factor is a member of the tumor necrosis factor superfamily that regulates the proliferation and activation of B-cells. NFκB is related to the transcription of these cytokines. Aberrant B and T cells activation will induce and promote local and systemic autoimmunity in the patients with primary Sjögren's syndrome.

Mutations in *IkBα* are associated with some autoimmune diseases. An 8-bp insertion in the promoter region of *IkBα* (*IkBα* −708 ins 8) protected individuals from the development of primary progressive multiple sclerosis [20]. Klein et al. showed that the *IkBα* polymorphisms might also be associated with Crohn's disease. The single-nucleotide polymorphisms in the 3′-untranslated region were significantly increased in patients with Crohn's disease [21].

Our previous study also showed that the *IκBα* −826T-550A-519C haplotype and *IκBα* −881A-826T-550A-519C-297C haplotype were associated with susceptibility to rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), respectively, in Taiwan [14, 22]. This study showed that *IkBα* −826T was also associated with susceptibility to primary Sjögren's syndrome. We also found that the *IkBα* −881A-826T-550A-519C-297C haplotype might play a significant

Table IV The Estimated Haplotype Frequencies of *IkBα* Promoter Polymorphisms in the Patients with Sjögren's Syndrome and the Controls

IkBα haplotype	Primary Sjögren's	Controls	OR (95% CI)	P value	Pc
IkBα −881A-826C-550A-519C-297C	0.123	0.753	0.05 (0.03–0.08)	<0.001	<0.007
IkBα −881A-826C-550A-519C-297T	0.002	0.01	–	–	–
IkBα −881A-826C-550A-519T-297C	0.041	0.097	4.64 (0.97–22.11)	0.051	NS
IkBα −881A-826C-550T-519C-297C	0.006	0.015	–	–	–
IkBα −881A-826T-550A-519C-297C	0.661	0.054	34.14 (17.77–65.59)	<0.001	<0.007
IkBα −881A-826T-550A-519C-297T	0.046	0.004	10.54 (1.32–83.96)	0.008	NS
IkBα −881A-826T-550T-519C-297C	0.019	0.008	2.27 (0.41–12.54)	NS	NS
IkBα −881G-826T-550A-519C-297C	0.021	0.045	0.44 (0.14–1.42)	NS	NS
IkBα −881G-826T-550A-519C-297T	0.017	0.045	0.33 (0.09–1.20)	NS	NS

En dash the p value was not calculated due to the fact that the number of cases is too small, Pc corrected p value
NS: not significant

role in susceptibility to primary Sjögren's syndrome, although the association between this haplotype and primary Sjögren's syndrome may be not be specific. This haplotype may be related to the promoter activity of *IkBα* and the production of IkBα, and then influence the function of NFkB, which plays an important role in the inflammatory and immune responses. An experiment about the effect of *IkBα* −826 C/T polymorphisms on the promoter function of *IkBα* is being conducted. Moreover, this study revealed that *IkBα* −881G might be a protective factor for the development of primary Sjögren's syndrome. Primary Sjögren's syndrome is an autoimmune disease with female predominance, which is also found in the patients with RA and SLE. Although the association of *IkBα* −826T with primary Sjögren's syndrome could also be noted in RA and SLE, these associations were not sex-dependent. A similar finding could also be found in the patients with ankylosing spondylitis (unpublished data), which is an autoimmune disease with male predominance.

This study also demonstrated that the *IkBα* promoter polymorphisms were not associated with the extra-glandular manifestations of primary Sjögren's syndrome (data not shown). Although the sample size is limited in this study, the power for the *IkBα* −826 C/T polymorphisms is more than 95%. The numbers of patients and controls may be enough for this study. A further study may be needed to confirm these findings in a larger group of patients and controls. In conclusion, the *IkBα* −826T allele and *IkBα* −881A-826T-550A-519C-297C haplotype are associated with susceptibility to primary Sjögren's syndrome in Taiwan, which also suggests they may be related to inflammatory responses despite the fact that this finding may not be disease-specific.

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