$I\kappa B\alpha$ promoter polymorphisms in patients with Behçet's disease

Yu-Hung Hung^a, Cheng-Chin Wu^b, Tsan-Teng Ou^b, Chia-Hui Lin^b, Ruei-Nian Li^c, Yu-Chih Lin^d, Wen-Chan Tsai^b, Hong-Wen Liu^b and Jeng-Hsien Yen^{a,b,e,*}

^aGraduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan

^bDivision of Rheumatology, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan

^cDepartment of Biomedical Science and Environmental Biology, College of Life Science, Kaohsiung Medical University, Kaohsiung, Taiwan

^dDivision of General Internal Medicine, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan

^eCenter of Excellence for Environmental Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan

Abstract. To investigate the role of $I\kappa B\alpha$ promoter polymorphisms in the development of Behçet's disease, eighty-six patients with Behçet's disease and 120 healthy controls were enrolled in this study. The $I\kappa B\alpha$ -881A/G, -826C/T, -550A/T, -519C/T, and -297C/T polymorphisms were measured by the method of polymerase chain reaction/restriction fragment length polymorphism. This study demonstrated that the genotype frequencies of $I\kappa B\alpha$ -826C/T and -826T/T were significantly higher in the patients with Behçet's disease than in the controls. Both in the dominant and in the recessive models, the patients with Behçet's disease have higher frequencies of the $I\kappa B\alpha$ -826T containing genotype than the controls. The allele frequency of $I\kappa B\alpha$ -826T was significantly increased in the patients with Behçet's disease. The frequencies of the $I\kappa B\alpha$ -881A -826T -550A -519C -297C and $I\kappa B\alpha$ -881A -826T -550A -519T -297C haplotypes were significantly higher in the patients with Behçet's disease than in the controls. In contrast, the haplotype frequency of $I\kappa B\alpha$ -881A -826C -550A -519C -297C in the patients with Behçet's disease was significantly decreased. This study also revealed that the Behçet's disease patients with $I\kappa B\alpha$ -826T/T have higher prevalence of skin lesions than those without $I\kappa B\alpha$ -826T/T. In summary, the $I\kappa B\alpha$ -826T allele, $I\kappa B\alpha$ -881A -826T -550A -519C -297C and $I\kappa B\alpha$ -881A -826T -550A -519T -297C haplotypes might be associated with susceptibility to Behçet's disease. The $I\kappa B\alpha$ -826T/T genotype was related to the development of skin lesions in the patients with Behçet's disease.

Keywords: $I\kappa B\alpha$, NF κ B inhibitor, polymorphisms, Behçet's disease

1. Introduction

Behçet's disease is a chronic inflammatory systemic autoimmune disease characterized primarily by recurrent oral ulcers, genital ulcers, ocular inflammation, skin lesions, and vasculitis. Although the etiology of Behçet's disease is still unknown, multiple genes and

environmental factors are involved in the pathogenesis of this disease. HLA-B51 is strongly associated with susceptibility to Behçet's disease in different ethnic groups [1]. The positive rate of HLA-B51 in Behçet's disease is about 60% [2]. However, the contribution of this allele to the overall genetic susceptibility to Behçet's disease is only about 19% [3]. Therefore, non-HLA genes may also be related to the pathogenesis of this disease [4–13]. A whole-genome screening also revealed the association of several non-HLA susceptibility loci with Turkish patients with Behçet's disease [14].

*Corresponding author: Dr. Jeng-Hsien Yen, Division of Rheumatology, Department of Internal Medicine, Kaohsiung Medical University Hospital, No. 100 Zih-You 1st Road, Kaohsiung City 807, Taiwan. Fax: +886 7 3118141; E-mail: jehsye@kmu.edu.tw.

Many pro-inflammatory cytokines are involved in the inflammatory process of Behçet's disease [13,15–18]. NF κ B is related to the transcription of these pro-inflammatory cytokines, immune response, and anti-apoptotic genes [19–22]. Therefore, NF κ B plays an important role in inflammatory diseases and in the development of autoimmunity [21,23]. I κ B is an inhibitor of NF κ B, which binds with NF κ B in the cytoplasm and influences the transcriptional activity of NF κ B. Therefore, I κ B may also play an important role in inflammatory immunological diseases.

The I κ B family includes I κ B α , I κ B β , I κ B γ , I κ B δ , I κ B ϵ , I κ B ζ , I κ B-R, Bcl-3, p100, and p105 [24,25]. All these proteins are characterized by the presence of multiple ankyrin repeats. I κ B α , a classic form of the I κ B family, consists of six ankyrin repeats, and can be found in cytoplasm and nucleus [25].

Several polymorphisms in the promoter region of *I κ B α* including -881A/G (rs 3138053), -826C/T (rs 2233406), -550A/T (rs 2233407), -519C/T (rs 2233408), and -297C/T (rs 2233409) have been identified [26]. Several transcription factor binding sites have been demonstrated in the promoter region of *I κ B α* (TFsearch web). The *I κ B α* promoter polymorphisms may affect I κ B α expression and influence the regulation of inflammatory response. A mutation in the coding region of *I κ B α* might result in the over-expression of I κ B α , which was implicated in the development of lymphoma [27].

Our previous studies showed that *I κ B α* -826T might be associated with the development of rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) in Taiwan [28,29]. However, the correlation between *I κ B α* promoter polymorphisms and Behçet's disease is still unknown. The purpose of the present study is to investigate the association of *I κ B α* promoter polymorphisms with the development of Behçet's disease.

2. Material and methods

Eighty-six patients with Behçet's disease (59 females and 27 males; mean $\pm$ SD = 47.2 $\pm$ 12.9 years) and 120 unrelated healthy controls (84 females and 36 males; mean $\pm$ SD = 44.6 $\pm$ 10.1 years) were enrolled in this study. All of the patients and controls are Taiwanese. This study has been approved by the IRB of Kaohsiung Medical University Hospital. The diagnosis of Behçet's disease was made according to the International Study Group criteria for diagnosis of Behçet's disease [30]. The linkage disequilibrium in

the promoter of *I κ B α* could not be found in Chinese (HapMap). Therefore, the *I κ B α* -881A/G, -826C/T, -550A/T, -519C/T, and -297C/T polymorphisms were detected in this study.

To determine the *I κ B α* -881A/G and -826C/T polymorphisms, a set of primers with the following sequences: 5'-GGTCCTTAAGGTCCAATCG-3' and 5'-GTTGTGGATACCTTGCACTA-3' (underlined: mismatched nucleotide) were used. PCR was carried out under the following conditions: initial denaturation at 95°C for 3 min and 5 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 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. A final extension phase was also performed at 72°C for 7 min. The restriction enzymes, *Tsp*R I and *Bfa* I, were used to determine the *I κ B α* -881A/G and *I κ B α* -826C/T polymorphisms, respectively.

The primers 5'-GCTTTCACAACCTTCTACCTG-3' and 5'-AGAGTGGAAA TGATGGCTG-3' were used to determine the *I κ B α* -519C/T polymorphisms. The amplification conditions consisted of initial denaturation at 96°C for 3 min, followed by 5 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 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 phase at 72°C for 7 min. Then the PCR product was digested with *Mnl* I.

To determine the *I κ B α* -550A/T polymorphisms, a nested PCR was performed with the PCR product for determining -519C/T polymorphisms and a set of new primers. The sequences of primers were 5'-TTGCTGCAAAGAGCCTGCT-3' (underlined: mismatched nucleotide) and 5'-AGAGTGGAAATGATGGCTG-3'. The amplification conditions were as follows: initial denaturation at 96°C for 3 min, followed by 5 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 digested with *Sfc* I.

The primers 5'-GAAAGGACCGGCAGTTGG-3' and 5'-GTACTTCCCTG CAGCCTG-3' were used to determine the polymorphisms of *I κ B α* -297C/T. The PCR was performed under the following conditions: initial denaturation at 96°C for 3 min and 5 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

Table 1
The genotype frequencies of *IκBα* promoter polymorphisms in the patients with Behçet's disease and the controls

<i>IκBα</i> genotype	Behçet's <i>n</i> = 86 (%)	Controls <i>n</i> = 120 (%)	OR (95% CI)	<i>p</i>
-881 A/A	73 (84.9)	92 (76.7)	1	
A/G	12 (13.9)	26 (21.7)	0.6 (0.3–1.2)	NS
G/G	1 (1.2)	2 (1.7)	0.6 (0.1–7.1)	NS
-826 C/C	1 (1.2)	79 (65.8)	1	
C/T	13 (15.1)	35 (29.2)	29.3 (3.7–233.1)	< 0.001
T/T	72 (83.7)	6 (5.0)	948 (111.4–8065.2)	< 0.001
Dominant model				
C/C	1 (1.2)	79 (65.8)	1	
C/T + T/T	85 (98.8)	41 (34.2)	44.1 (5.9–328.3)	< 0.001
Recessive model				
C/C + C/T	14 (16.3)	114 (95.0)	1	
T/T	72 (83.7)	6 (5.0)	97.7 (35.9–265.8)	< 0.001
-550 A/A	83 (96.5)	108 (90.0)	1	
A/T	3 (3.5)	11 (9.2)	0.4 (0.1–1.3)	NS
T/T	0 (0)	1 (0.8)	0.4 (0.04–4.9)	NS
-519 C/C	74 (86.0)	107 (89.2)	1	
T/C	11 (12.8)	12 (10.0)	1.3 (0.6–3.2)	NS
T/T	1 (1.2)	1 (0.8)	1.4 (0.1–23.5)	NS
-297 C/C	73 (84.9)	101 (84.2)	1	
C/T	12 (13.9)	17 (14.2)	1.0 (0.4–2.1)	NS
T/T	1 (1.2)	2 (1.7)	0.7 (0.1–7.8)	NS

NS: not significant.

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 *Hpy8 I* was used.

The polymorphisms of *IκBα* -826C/T measured by the PCR/RFLP method were also confirmed by direct sequencing.

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 (*P_c*). The OR was calculated by the method of Woolf and by a modification of the method of Haldane. The estimated haplotype frequencies were determined by the EH program (Web Resources of Genetic Linkage Analysis).

3. Results

The distributions of the genotypes in the controls and the patients were compatible with the Hardy-Weinberg equilibrium. In comparison with *IκBα* -826C/C, this study demonstrated that the genotype frequency of *IκBα* -826C/T was significantly higher in the patients with Behçet's disease than in the controls (Table 1, *p* < 0.001, OR = 29.3, 95% CI = 3.7–233.1). A similar finding could also be observed in the genotype frequency of *IκBα* -826T/T (Behçet's vs controls: *p* < 0.001,

OR = 948, 95% CI = 111.4–8065.2). The genotype frequencies of *IκBα* -826C/T polymorphisms, both in the dominant model (C/T + T/T vs C/C) and in the recessive model (T/T vs C/C + C/T), were significantly different between the patients with Behçet's disease and the controls. In the dominant model, the genotype frequency of *IκBα* -826 C/T + T/T was significantly higher in the patients with Behçet's disease than in the controls (*p* < 0.001, OR = 44.1, 95% CI = 5.9–328.3). A similar finding could also be observed in the recessive model (T/T vs C/C + C/T: *p* < 0.001, OR = 97.7, 95% CI = 35.9–265.8).

We also found that the patients with Behçet's disease had a significantly higher allele frequency of *IκBα* -826T than the controls (Table 2, *p* < 0.001, OR = 43.0, 95% CI = 23.2–79.8).

Direct sequencing was performed to verify the genotypes measured by the method of PCR/RFLP (Fig. 1). The results of PCR/RFLP were compatible with those of direct sequencing.

This study demonstrated that the estimated haplotype frequency of *IκBα* -881A -826T -550A -519C -297C in the patients with Behçet's disease was significantly higher than that of the controls (*p* < 0.001, *P_c* < 0.006, OR = 34.14, 95% CI = 17.77–65.59). A similar finding could also be observed in the estimated haplotype frequency of *IκBα* -881A -826T -550A -519T -297C (Behçet's disease vs controls: *p* < 0.001, *P_c* < 0.006,

Table 2
The allele frequencies of *I κ B α* promoter polymorphisms in the patients with Behçet's disease and the controls

<i>IκBα</i> polymorphisms	Behçet's 2n = 172 (%)	Controls 2n = 240 (%)	OR (95% CI)	p
-881 A	158 (91.9)	210 (87.5)	1.6 (0.8–3.1)	NS
G	14 (8.1)	30 (12.5)		
-826 C	15 (8.7)	193 (80.4)	43.0 (23.2–79.8)	< 0.001
T	157 (91.3)	47 (19.6)	3.2 (0.9–11.5)	NS
-550 A	169 (98.3)	227 (94.6)		
T	3 (1.7)	13 (5.4)		
-519 C	159 (92.4)	226 (94.2)	0.8 (0.3–1.7)	NS
T	13 (7.6)	14 (5.8)		
-297 C	158 (91.9)	219 (91.3)	1.1 (0.5–2.2)	NS
T	14 (8.1)	21 (8.7)		

NS: not significant.

Sequences of *I κ B α* -826 C/T polymorphisms:

RFLP results of *I κ B α* -826 C/T polymorphisms:

Fig. 1. The results of RFLP in *I κ B α* -826C/T polymorphisms were confirmed by direct sequencing.

Table 3

The estimated haplotype frequencies of *I κ B α* promoter polymorphisms in the patients with Behçet's disease and the controls

<i>IκBα</i> haplotype	Behçet's disease	Controls	OR(95% CI)	<i>p</i>	<i>P_c</i>
-881A -826C -550A -519C -297C	0.063	0.753	0.02 (0.01–0.04)	< 0.001	< 0.006
-881A -826C -550A -519C -297T	0.006	0.01	—	—	—
-881A -826C -550A -519T -297C	0.002	0.01	—	—	—
-881A -826C -550T -519C -297C	0.000	0.015	—	—	—
-881A -826T -550A -519C -297C	0.779	0.054	34.14 (17.77–65.59)	< 0.001	< 0.006
-881A -826T -550A -519C -297T	0.000	0.004	—	—	—
-881A -826T -550A -519T -297C	0.057	0.000	30.48 (3.93–236.52)	< 0.001	< 0.006
-881A -826T -550T -519C -297C	0.006	0.008	—	—	—
-881G -826T -550A -519C -297C	0.013	0.045	0.22 (0.05–1.00)	NS	NS
-881G -826T -550A -519C -297T	0.047	0.045	1.02 (0.42–2.52)	NS	NS
-881G -826T -550A -519T -297T	0.000	0.022	0.11 (0.01–0.93)	NS	NS

—: The *p* value was not calculated due to the fact that the number of cases is too small. *P_c*: corrected *p* value.

Table 4

Associations between the *I κ B α* -826T/T genotype and clinical manifestations of Behçet's disease

		<i>IκBα</i> -826T/T		<i>p</i>	OR (95% CI)
		+, <i>n</i> = 72 (%)	–, <i>n</i> = 14 (%)		
Uveitis	+	13 (18.1)	6 (42.9)	NS	
	–	59 (81.9)	8 (57.1)		
Cutaneous vasculitis	+	16 (22.2)	2 (14.3)	NS	
	–	56 (77.8)	12 (85.7)		
Peripheral Neuropathy	+	10 (13.9)	2 (14.3)	NS	
	–	62 (86.1)	12 (85.7)		
Skin lesion	+	60 (83.3)	5 (35.7)	0.001	9.0 (2.6–31.6)
	–	12 (16.7)	9 (64.3)		

Skin lesions include erythema nodosum, pseudofolliculitis, papulopustular lesion or acneiform nodule.

OR = 30.48, 95% CI = 3.93–236.52). In contrast, the estimated haplotype frequency of *I κ B α* -881A -826C -550A -519C -297C was significantly decreased in the patients with Behçet's disease when compared with that of the controls (*p* < 0.001, *P_c* < 0.006, OR = 0.02, 95% CI = 0.01–0.04).

The associations of the *I κ B α* -826T/T genotype with the clinical manifestations of Behçet's disease were shown in Table 4. The patients with Behçet's disease, carrying the *I κ B α* -826T/T genotype, have higher prevalence of skin lesions including erythema nodosum, pseudofolliculitis, papulopustular lesion or acneiform nodules than the patients without *I κ B α* -826T/T.

4. Discussion

This study demonstrated that the *I κ B α* -826T allele, *I κ B α* -881A -826T -550A -519C -297C haplotype and *I κ B α* -881A -826T -550A -519T -297C haplotype might be related to susceptibility to Behçet's disease in Taiwan.

NF κ B activation may modulate the expression of anti-apoptotic genes leading to apoptosis resistance in T-cell subsets of Behçet's disease, and plays an important role in the pathogenesis of Behçet's disease [31]. The anti-apoptogenic effect of NF κ B is caused by the expression of anti-apoptogenic molecules including Bcl-xL, XIAP, IAP, and TRAFs [32]. *I κ B* inhibits the transcription function of NF κ B. Different *I κ B* molecules preferentially inhibit the distinct NF κ B/Rel protein dimer [24]. The central portions of *I κ B* molecules contain several ankyrin repeats. The ankyrin repeats bind to the Rel homology domain of NF κ B/Rel, which causes NF κ B to remain in the cytoplasm by masking the nuclear localization sequence of NF κ B. Nuclear import of *I κ B α* is also found [33,34]. When *I κ B α* is expressed in the nucleus, it can inhibit the interaction of NF κ B with DNA and promote the export of NF κ B from the nucleus to the cytoplasm [35]. The C-terminal domain of *I κ B* may block DNA binding by NF κ B and dissociate DNA-bound NF κ B dimers [36].

Three NF κ B binding sites, which are required for induction of gene expression, have been demonstrated in the promoter of *I κ B α* [37]. A putative binding

site for transcription factors ROR alpha 1 and ROR alpha 2 is in the position of *I κ B α* -881 (TFsearch web). The *I κ B α* -826C/T polymorphisms are near a putative binding site of transcription factor GATA-2 (TFsearch web). Another putative binding site for transcription factor C/EBP is in the position of *I κ B α* -519 (TFsearch web). Therefore, the polymorphisms in the *I κ B α* promoter may affect the binding of transcriptions, and then influence the expression of *I κ B α* .

This study demonstrated that the *I κ B α* -826T allele might be related to susceptibility to Behçet's disease. We also found that the *I κ B α* -881A -826T -550A -519C -297C and *I κ B α* -881A -826T -550A -519T -297C haplotypes played significant roles in the development of Behçet's disease. Our previous study showed that the *I κ B α* -826T -550A -519C haplotype and *I κ B α* -881A -826T -550A -519C -297C haplotype were associated with susceptibility to other autoimmune diseases in Taiwan [28,29,38]. These haplotypes may be related to the promoter activity of *I κ B α* and the production of *I κ B α* , and then influence the function of NF κ B, which plays an important role in the inflammatory and immune responses. The promoter activities of various *I κ B α* promoter polymorphisms were detected with a luciferase reporter assay. It showed that individuals with *I κ B α* -826T allele had a lower promoter activity than those with *I κ B α* -826C (unpublished data). Although the sample size of this study is limited, the power for the *I κ B α* -826C/T polymorphisms is more than 95%. The numbers of patients and controls may suffice for this study.

Mutations in the *I κ B α* are associated with other autoimmune diseases. An 8 bp insertion in the promoter region of *I κ B α* (*I κ B α* -708 ins 8) prevented the development of primary progressive multiple sclerosis [39]. The *I κ B α* polymorphisms may also be associated with Crohn's disease or autoimmune diabetes mellitus [40,41]. The single nucleotide polymorphisms in the 3'-UTR were significantly increased in these patients. Mutations of *I κ B α* may also be associated with the development of other inflammatory diseases and malignancies [42,43]. Mozzato-Chamay showed that the *I κ B α* -881G/ -826T haplotype protected the development of scarring trachoma, an inflammatory disease, in Gambia [26]. The *I κ B α* -881G -826T -297T haplotype was significantly associated with sarcoidosis in the UK and the Netherlands, and the *I κ B α* -826T allele was related to the stage of sarcoidosis [44]. *I κ B α* -deficient mice died of a wasting disease that was attributed to over-expression of TNF α [45]. Klement showed that *I κ B α* deficiency also resulted in a sustained NF κ B response and severe widespread dermatitis in mice [46].

Table 5

Associations of *I κ B α* polymorphisms with various autoimmune diseases

Diseases	Associated <i>IκBα</i>	Population	Ref.
Multiple sclerosis (primary progressive type)	decreased -708ins8	German	[39]
LADA	increased AA genotype (rs696 in 3'-UTR)	Czech	[41]
Crohn's	increased AA genotype (rs696 in 3'-UTR)	German	[40]
PsA	no	Canadian	[47]
RA	increased -826T (rs 2233406)	Taiwanese	[28]
Behcet's	increased -826T (rs 2233406)	Taiwanese	present study

LADA: latent autoimmune diabetes in adult; PsA: psoriatic arthritis. RA: rheumatoid arthritis; SLE: systemic lupus erythematosus.

This study also demonstrated that the *I κ B α* -826T/T genotype was associated with the development of skin lesions in the patients with Behçet's disease.

The polymorphisms of *I κ B α* have been demonstrated to be associated with some autoimmune diseases. The associations of these polymorphisms with various autoimmune diseases were summarized in Table 5.

In conclusion, the *I κ B α* -826T allele, *I κ B α* -881A -826T -550A -519C -297C haplotype, and *I κ B α* -881A -826T -550A -519T -297C haplotype may be related to susceptibility to Behçet's disease. In contrast, the *I κ B α* -881A -826C -550A -519C -297C haplotype may prevent the development of Behçet's disease in Taiwan. Moreover, the *I κ B α* -826T/T genotype is associated with skin lesions in patients with Behçet's disease.

References

- [1] S. Ohno, M. Ohguchi, S. Hirose, H. Matsuda, A. Wakisaka and M. Aizawa, Close association of HLA-Bw51 with Behçet's disease, *Arch Ophthalmol* **100** (1982), 1455–1458.
- [2] N. Mizuki, M. Ota, Y. Katsuyama, K. Yabuki, H. Ando, T. Shiina, E. Nomura, K. Onari, S. Ohno and H. Inoko, HLA-B*51 allele analysis by the PCR-SBT method and a strong association of HLA-B*5101 with Japanese patients with Behçet's disease, *Tissue Antigens* **58** (2001), 181–184.
- [3] S. Hirohata and H. Kikuchi, Behçet's disease, *Arthritis Res Ther* **5** (2003), 139–146.
- [4] R. Gunesacar, E. Erken, B. Bozkurt, H.T. Ozer, S. Dinkci, E.G. Erken and Z. Ozbalkan, Analysis of CD28 and CTLA-4 gene polymorphisms in Turkish patients with Behçet's disease, *Int J Immunogenet* **34** (2007), 45–49.
- [5] J. Karasneh, A.H. Hajeer, J. Barrett, W.E. Ollier, M. Thornhill and A. Gul, Association of specific interleukin 1 gene cluster polymorphisms with increased susceptibility for Behçet's disease, *Rheumatology (Oxford)* **42** (2003), 860–864.
- [6] A. Meguro, M. Ota, Y. Katsuyama, A. Oka, S. Ohno, H. Inoko and N. Mizuki, Association of the toll-like receptor 4 gene polymorphisms with Behçet's disease, *Ann Rheum Dis* **67** (2008), 725–727.

- [7] K. Durrani and G.N. Papaliodis, The genetics of Adamantiades-Behcet's disease, *Semin Ophthalmol* **23** (2008), 73–79.
- [8] J.H. Yen, W.C. Tsai, C.H. Lin, T.T. Ou, C.J. Hu and H.W. Liu, Cytochrome P450 1A1 and manganese superoxide dismutase gene polymorphisms in Behcet's disease, *J Rheumatol* **31** (2004), 736–740.
- [9] I. Krause and A. Weinberger, Behcet's disease, *Curr Opin Rheumatol* **20** (2008), 82–87.
- [10] Y.J. Lee, S.W. Kang, J.J. Park, Y.D. Bae, E.Y. Lee, E.B. Lee and Y.W. Song, Interleukin-18 promoter polymorphisms in patients with Behcet's disease, *Hum Immunol* **67** (2006), 812–818.
- [11] T. Ahmad, G.R. Wallace, T. James, M. Neville, M. Bunce, K. Mulcahy-Hawes, A. Armuzzi, J. Crawshaw, F. Fortune, R. Walton, M.R. Stanford, K.I. Welsh, S.E. Marshall and D.P. Jewell, Mapping the HLA association in Behcet's disease: a role for tumor necrosis factor polymorphisms? *Arthritis Rheum* **48** (2003), 807–813.
- [12] K. Nakao, Y. Isashiki, S. Sonoda, E. Uchino, Y. Shimonagano and T. Sakamoto, Nitric oxide synthase and superoxide dismutase gene polymorphisms in Behcet disease, *Arch Ophthalmol* **125** (2007), 246–251.
- [13] J.A. Karasneh, A.H. Hajeer, A. Silman, J. Worthington, W.E. Ollier and A. Gul, Polymorphisms in the endothelial nitric oxide synthase gene are associated with Behcet's disease, *Rheumatology (Oxford)* **44** (2005), 614–617.
- [14] J. Karasneh, A. Gul, W.E. Ollier, A.J. Silman and J. Worthington, Whole-genome screening for susceptibility genes in multicase families with Behcet's disease, *Arthritis Rheum* **52** (2005), 1836–1842.
- [15] K. Hamzaoui, A. Hamzaoui, I. Ghorbel, M. Khanfir and H. Houman, Levels of IL-15 in serum and cerebrospinal fluid of patients with Behcet's disease, *Scand J Immunol* **64** (2006), 655–660.
- [16] U. Musabak, S. Pay, H. Erdem, I. Simsek, A. Pekel, A. Dinc and A. Sengul, Serum interleukin-18 levels in patients with Behcet's disease. Is its expression associated with disease activity or clinical presentations? *Rheumatol Int* **26** (2006), 545–550.
- [17] H. Yanagihori, N. Oyama, K. Nakamura, N. Mizuki, K. Oguuma and F. Kaneko, Role of IL-12B promoter polymorphism in Adamantiades-Behcet's disease susceptibility: An involvement of Th1 immunoreactivity against Streptococcus Sanguinis antigen, *J Invest Dermatol* **126** (2006), 1534–1540.
- [18] A. Kulaber, I. Tugal-Tutkun, S.P. Yentur, G. Akman-Demir, F. Kaneko, A. Gul and G. Saruhan-Direskeneli, Pro-inflammatory cellular immune response in Behcet's disease, *Rheumatol Int* **27** (2007), 1113–1118.
- [19] P.J. Barnes and M. Karin, Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases, *N Engl J Med* **336** (1997), 1066–1071.
- [20] P.A. Baeuerle, Pro-inflammatory signaling: last pieces in the NF-kappaB puzzle? *Curr Biol* **8** (1998), R19–R22.
- [21] P.P. Tak and G.S. Firestein, NF-kappaB: a key role in inflammatory diseases, *J Clin Invest* **107** (2001), 7–11.
- [22] S. Castro-Alcaraz, V. Miskolci, B. Kalasapudi, D. Davidson and I. Vancurova, NF-kappa B regulation in human neutrophils by nuclear I kappa B alpha: correlation to apoptosis, *J Immunol* **169** (2002), 3947–3953.
- [23] E. Dale, M. Davis and D.L. Faustman, A role for transcription factor NF-kappaB in autoimmunity: possible interactions of genes, sex, and the immune response, *Adv Physiol Educ* **30** (2006), 152–158.
- [24] M.J. May and S. Ghosh, Rel/NF-kappa B and I kappa B proteins: an overview, *Semin Cancer Biol* **8** (1997), 63–73.
- [25] S.T. Whiteside and A. Israel, I kappa B proteins: structure, function and regulation, *Semin Cancer Biol* **8** (1997), 75–82.
- [26] N. Mozzato-Chamay, E.L. Corbett, R.L. Bailey, D.C. Mabey, J. Raynes and D.J. Conway, Polymorphisms in the IkappaB-alpha promoter region and risk of diseases involving inflammation and fibrosis, *Genes Immun* **2** (2001), 153–155.
- [27] F. Emmerich, M. Meiser, M. Hummel, G. Demel, H.D. Foss, F. Jundt, S. Mathas, D. Krappmann, C. Scheidereit, H. Stein and B. Dorken, Overexpression of I kappa B alpha without inhibition of NF-kappaB activity and mutations in the I kappa B alpha gene in Reed-Sternberg cells, *Blood* **94** (1999), 3129–3134.
- [28] C.H. Lin, T.T. Ou, C.C. Wu, W.C. Tsai, H.W. Liu and J.H. Yen, IkappaBalpha promoter polymorphisms in patients with rheumatoid arthritis, *Int J Immunogenet* **34** (2007), 51–54.
- [29] C.H. Lin, S.C. Wang, T.T. Ou, R.N. Li, W.C. Tsai, H.W. Liu and J.H. Yen, IkappaBalpha Promoter Polymorphisms in Patients with Systemic Lupus Erythematosus, *J Clin Immunol* **11** (2007), 11.
- [30] Criteria for diagnosis of Behcet's disease. International Study Group for Behcet's Disease, *Lancet* **335** (1990), 1078–1080.
- [31] M. Todaro, M. Zerilli, G. Triolo, F. Iovino, M. Patti, A. Accardo-Palumbo, F. di Gaudio, M.C. Turco, A. Petrella, R. de Maria and G. Stassi, NF-kappaB protects Behcet's disease T cells against CD95-induced apoptosis up-regulating anti-apoptotic proteins, *Arthritis Rheum* **52** (2005), 2179–2191.
- [32] A. Kawakami and K. Eguchi, Involvement of apoptotic cell death in autoimmune diseases, *Med Electron Microsc* **35** (2002), 1–8.
- [33] F. Arenzana-Seisdedos, J. Thompson, M.S. Rodriguez, F. Bachelier, D. Thomas and R.T. Hay, Inducible nuclear expression of newly synthesized I kappa B alpha negatively regulates DNA-binding and transcriptional activities of NF-kappa B, *Mol Cell Biol* **15** (1995), 2689–2696.
- [34] P. Turpin, R.T. Hay and C. Dargemont, Characterization of IkappaBalpha nuclear import pathway, *J Biol Chem* **274** (1999), 6804–6812.
- [35] F. Arenzana-Seisdedos, P. Turpin, M. Rodriguez, D. Thomas, R.T. Hay, J.L. Virelizier and C. Dargemont, Nuclear localization of I kappa B alpha promotes active transport of NF-kappa B from the nucleus to the cytoplasm, *J Cell Sci* **110** (Pt 3) (1997), 369–378.
- [36] M.K. Ernst, L.L. Dunn and N.R. Rice, The PEST-like sequence of I kappa B alpha is responsible for inhibition of DNA binding but not for cytoplasmic retention of c-Rel or RelA homodimers, *Mol Cell Biol* **15** (1995), 872–882.
- [37] C.Y. Ito, A.G. Kazantsev and A.S. Baldwin, Jr., Three NF-kappa B sites in the I kappa B-alpha promoter are required for induction of gene expression by TNF alpha, *Nucleic Acids Res* **22** (1994), 3787–3792.
- [38] T.T. Ou, C.H. Lin, Y.C. Lin, R.N. Li, W.C. Tsai, H.W. Liu and J.H. Yen, IkappaBalpha promoter polymorphisms in patients with primary Sjogren's syndrome, *J Clin Immunol* **28** (2008), 440–444.
- [39] B. Mitterski, S. Bohringer, W. Klein, E. Sindern, M. Haupts, S. Schimrigk and J.T. Epplen, Inhibitors in the NFkappaB cascade comprise prime candidate genes predisposing to multiple sclerosis, especially in selected combinations, *Genes Immun* **3** (2002), 211–219.
- [40] W. Klein, A. Tromm, C. Folwaczny, M. Hagedorn, N. Duerig, J.T. Epplen, W.H. Schmiegel and T. Griga, A polymorphism of the NFKBIA gene is associated with Crohn's disease pa-

- tients lacking a predisposing allele of the CARD15 gene, *Int J Colorectal Dis* **19** (2004), 153–156.
- [41] K. Katarina, P. Daniela, N. Peter, R. Marianna, C. Pavlina, P. Stepanka, L. Jan, T. Ludmila, A. Michal and C. Marie, HLA, NFKB1 and NFKBIA gene polymorphism profile in autoimmune diabetes mellitus patients, *Exp Clin Endocrinol Diabetes* **115** (2007), 124–129.
- [42] J. Gao, D. Pfeifer, L.J. He, F. Qiao, Z. Zhang, G. Arbman, Z.L. Wang, C.R. Jia, J. Carstensen and X.F. Sun, Association of NFKBIA polymorphism with colorectal cancer risk and prognosis in Swedish and Chinese populations, *Scand J Gastroenterol* **42** (2007), 345–350.
- [43] X.F. Sun and H. Zhang, NFKB and NFKBI polymorphisms in relation to susceptibility of tumour and other diseases, *Histol Histopathol* **22** (2007), 1387–1398.
- [44] A. Abdallah, H. Sato, J.C. Grutters, S. Veeraraghavan, P.A. Lympny, H.J. Ruven, J.M. van den Bosch, A.U. Wells, R.M. du Bois and K.I. Welsh, Inhibitor kappa B-alpha (IkappaB-alpha) promoter polymorphisms in UK and Dutch sarcoidosis, *Genes Immun* **4** (2003), 450–454.
- [45] A.A. Beg, W.C. Sha, R.T. Bronson and D. Baltimore, Constitutive NF-kappa B activation, enhanced granulopoiesis, and neonatal lethality in I kappa B alpha-deficient mice, *Genes Dev* **9** (1995), 2736–2746.
- [46] J.F. Klement, N.R. Rice, B.D. Car, S.J. Abbondanzo, G.D. Powers, P.H. Bhatt, C.H. Chen, C.A. Rosen and C.L. Stewart, IkappaBalpha deficiency results in a sustained NF-kappaB response and severe widespread dermatitis in mice, *Mol Cell Biol* **16** (1996), 2341–2349.
- [47] C. Butt, S. Sun, L. Peddle, C. Greenwood, S. Hamilton, D. Gladman and P. Rahman, Association of nuclear factor-kappaB in psoriatic arthritis, *J Rheumatol* **32** (2005), 1742–1744.