Histol Histopathol (2007) 22: 1387-1398
DOI: 10.14670/HH-22.1387

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Review

NFKB and *NFKBI* polymorphisms in relation to susceptibility of tumour and other diseases

X-F. Sun[1] and H. Zhang[2]
Departments of [1]Oncology and [2]Dermatology, Institute of Biomedicine and Surgery, University of Linköping, Linköping, Sweden

Summary. Nuclear factor-κB (NF-κB) is responsible for the expression by regulating many genes for immune response, cell adhesion, differentiation, proliferation, angiogenesis and apoptosis. The function of NF-κB is inhibited by binding to NF-κB inhibitor (IκB), and imbalance of NF-κB and IκB has been associated with development of many diseases, including tumours. In this review, we focus on polymorphisms of the *NFKB* and *NFKBI* genes in relation to development of common inflammatory diseases including ulcerative colitis (UC), Crohn's disease (CD), rheumatoid arthritis, systemic lupus erythematosus, psoriatic arthritis, giant cell arthritis, type 1 diabetes, multiple sclerosis, celiac disease, and Parkinson's disease, as well as susceptibility of several cancers, such as oral squamous cell carcinoma, colorectal cancer (CRC), hepatocellular carcinoma, breast cancer and myeloma.

Key words: NFKB, NFKBI, Polymorphism, Inflammatory disease, Susceptibility, Tumour

Introduction

Nuclear factor-κB (NF-κB) regulates the transcription of many genes for immune response, cell adhesion, differentiation, proliferation, angiogenesis and apoptosis (Beinke and Ley, 2004). Abnormalities in the NF-κB regulation are involved in multiple human pathologies including inflammatory diseases, immune deficiencies, diabetes and atherosclerosis as well as tumours. The function of NF-κB is inhibited by binding to NF-κB inhibitor (IκB) and release of activated NF-κB follows proteasome-mediated degradation of IκB resulting from phosphorylation of the inhibitor and finally conjugation with ubiquitin (Beinke and Ley, 2004; Berenson et al., 2001).

Since NF-κB is responsible for the regulation of many other genes in disease progression, variants in the genes coding for the NF-κB and IκB proteins of could be potentially involved in disease development. In this review, we focus on polymorphisms of the *NFKB* (encoding for NF-κB) and *NFKBI* (encoding for IκB) in relation to development of inflammatory and other diseases including ulcerative colitis (UC), Crohn's disease (CD), rheumatoid arthritis, systemic lupus erythematosus, psoriatic arthritis, giant cell arthritis, type 1 diabetes, multiple sclerosis, celiac disease, Parkinson's disease, and susceptibility of tumours including oral squamous cell carcinoma, colorectal cancer (CRC), hepatocellular carcinoma, breast cancer and myeloma.

NFKB genes and proteins

Five members in the NF-κB family have been identified, and they are NF-κB1 (p105/p50), NF-κB2 (p100/p52), RelA (p65), RelB and c-Rel. *NFKB1* gene mapped on 4q23-q24, is composed of 24 exons and introns varying between 40 000 and 323 bp, spansing 156 kb. The gene encoding p105 and p50 proteins, p105 is a non-DNA-binding cytoplasmic molecule and p50 is a DNA-binding protein corresponding to the N-terminus of p105 (OMIN, 164011) (Le Beau et al., 1992; Liptay et al., 1992; Mathew et al., 1993; Heron et al., 1995). *NFKB2* gene, mapped on 10q24, encodes p100 and p52 proteins (OMIN, 164012) (Liptay et al., 1992; Mathew et al., 1993). *RelA* (*NFKB3*) gene, at 11q12-q13 with 10 exons, encodes p65 (OMIN, 164014) (Mathew et al., 1993; Deloukas et al., 1994). *RelB* gene is assigned to chromosome 19 (OMIM, 604758, MI-12248), and *c-Rel* gene is located on 2p13-p12 (Brownell et al., 1986).

NF-κB proteins share highly conserved Rel homology domain (RHD) responsible for DNA binding, dimerization and interaction with IκB. Members in the NF-κB family (NF-κB1, NF-κB2, RelA, RelB and c-Rel) form homo- and heterodimers including p50/RelA,

Offprint requests to: Prof. Xiao-Feng Sun, MD, PhD., Department of Oncology, Institute of Biomedicine and Surgery, University of Linköping, SE-581 85 Linköping, Sweden. e-mail: xiasu@ibk.liu.se

Abbreviations: CD, Crohn's disease; CRC, colorectal cancer; IBD, inflammatory bowel disease; IκB, nuclear factor-κB inhibitor; IKK, IκB kinase; NF-κB, nuclear factor-κB; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SSCP, single strand conformation polymorphism; UC, ulcerative colitis.

p50/RelB, p50/c-Rel, p52/RelA, p52/RelB, p52/c-Rel, RelA/RelB, RelA/c-Rel, p50/p50, p52/p52 and RelA/RelA. Among them, NF-κB1 (p50)/RelA (p65) heterodimer is the common NF-κB/Rel complex. RelB acts as a transcriptional activator and a repressor of NF-κB dependent gene expression. It acts as an activator when binding to NF-κB1 (p50) or NF-κB2 (p52) (Fig. 1), and as inhibitor formating the RelA (p65)/RelB heterodimer that does not bind to IκB sites in the cytoplasm or the nucleus. Moreover, the functions of NF-κB1 (p105) and NF-κB2 (p100) are distinct although their structures are similar. It has been shown that processing from p105 to p50 is constitutive, and p100 processing to p52 is critical for the organogenesis of peripheral lymphoid tissues and B-cell development. Induction of the p100 processing is tightly regulated by a subset of ligands which activate NF-κB (Berenson et al., 2001; Beinke and Ley, 2004).

NFκBI genes and proteins

The IκB proteins belong to the functionally related family comprising IκBα, IκBß, IκBγ, IκBε, BCL3, p105 and p100. *NFKBIA* (*IKBA*) gene located at locus 14q13 contains 6 exons spanning 3.5kb (OMIN, 164008) (Le Beau et al., 1992). The gene includes three regions: N-terminal region with phosphorylation sites to regulate signal-dependent degradation of IκBα through the ubiquitin-proteasome pathway; ankyrin repeat domain physically associated with NF-κB proteins, and C-terminal PEST region (rich in prolin (P), glutamate (E), serine (S) and threonine (T)) regulating basal degradation. Exon 1 encodes the N-terminal region containing the serine residues. The ankyrin repeat domain is located in exons 2 to 5, and C-terminal PEST region is found in exon 6 (OMIN, 164008) (Le Beau et al., 1992). *NFKBIB* gene mapped at 19q13.1, has 10 exons, and 1,940 bp cDNA (OMIN, 604495) (Okamoto et al., 1998). These two genes share the same ancestral origin and are generated by localized gene duplication. The common feature of these proteins is the ankyrin repeat of 30-33 amino acids.

NF-κB signalling pathways

Phosphorylation of the IκB proteins is regulated by IκB kinases (IKK), and activity of IKK is strongly induced by stimuli in the NF-IκB pathway such as tumour necrosis factor-alpha (TNFα), interleukin 1-beta (IL-1ß), growth factors, B or T cell activation, lymphokines, oxidant-free radicals, inhaled particles, certain products of viral or bacterial genes, UV irradiation, and by other physiological and pathological stimuli (Berenson et al., 2001; Beinke and Ley, 2004). Moreover, the IKK complex contains two kinase subunits, IKKα (or IKK1) and IKKß (IKK2) and a structural subunit, IKKγ (also known as NF-κB essential modulator, NEMO), which is required for coupleing the

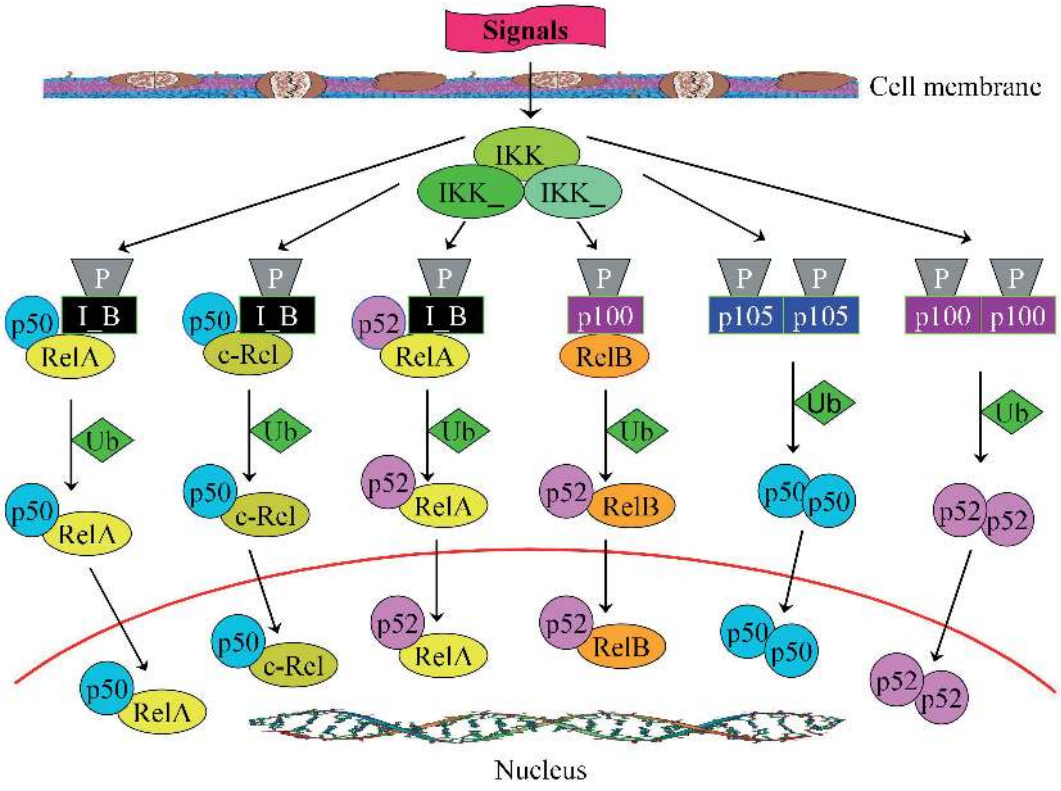

Fig. 1. Model of different NF-κB signalling pathways.

IKK complex. The IKK complex of IKKα–IKKß–IKKγ phosphorylates the IκB and furthewr induces its rapid degradation, which is essential for immune responses, inflammation and promoting cell survival. There is another alternative for NF-κB pathway to activate agonists, which plays an important role in secondary lymphoid organogenesis, maturation of B-cells and adaptive humoral immunity. This process requires NIK (NF-κB interacting kinase) and IKKα which induces the slow processing of p100 to p52, resulting in nuclear translocation of p52/RelB heterodimers. Moreover, the p105 pathway is involved in immune and inflammatory responses. In addition, agonist activation in this pathway induces phosphorylation of the p105 PEST region by the classical IKK complex, and this process also triggers p105 polyubiquitination and subsequent degradation, releasing p50 homodimers which undergo nuclear translocation and gene transcription (Berenson et al., 2001; Beinke and Ley, 2004) (Fig. 1).

In addition to modulation of IKK activity, it is also shown that NF-κB transcription can be activated by p65 phosphorylation, which subunit contains the Rel homology domain in its amino terminus and two transactivation domains in its carboxy-terminus. Phosphorylation of serine residue 276 in p65 by protein kinase A facilitates its interaction with the nuclear coactivator cAMP-responsive element-binding protein-binding protein (CBP) resulting in enhanced p65 transcriptional activity. TNFα treated cells can also induce p65 phosphorylation, although it occurs on serine residue 529 and enhances p65 transactivation. The IKKs can directly phosphorylate residues 536 in the transactivation domain of p65. In addition, the IKKs contribute either directly or indirectly with the ability of the PI-3 inducible kinase AKT to stimulate p65 transactivation. Several studies suggest that C-terminal truncation of p100 in lymphomas induce nuclear translocation of the mutant p100 molecule, which directly activate transcription, without requiring processing to p52. Recently, it has been reported that p100 deletion is constitutively processed to p52 in the nucleus due to removal of inhibitory p100 death domain (Berenson et al., 2001; Yamamoto and Gaynor, 2001; Beinke and Ley, 2004) (Fig. 1).

After the complete degradation of IκB, NF-κB is translocated to the nucleus and binds to the enhancer or promoter regions and further regulates their transcription, where the activation is decided by acetylation of NF-κB and, p300 and CBP acetyltransferases play a major role in the acetylation of RelA (p65). Acetylated NF-κB is active and resistant to the inhibitory effects of IκB. However, when histone deacetylase 3 (HDAC3) deacetylates NF-κB, IκB readily binds to NF-κB and causes its translocation. HDAC3 is triggered by NF-κB to turn off the biological processes. IκBα is one of the target genes activated by NF-κB. The newly synthesized IκBα enters in the nucleus, removes NF-κB from DNA, and exports the complex back to the cytoplasm to restore its original latent state (Berenson et al., 2001; Yamamoto and Gaynor, 2001; Beinke and Ley, 2004).

NF-κB and IκB proteins in relation to diseases

Increased NF-κB activation is involved in inflammatory response. It has been reported that levels of NF-κB are increased in lamina propria biopsy from patients with inflammatory bowel disease (IBD), such as UC and CD. Inhibition of NF-κB activation represents a mechanism behind steroids anti-inflammatory effect in IBD. Some *E. coli* bacteria from inflamed IBD tissues can activate NF-κB in intestinal epithelial cells, while the *E. coli* from noninflamed tissues or wild-type *E. coli* have weaker or no such effects (La Ferla et al., 2004). Furthermore, the NF-κB ctivation in IBD is mainly induced by cytokines, such as IL-1, IL-2, IL-6, IL-8, IL-12 and TNF-α, which play important roles in the IBD pathogenesis (Murata et al., 1995; Neurath et al., 1998; Al-Mohanna et al., 2002; Chang et al., 2005). In a model system of intestinal epithelial cells, nontyphoidal salmonellae induce secretion of proinflammatory cytokines, such as IL-8, while the proinflammatory cytokines are not secreted if not present the bacterial origin (Gewirtz et al., 2001; Nadeau et al., 2002).

NF-κB signalling plays a essential role in tumour development and aggressiveness by enhancing tumour angiogenesis, proliferation, anti-apoptosis, and repressing immune response. Thus, NF-κB provides a critical mechanistic link between inflammation and tumour. Further, various inflammatory cytokines, chemokines, necrotic cell products, bacteria and viruses stimulate NF-κB activation. On the other hand, the NF-κB proteins enhance the expression of some cellular genes involving cytokines, chemokines, the major histocompatibility complex (MHC), and receptors required for neutrophil adhesion and migration (Dejardin et al., 1998; Dong et al., 2005). In cancer cells, NF-κB is constitutively active and resides in the nucleus. However, this activation is due to chronic stimulation in the IKK pathway, or in other defective genes encoding IκBα (Berenson et al., 2001; Beineky and Ley, 2004). Notably, NF-κB promotes or inhibits carcinogenesis, depending on the cell types. Imbalance of NF-κB and IκB has been involved in various diseases, however, mechanisms behind how specific polymorphisms of various genes associate with disease development are unclear.

NFKB polymorphisms in relation to diseases

UC

UC is a chronic inflammatory disorder in the gastrointestinal tract. The exact causes remain elusive although it is thought to be the result of an inappropriate and ongoing activation of the mucosal immune system driven by the presence of normal luminal flora in a genetically susceptible host (Bouma and Strober, 2003).

Increased NF-κB activation has been found in the intestinal mucosa of UC patients (Rogler et al., 1998).

The *NFKB*1 promoter with exon 1 and all 23 coding exons and their flanking introns are sequenced in 10 IBD probands and two controls (Karban et al., 2004). Several polymorphisms such as -94int/delATTG in the *NFKB1* promoter, exon 1+252C→G, exon 12+77C→T, IVS15 -25G→T, IVS22 +15C→T, and IVS22+22C→G have been recently identified (Miterski et al., 2002; Wintermeyer et al., 2002). Further genotypinf the -94ins/delATTG shows that homozygote the -94delATTG allele is more frequent in 350 unrelated, non-Jewish UC cases and 802 non-Jewish controls from a North American population (Table 1). Shortly after, the -94ins/delATTG polymorphism is examined in 127 unrelated Dutch UC patients and 155 matched healthy controls (Borm et al., 2005), and found the homozygous for -94 del ATTG allele is a significant risk factor for UC development (Table 1). They further found that this polymorphism was linked to particular clinical phenotypes in the male but not in female patients. The mean age of onset was found significantly lower in the patients with homozygous for the -94 delATTG allele, indicating that this homozygosity is a risk factor for early onset of the disease.

Further functional study shows that nuclear proteins from normal colon tissue and colonic cell lines bind to -94insATTG but not to the -94delATTG. *NFKB1* promoter/exon 1 luciferase reporter plasmid containing the -94delATTG allele showed less promoter activity than the comparable constructs containing the -94insATTG allele when transfected into either HeLa or HT-29 cell lines, suggesting that the -94ins/delATTG polymorphism influences promoter activity of the *NFKB1* gene (Karban et al., 2004). They proposed that the -94ins/delATTG polymorphism differentially bind to an unidentified nuclear protein. However, whether the potential up-regulation of *NFKB1* promoter activity by nuclear protein binding to the -94insATTG and not -94delATTG allele accounts for the differences in

Table 1. Genotypes of the -94ins/delATTG in NFKBA promoter region in controls and patients.

Studies	Controls (%)			Patients (%)			P
	WW	WD	DD	WW	WD	DD	
Ulcer colitis							
Borm et al. (2005)[25]	64 (41)	67 (43)	24 (16)	34 (27)	61 (48)	32 (25)	0.02
Glas et al. (2006)[32]	358 (37)	458 (47)	158 (16)	121 (33)	181 (50)	63 (17)	NS
Karban et al. (2004)[22]	298 (37)	385 (48)	119 (15)	108 (31)	168 (48)	74 (21)	0.004
Mirza et al. (2006)[33]	231 (35)	330 (50)	96 (15)	170 (36)	225 (48)	77 (16)	NS
Oliver et al. (2005)[31]	114 (43)	113 (43)	37 (14)	107 (41)	122 (47)	29 (11)	NS
Crohn's disease							
Borm et al. (2005)[25]	64 (41)	67 (43)	24 (16)	53 (38)	70 (50)	16 (12)	NS
Glas et al. (2006)[32]	358 (37)	458 (47)	158 (16)	231 (37)	301 (48)	98 (15)	NS
Karban et al. (2004)[22]	58 (39)	70 (47)	21 (14)	23 (31)	39 (53)	12 (16)	NS
Rheumatoid arthritis							
Orozco et al. (2005)[46]	114 (43)	113 (43)	37 (14)	112 (41)	131 (48)	29 (11)	NS
Systemic lupus erythematosus							
Orozco et al. (2005)[46]	114 (43)	113 (43)	37 (14)	73 (40)	89 (49)	19 (11)	NS
Psoriatic arthritis							
Butt et al. (2005)[47]	26 (31)	45 (54)	12 (15)	75 (33)	111 (50)	38 (17)	NS
Giant cell arteritis							
Martin et al. (2006)[48]	78 (38)	96 (47)	30 (15)	28 (29)	49 (51)	19 (20)	NS
Celiac disease							
Rueda et al. (2006)[49]	282 (40)	321 (45)	108 (15)	193 (40)	224 (47)	64 (13)	NS
Type 1 diabetes							
Kosoy et al. (2005)[50]	Ins (0.631)	Del (0.369)		Ins (0.598)	Del (0.402)		0.047**
Martinez et al. (2006)[54]	170 (37)	214 (47)	74 (16)	110 (41)	120 (45)	39 (14)	NS
Oral submucous fibrosis							
Lin et al. (2006)[42]	43 (21)	100 (50)	58 (29)	23 (27)	45 (53)	17 (20)	NS
Oral squamous cell carcinoma							
Lin et al. (2006)[42]	43(21)	100 (50)	28 (29)	59 (28)	103 (49)	50 (23)	NS (0.04*, 0.005*)
Colorectal cancer***							
Lewander et al. (Chinese)	113 (24)	266 (58)	79 (17)	50 (26)	101 (52)	42 (22)	NS
Lewander et al. (Swedish)	116 (26)	256 (58)	67 (15)	16 (8)	134 (69)	43 (22)	<0.0001

DD, deletion homozygote; NS, non-significance , WD, heterozygote; WW, wild homozygote; *, ≥ 50 years old, WD versus DD, p=0.04, WW versus DD, p=0.005; **, allele analysis; ***, unpublished data.

NFKB1 or the different activity is independent of this binding are required further investigation.

The mechanism behind NFKB1 in relation to disease susceptibility remains unclear although -94 del ATTG has been proven to reduce activation of NF-κB1 transcription. The -94delATTG allele may result in decreased *NFKB1* message and hence decreased p50/p105 NF-κB protein production, for example the CD associated with NOD2 mutations results in a decrease in NF-κB activity. The NOD2 is a key component in innate mucosa as an antibacterial factor, and lacl of NOD2 activity contributes to the development of CD. Moreover, mutation in NOD2 weakens hosts to clean invasive bacteria (Bonen et al., 2003; Hisamatsu et al., 2003). It is possible, therefore, that activation of NF-κB may maintain the normal cellular defences against intestinal bacteria by the innate immune system. Defect of the NF-κB allows bacteria to cross the intestinal lumen and escape the clean by immune system, and hence contribute to on-going intestinal inflammation. Decreased -94delATTG allele expression reduces NF-κB p50/p65 heterodimers, major mediators of inflammation, leading to decreased ability to protect the bacteria (Tomczak et al., 2003; Karban et al., 2004). Further, experiments in mouse macrophages, human monocytic cells and blood monocytes show that p50 blocks expression of the TNF-α gene. During this process, the p50 homodimer rather than p50/p65 heterodimers is increased with an enhanced p105 mRNA expression, indicating up-regulation of p50 transcription. The p50, unlike p65, does not contain transactivation domain, and p50 homodimers functions to block p65 dimers from binding to promoters and activating inflammatory related genes (Ziegler-Heitbrock et al., 1994; Erdman et al., 2001) Diminished *NFKB1* transcription results in less amount of the available p50, and fewer p50/p50 homodimers will be formed. The reduced amount of the p50/p50 homodimers then, in turn, leads to activation of inflammatory genes, and NF-κB induced immune response, as seen in UC. Clearly, any change in NF-κB signalling pathway can alter the immune response, and enhance susceptibility of diseases. Obviously, further studies on molecular promoter are needed to define the overall function of the -94ins/delATTG *NFKB1* polymorphism, and other polymorphisms influencing the promoter activity (Oliver et al., 2005).

In a Spanish population including 258 UC patients and 264 healthy controls, the genotype and allele frequencies of the -94ins/delATTG are not significantly difference between the patients and controls (Table 1). In addition, no association of the polymorphism was found with clinical parameters such as gender, age, smoking habits and disease location (Oliver et al., 2005). Recently, a German study comprising 365 UC patients and 974 healthy controls did not show association of the polymorphism with UC risk (Table 1) and disease localization (Glas et al., 2006). In 472 British UC patients and 657 ethnically matched healthy controls Mirza et al. did not find a significant impact of the polymorphism in UC risk (Mirza et al., 2005, Table 1). Taken together, the -94ins/delATTG polymorphism isassociated with UC development in American and Dutch populations, but not in Spanish, German and British populations.

The discrepancy in the relationships between the polymorphism and susceptibility of the dresses is not uncommon, and could be explained by several reasons. Firstly, it is caused by various populations studied, namely specific ethnic background of populations influence the distributions of the polymorphism genotypes. It is, therefore, necessary to analyse associations of the -94ins/delATTG *NFKB1* polymorphism with UC in the populations with similar ethnic and geographic origin. Apart from the ethnic and geographic origin, family background has also associated with the -94ins/delATTG polymorphism. A linkage analysis by genome-wide screen using 377 autosomal markers in 297 CD, UC, or mixed relative pairs from 174 families show that the region on chromosome 4q containing the *NFKB1* locus is much greater for the limited subset of mixed UC and CD families but not in the families without UC (Cho et al., 1998). Secondly, the differences in environmental factors, diet and lifestyle are known to exist in different ethnic groups. Thirdly, the numbers of cases/controls, and their characteristics such as gender and age studied also affect the results. Fourthly, frequency of the polymorphisms are influenced by different techniques in the various studies. Finally, it can not exclude that other genes encoding for protein in NF-κB pathway contribute to the pathogenesis of UC (Glas et al., 2006). For such example, we see that both p50 and p65 of NF-κB are increased in patients with CD and UC, but p65 is the one with most striking increased expression (Neurath et al., 1998). So, it is of importance to examine the polymorphism, such as *NFKB2*, *RelA*, *RelB* and *c-rel*, or the genes encoding other components of the NF-κB cascade such as inhibitors of NF-κB in IBD. Therefore, it is necessary to perform the study in a larger number of case-control, strict ethical population and reliable multiple testing for environment factors to further identify the role of this polymorphism in the development of UC and other diseases.

Crohn's disease

CD is a chronic inflammatory disorder in the gastrointestinal tract with inappropriate activation of the mucosal immune system driven by normal luminal flora in susceptible host (Bouma and Strober, 2003). NF-κB activation are elevated in the intestinal mucosa of CD patients (Rogler et al., 1998). However, examined the -94ins/del ATTG polymorphism did not relate to CD risk either in Jewish (cases=28 and controls=142) or non-Jewish (cases=74 and controls=149) subjects (Karban et al., 2004, Table 1). Borm et al. examined the -94ins/delATTG polymorphism in 139 unrelated Dutch

Caucasian patients with CD, and 155 matched healthy controls, and find that frequency of allele deletion was similar in CD patients and the controls (Borm et al., 2005, Table 1), even after the stratification of gender. Moreover, using PCR-RFLP, also genotyping the -94ins/delATTG in 630 patients with CD and 974 healthy controls in a population of German origin did not show significant association of the polymorphism with CD risk (Glas et al., 2006, Table 1). However, significant association of the NFKB1 polymorphism with gender, age and disease location was found in CD patients.

CRC

A link between inflammation, such as UC, and CRC has been suspected. Thus, the -94ins/delATTG was related to an increased risk of UC, and further to CRC risk. To test this hypothesis, we performed a case-control study in two general populations from Sweden and China. There are 193 unselected patients, 90 patients with ≥ 3 affected 1st degree relatives, 85 patients with 2 affected 1st degree relatives, and 109 sporadic cancer patients in the Swedish group, and 193 unselected patients in Chinese group. The study also includes 439 Swedish and 458 Chinese healthy individuals as controls. Genotypes of the -94ins/delATTG are determined by PCR-RFLP, and results show that either heterozygous or homozygous of the -94 del ATTG allele is associated with an increased susceptibility to Swedish sporadic CRC, but not to the Swedish patients with a family history of CRC or Chinese patients (unpublished data, Table1).

It has been noticed that these two populations share similar frequencies of the genotypes in the controls (26%, 58%, 15%, versus 24%, 58%, 17%, Table 1), indicating that the individuals with the same genotype in the different populations, had different risk for developing CRC (unpublished data). Among these groups of Swedish sporadic, hereditary and familiar CRC, there was an increased risk of the heterozygous or homozygous -94 del ATTG allele in the sporadic CRC patients, but not in the other groups. All three groups of the patients were recruited from the same residential area lowering the impact of the differences in lifestyle and environmental exposures on the results. Therefore, the difference could be explanined by the sporadic cancer developed through different mechanisms, for example, more dependent on NF-κB pathway than hereditary/familiar cancer. Significant relationship of the genotypes with cancer risk was found among the unselected Swedish patients, but not hereditary/familiar CRC. Although there was no information concerning patient family history in the Swedish unselected patients, a previous study has revealed that about 10% of the total patients had a family history of CRC (Olsson and Lindblom, 2003). It is possible that this polymorphism does not directly promote carcinogenesis, rather than involving the susceptibility to carcinogenes. We therefore propose that appropriate activation of NF-κB protects against carcinogens Swedish population, but not in Chinese population, and not relevant to the patients with a family history of CRC (unpublished data).

CRC is a multifactor caused disease with different incidence in different countries. Although the reasons for this difference are still under debate there are several studies showing that it depends on a combination of differences in polymorphism distributions with environmental factors. It has been generally accepted that lifestyle and environment influence the various incidence, which has been proven by migration studies showing an adaptation after few generations (Geddes et al., 1991; Iscovich and Howe, 1998; Maskarinec and Noh, 2004). Environmental factors may influence the cancer risk by somatic mutations while polymorphisms may increase the sensitivity to the environmental exposures. It is, therefore, interesting to study cancer patients from populations with different cancer incidence such as high CRC in Sweden and a relatively low incidence in China (Parkin, 2004).

Oral squamous cell carcinoma

Areca (betel)-chewing is tightly associated with the high prevalence of oral squamous cell carcinoma, and oral submucous fibrosis as a precancerous condition (Tilakaratne et al., 2006). NF-κB protein is highly expressed in oral submucous fibrosis, and the treatment of the disease decrease NF-κB expression, suggesting that chewing areca quid may activate NF-κB, and is involved in the pathogenesis of oral submucous fibrosis (Ni et al., 2006). Lin et al. investigated the -94ins/delATTG polymorphism in NFKB1 promoter in 212 primary oral squamous cell carcinoma patients, 85 oral submucous fibrosis from male areca chewers, and 201 male areca chewer controls in Taiwan, and they did not find a significant difference in NFKB1 genotype among oral squamous cell carcinoma, oral submucous fibrosis and controls (Table 1). However, after stratification with age, oral squamous cell carcinoma was shown a higher frequency of the insertion allelotype in the patients >50 years old. There is no significant difference in the NFKB1 allelotype or genotype in patients with oral squamous cell carcinoma exhibiting different status of lymph node metastasis or clinical stage, suggesting that the functional NFKB1 promoter polymorphism is valuable for assessment of oral squamous cell carcinoma.

Rheumatoid arthritis and systemic lupus erythematosus

Rheumatoid arthritis is complex disease, with contributions from systemic autoimmunity and local inflammation. Further, persistent synovial joint inflammation and invasive synovial pannus tissue lead to joint destruction. Systemic lupus erythematosus is a chronic inflammatory autoimmune disorder. NF-κB has been activated in rheumatoid arthritis synovium, and

resembled in inflammation mediators from rheumatoid arthritis, suggesting a role in the control of inflammation (Miagkov et al., 1998; Makarov, 2001). However, the activation of NF-κB is significantly decreased in systemic lupus erythematosus patients (Wong et al., 1999). These observations indicate that the mechanism for NF-κB regulation differs between these autoimmune diseases (Orozco et al., 2005). They analyzed the distribution of the -94ins/delATTG NFκB1 in 272 rheumatoid arthritis patients, and 181 systemic lupus erythematosus patients, and 264 healthy controls from Southern Spain. They did not find significant difference in the distribution of the -94ins/delATTG NFKB1 genotypes and alleles between rheumatoid arthritis patients, systemic lupus erythematosus patients and controls (Table 1), suggesting that the -94ins/delATTG on NFKB1 dose not play a critical role in the development of rheumatoid arthritis or systemic lupus erythematosus.

Psoriatic Arthritis

Psoriatic Arthritis is a complex immunological mediated disorder resulting from interplay between multiple genetic and environmental factors. However, Genotypes of the -94ins/delATTG (Table 1) and 5 SNPs (rs4648065, rs4648072, rs4648085, rs4648086 and rs4648099) in *NFKB1*, 4 SNPs (promoter SNP rs11568292, coding SNP rs7116571, and 3' SNP rs2009453 and rs6591183) in RelA and 7 SNPs (promoter SNP -410 (rs2233409), -642 (rs2233408), -673 (rs2233407), -949 (rs2233406) and 3' SNP 2643 (rs8904), 2758 (rs696) and 3053 (rs2273650)) in NFKBIA in 224 patients with psoriatic arthritis and 88 ethnically matched controls in Canada did not show any of the SNPs associated with *NFKB1*, *RelA* and *NFKBIA* or with multi-locus haplotypes in the disease (Butt et al., 2005).

Giant cell arthritis

Giant cell arthritis is a polygenic disease with vascularise in large and middle-sized arteries in the elderly. Martin et al. analysed the -94ins/delATTG NFKB1 polymorphism in 96 patients with biopsy-proven giant cell arthritis and 204 ethnically matched controls in Spain, and they did not found difference in allele or genotype between the patients and controls (Martin et al., 2006, Table 1), even though the patients were stratified with gender, presence of polymyalgia rheumatics, severe ischemic manifestations, or other clinical manifestations of giant cell arthritis. The results do not support a role for the -94ins/delATTG NFKB1 polymorphism in susceptibility and clinical expression of giant cell arthritis.

Celiac disease

Celiac disease is a digestive disorder from poor absorption of certain nutrients and the patients can not tolerate gluten in wheat, rye, and barley. Rueda et al. examined the -94ins/delATTG NFKB1 polymorphism, in 478 patients with celiac disease, a panel of 196 celiac families and 711 unrelated healthy individuals from both familial and case-control analysis from Spanish-Caucasian origin. Neither the -94ins/delATTG genotype nor allele distribution had significant difference between celiac disease patients and controls (Rueda et al., 2006, Table 1). Accordingly, familial analysis did not reach statistical significance in the transmission of the -94ins/delATTG alleles, suggesting that the -94ins/delATTG NFKB1 polymorphism does not play a role in celiac disease susceptibility.

Type 1 diabetes

Type 1 diabetes is a disorder of glucose homeostasis that arises as a consequence of autoimmune destruction of the pancreatic ß-cells, the sole source of insulin. A study of the -94 ins/delATTG polymorphism in 777 affected siblings and 308 unaffected ones from 375 type 1 diabetes multiplex families in the UK and US showes that the affected members had significantly lower frequency of the -94 delATTG than that in the unaffected members (Kosoy and Concannon, 2005, Table 1), suggesting its potential role as candidate gene increasing type 1 diabetes predisposition. *NFKB1* polymorphism may influence type 1 diabetes in several ways such as the modulation of T-cell response, aberrant regulation of cytokine production by macrophages and altered dendritic cell development (Hilliard et al., 2002; Ouaaz et al., 2002; Liu and Beller, 2003). However, the same polymorphism examined in 269 type 1 diabetes patients and 458 healthy controls in Spain did not reveal the difference (Martinez et al., 2006, Table 1).

Based on these studies, it seems that the -94ins/delATTG polymorphism polymorphism dose not correlate to clinicopathological variables such as tumour location, stage, differentiation and patient survival, in various diseases including UC (Oliver et al., 2005; Glas et al., 2006), CD (Glas et al., 2006), CRC (unpublished data), oral squamous cell carcinoma (Lin et al., 2006), rheumatoid arthritis or systemic lupus erythematosus patients (Orozco et al., 2005) and giant cell arteritis (Martin et al., 2006). Thus, there is no evidence that *NFKB1* polymorphism is important in the pathogenesis of these diseases.

Multiple sclerosis

Multiple sclerosis is an autoimmune disease with perivascular inflammation and localized myelin destruction in the central nervous system (Sospedra and Martin, 2005). Misterski et al., examined polymorphisms of *NFKB1* promoter, *NFKB1* exons 1, 2, 12, 17, as well as *NFKB3* promoter and exons 1-10, in more than 800 unrelated multiple sclerosis patients and more than 400 healthy controls in Germany, and they found a C to T

transition in exon 12 of *NFKB1* but no changes in amino acid (A380A). Furthermore, the allele and genotype frequencies of this polymorphism are similar between the patients and controls. They also found a rare variant in exon 17 (L614F) of *NFKB1* (Miterski et al., 2002).

Parkinson's disease

Parkinson's disease is a degenerative movement disorder characterized by progressive cell loss confined mostly to dopaminergic neurons of the substantia nigra. Several studies show that formation of reactive oxygen species is a key step in selective neuronal vulnerability in Parkinson's disease. Reactive oxygen species can directly damage neuron by the alterations of nucleic acids or proteins, and also take part in signal transduction leading to apoptosis (Kolesnick and Golde, 1994; Przedborski and Ischiropoulos, 2005). NF-κB can be activated by TNFα, which is known to be implicated in oxidative stress-induced apoptosis (Hunot et al., 1997).

A mutation analysis in exons 3-24 of *NFKB1* gene in 96 sporadic Parkinson's disease patients and 414 healthy controls in German population has identified three base changes: exon 8: C1096T, exon 12: C1537T, and intron 12: C+10T. However, the frequency of these changes was similar in the patients and controls, and they did not affect the amino acid sequence, suggesting that the base changes may not be directly involved in Parkinson's disease development (Wintermeyer et al., 2002).

CA repeats on *MANBA* gene in relation to diseases

Ota et al. analysed a polymorphic dinucleotide CA repeat using primers CTT CAG TAT CTA AGA GTA TCC T and CAA GTA AGA CTC TAC GGA GTC. This CA repeat was thought to be located on *NFKB1* gene (4q23–24) (Ota et al., 1999). Shortly after, several groups used the same primers and analysed the CA repeat in various kinds of diseases. Hegazy et al. reported that the CA repeat had a strong association with type 1 diabetes patients in UK (Hegazy et al., 2001), but it is not confirmed in Danish diabetes families (Gylvin et al., 2002). The CA repeat was not related to breast cancer in an Australian population (Curran et al., 2002), and it did not play a major role in susceptibility of celiac disease in Spanish families (Rueda et al., 2004). Orozco et al. analyzed the CA repeat in rheumatoid arthritis and systemic lupus erythematosus patients from Southern Spain, and did not find any asoociation with the diseases (Orozco et al., 2005). Recently, a study from UK has demonstrated that the CA repeat is close proximity to the coding region (approximately 300 bp downstream to exon 17) of *MANBA* gene (GenBank accession no. AF224669) but not in the regulatory region of the NFKB1 (Ota et al., 1999; Hegazy et al., 2001; Curran et al., 2002; Gylvin et al., 2002; Rueda et al., 2004; Orozco et al., 2005).

MANBA is located on chromosome 4q22-q25 encoding for ß-mannosidase as an exoglycosidase which is involved in the degradation of N-linked oligosaccharide moieties of glycoproteins. Genetic deficiency of this enzyme results in beta-mannosidosis, a lysosomal storage disease with a wide spectrum of neurological involvement (Alkhayat et al., 1998). Moreover, it has been demonstrated that serum level of ß-mannosidase is increased in rats bearing transplantable Reuber H-35 hepatoma or mice bearing L1210 murine leukaemia (Bosmann et al., 1975). Furthermore, expression of the ß-mannosidase has been found to be increased in oesophageal dysplasia and squamous cell carcinoma (Sud et al., 2004).

We recently studied the relationship of the CA repeat with CRC risk in Swedish (152 CRC patients and 441 controls) and Chinese populations (196 CRC patients and 577 controls), and the clinicopathological significance of this polymorphism on CRC patients, by using capillary electrophoresis. The MANBA genotypes were found to be related to CRC risk in the Swedish population, where individuals with <22 CAn/<22 CAn had significantly increased risk for CRC compared with those with ≥ 22 CAn/≥ 22 CAn. Genotype <22 CAn/<22 CAn was also significantly associated with increased risk for CRC in the Chinese population when compared with <22 CAn/≥ 22 CAn. These findings suggested the involvement of this polymorphism in CRC predisposition (unpublished data).

NFKBI polymorphisms in relation to diseases

Psoriatic arthritis

Butt et al. examined seven SNPs in NFKBIA: promoter SNP –410 (rs2233409), -642 (rs2233408), -673 (rs2233407), -949 (rs2233406), and 3' SNP 2643 (rs8904), 2758 (rs696), and 3053 (rs2273650) in 224 patients with psoriatic arthritis and 88 matched controls in Canadian population, and they did not find the SNPs tested for the single locus associations in *NFKBIA* or with multi-locus haplotypes (Butt et al., 2005).

Multiple sclerosis

Misterski et al. examined polymorphisms of *NFKBIA* promoter, exons 1-6 and intron 3 including -1256 c/t, -1169 a/g, -1001 a/g, -708ins8, -420 c/t, D27D, A102A, g49a, g262a and 3'UTR t2c, in more than 800 unrelated multiple sclerosis patients and more than 400 healthy blood donors in a German population. They found that an allele (a -708ins8) occurred at lower frequency in the patients with primary progressive course of multiple sclerosis than in the controls, indicating that this polymorphism affects the function of the IκB protein (Miterski et al., 2002).

Multiple myeloma

Parker et al. examined the DNA sequence of the NFKBIA from bone marrow mononuclear cells from 18 American multiple myeloma patients and 24 healthy

individuals as well as two multiple myeloma cell-lines (Parker et al., 2002). Two novel polymorphisms, 104149 (A→T) of exon 1 and 102658 of exon 4 (T→G), have been identified and six previous identified polymorphisms observed, 104352 (C→T) of exon 1, 103508 (C→T) of exon 2, 102889 (G→A) of exon 3, 102542 (C→T) of exon 4, 101799 (T→C) and 101675 (A→G) of exon 6 (Jungnickel et al., 2000). Among these polymorphisms, the prevalence of 104149 (A→T), 101799 (T→C) and 101675 (A→G) were significantly higher in the patients compared with the controls. The others were more prevalent in the patients, but did not reach a statistical levels. Because the polymorphisms identified in the patients are located near critical parts that determine the degradation and function of IκB protein, they are likely to lead to changes in the amount, structure, and/or function of IκB. These changes may lead to less IκB binding to NF-κB, resulting in the activation of NF-κB protein.

Chronic hepatitis B and hepatocellular carcinoma

Kim et al. used direct DNA sequencing to screen 16 healthy Korean individuals to identify polymorphisms in the *NFKBIA*, and found 10 variants within 3.5 kb full genomic DNA of *NFKBIA*; two SNPs in promoter region (*c.-673A→T, c.-642C→T*), two synonymous SNPs in exon 1 (c.78G→A, c.81C→T), three SNPs in introns (c.284T→A, c.1952A→G and c.2444C→T) and one deletion and two SNPs in 3'UTR (c.2710-2712delGAA, c.2758G→A and c.3053G→A). The frequencies of each SNPs were 0.247 (c.-673A→T), 0.129 (c.-642C→T), 0.031 (c.78G→A), 0.228 (c.81C→T), 0.094 (c.284T→A), 0.031 (c.1952A→G), 0.316 (c.2444C→T), 0.312 (c.2710-2712delGAA), 0.329 (c.2758G→A) and 0.292 (c.3053G→A). Among these polymorphisms, six (c.-673A→T, c.-642C→T, c.81C→T, c.2444C→T, c.2758G→A and c.3053G→A) were selected for larger scale genotyping for hepatocellular carcinoma with 1,750 subjects dividing into three groups: chronic hepatitis B virus carriers, liver cirrhosis and hepatocellular carcinoma (Kim et al., 2003). However, they did not find any significant association of the *NFKBIA* variants with development of hepatocellular carcinoma among the chronic hepatitis B patients. Among these polymorphisms in *NFKBIA*, no functional significance has been reported although Glavac et al. have identified an A to G variation (rs696) in the 3' UTR of NFKBIA (Glavac et al., 1994). Accumulating evidence from other genes, such as DMPK, CCND1 and TNF, has suggested that variants in the 3' untranslated region (UTR) might have functional significance in the regulation of gene expression (Conne et al., 2000).

Breast cancer

This polymorphism was examined in 109 breast cancers, and 109 age matched controls in an Australia and they did not find difference in allele or genotype frequencies of the polymorphism between the patients and controls (Curran et al., 2002).

UC and UC

Klein et al. examined variations of the CARD15 gene in 145 UC patients, and 259 CD patients and 441 healthy controls in German population. The A allele and AA genotype frequencies were found significantly higher in CD patients without a variation in the CARD15 gene, but no difference between the UC patients and the controls (Klein et al., 2004).

CRC

We investigated the susceptibility of this variation in CRC in Swedish (155 CRCs and 438 controls) and Chinese population (199 CRCs and 577 controls). The frequency of AG genotype was increased in Chinese patients ≥ 50 years of age, even after adjusting of age, indicating that the susceptibilities of this polymorphism may vary among diverse populations and different types of cancers (Gao et al., 2007).

The A to G variation in 3' UTR of NFKBIA affects *NFKBIA* expression and further modulates the transcription activity of NF-κB. Moreover, the expression of *NFKBIA* is influenced by its interaction with other variants in close proximity to this polymorphism, as well as environmental exposures such as diet and smoking. This may explain why the *NFKBIA* polymorphism plyas different role in CRC development in different populations. Further analysis containing information of genetic linkage, gene expression and environmental exposure in different populations would contribute to our understanding the function of the *NFKBIA* polymorphism. Although this polymorphism was not associated with CRC risk in the Swedish population, the patients with GG genotype had a worse prognosis than those with AA/AG genotype. Even in multivariate analysis, the significance still remained, independent of gender, age, tumour location, Dukes' stage and differentiation, indicating that the NFKBIA polymorphism was not only involved in CRC risk but also in cancer progression (Gao et al., 2007).

The genotype frequencies of health individuals between Swedish and Chinese populations were significantly different (AA, 17%; AG, 50% and GG, 33% for Swedish; AA, 14%; AG, 45% and GG, 41% for Chinese). The distribution of the genotypes in the Swedish controls was similar to that in 441 German healthy controls (13%, 49% and 38%) (Klein et al., 2004), but not to that in Australian healthy controls, where 109 individuals were examined (AA, 6%; AG, 45% and GG, 49%) (Curran et al., 2002). Further studies with a larger series of strictly ethnic selective populations would help us to understand the genotype distribution among different groups.

NF-κB and IBκ in relation to tumour therapy

NF-κB is overexpressed in the bone marrow from multiple myeloma patients, especially in chemo-resistant

diseases (Hideshima et al., 2001). However, decreased expression of IκBα in patients with different types of diseases, such as in prostate cancer (Dong et al., 2002), suggesting that IκB is involved in controlling the oncogenic potential of NF-κB. Imbalance of NF-κB and IκB is a critical step in tumour development and response to treatments. Inhibiting IκBα phosphorylation or degradation has been linked to activation of NF-κB, leading to apoptosis and cancer cell growth arrest. Proteasome inhibitor PS-341, an inhibitor of NF-κB activity, directly inhibits proliferation and induces apoptosis in human multiple myeloma cell lines and freshly isolated multiple myeloma cells from the patients (Hideshima et al., 2001). In clinical trials, PS-341 has been proven to inhibit multiple myeloma growth, overcome drug resistance, reduce attendant toxicity and improve outcome of the patients (Richardson, 2003; Jagannath et al., 2004). Following this success, PS-341 has become of interest for novel treatment strategy of cancer, and shown its inhibition of NF-κB activity in a phase II trial of patients with metastatic CRC (Mackay et al., 2005). Several NF-κB and IKK inhibitors are under development, and a number of natural products in high doses also can inhibit NF-κB activation (Karin et al., 2004).

IκB and its signalling pathways that mediate NF-κB activation have become attractive targets for developing new chemopreventive and chemotherapeutic approaches. Designing anti-cancer agents to block NF-κB activity or to increase their sensitivity to conventional chemotherapy may have great therapeutic value. It is great of importance to study relationship between polymorphisms of NFKB and NFKBI with response/resistance to therapy in order to identify patients for better therapy.

Acknowledgements. The study was supported by grants from the Swedish Cancer Foundation and the Health Research Council in the Southeast of Sweden.

References

Al-Mohanna F., Saleh S., Parhar R.S. and Collison K. (2002). IL-12-dependent nuclear factor-kappaB activation leads to de novo synthesis and release of IL-8 and TNF-alpha in human neutrophils. J. Leukoc. Biol. 72, 995-1002.

Alkhayat A.H., Kraemer S.A., Leipprandt J.R., Macek M., Kleijer W.J. and Friderici K.H. (1998). Human beta-mannosidase cDNA characterization and first identification of a mutation associated with human beta-mannosidosis. Hum. Mol. Genet. 7, 75-83.

Beinke S. and Ley S.C. (2004). Functions of NF-kappaB1 and NF-kappaB2 in immune cell biology. Biochem. J. 382, 393-409.

Berenson J.R., Ma H.M. and Vescio R. (2001). The role of nuclear factor-kappaB in the biology and treatment of multiple myeloma. Semin. Oncol. 28, 626-633.

Bonen D.K., Ogura Y., Nicolae D.L., Inohara N., Saab L., Tanabe T., Chen F.F., Foster S.J., Duerr R.H., Brant S.R., Cho J.H. and Nunez G. (2003). Crohn's disease-associated NOD2 variants share a signaling defect in response to lipopolysaccharide and peptidoglycan. Gastroenterology 124, 140-146.

Borm M.E., van Bodegraven A.A., Mulder C.J., Kraal G. and Bouma G. (2005). A NFKB1 promoter polymorphism is involved in susceptibility to ulcerative colitis. Int. J. Immunogenet. 32, 401-405.

Bosmann H.B., Spataro A.C. and Myers M.W. (1975). Serum and host liver activities of glycosidases and sialyltransferases in animals bearing transplantable tumors. Res. Commun. Chem. Pathol. Pharmacol. 12, 499-512.

Bouma G. and Strober W. (2003). The immunological and genetic basis of inflammatory bowel disease. Nat. Rev. Immunol. 3, 521-533.

Brownell E., Kozak C.A., Fowle J.R. 3rd, Modi W.S., Rice N.R. and O'Brien S.J. (1986). Comparative genetic mapping of cellular rel sequences in man, mouse, and the domestic cat. Am. J. Hum. Genet. 39, 194-202.

Butt C., Sun S., Peddle L., Greenwood C., Hamilton S., Gladman D. and Rahman P. (2005). Association of nuclear factor-kappaB in psoriatic arthritis. J. Rheumatol. 32, 1742-1744.

Chang J., Zhang C., Tani-Ishii N., Shi S. and Wang C. Y. (2005). NF-kappaB activation in human dental pulp stem cells by TNF and LPS. J. Dent. Res. 8, 994-998.

Cho J.H., Nicolae D.L., Gold L.H., Fields C.T., LaBuda M.C., Rohal P.M., Pickles M.R., Qin L., Fu Y., Mann J.S., Kirschner B.S., Jabs E.W., Weber J., Hanauer S.B., Bayless T.M. and Brant S.R. (1998). Identification of novel susceptibility loci for inflammatory bowel disease on chromosomes 1p, 3q, and 4q: evidence for epistasis between 1p and IBD1. Proc. Natl. Acad. Sci. USA 95, 7502-7507.

Conne B., Stutz A. and Vassalli J.D. (2000). The 3' untranslated region of messenger RNA: A molecular 'hotspot' for pathology? Nat. Med. 6, 637-641.

Curran J.E., Weinstein S.R. and Griffiths L.R. (2002). Polymorphic variants of NFKB1 and its inhibitory protein NFKBIA, and their involvement in sporadic breast cancer. Cancer Lett. 188, 103-107.

Dejardin E., Deregowski V., Greimers R., Cai Z., Chouaib S., Merville M. P. and Bours V. (1998). Regulation of major histocompatibility complex class I expression by NF-kappaB-related proteins in breast cancer cells. Oncogene 16, 3299-3307.

Deloukas P., Dauwerse J.G., van Ommen G.J. and van Loon A.P. (1994). The human NFKB3 gene encoding the p65 subunit of transcription factor NF-kappa B is located on chromosome 11q12. Genomics 19, 592-594.

Dong C., Slattery M.J., Liang S. and Peng H.H. (2005). Melanoma cell extravasation under flow conditions is modulated by leukocytes and endogenously produced interleukin 8. Mol. Cell Biomech. 2, 145-159.

Dong Q.G., Sclabas G.M., Fujioka S., Schmidt C., Peng B., Wu T., Tsao M.S., Evans D.B., Abbruzzese J.L., McDonnell T.J. and Chiao P.J. (2002). The function of multiple IkappaB: NF-kappaB complexes in the resistance of cancer cells to Taxol-induced apoptosis. Oncogene 21, 6510-6519.

Erdman S., Fox J.G., Dangler C.A., Feldman D. and Horwitz B.H. (2001). Typhlocolitis in NF-kappa B-deficient mice. J. Immunol. 166, 1443-1447.

Gao J., Pfeifer D., He L.J., Qiao F., Zhang Z., Arbman G., Wang Z.L., Jia C.R., Carstensen J. and Sun X.F. (2007). Association of NFKBIA polymorphism with colorectal cancer risk and prognosis in Swedish and Chinese populations. Scand. J. Gastroenterol. 42, 345-350.

Geddes M., Balzi D., Buiatti E., Khlat M. and Parkin D. (1991). Cancer in Italian migrants. Cancer Causes Control 2, 133-140.

Gewirtz A.T., Simon P.O. Jr, Schmitt C.K., Taylor L.J., Hagedorn C.H., O'Brien A.D., Neish A.S. and Madara J.L. (2001). Salmonella typhimurium translocates flagellin across intestinal epithelia, inducing a proinflammatory response. J. Clin. Invest. 107, 99-109.

Glas J., Torok H.P., Tonenchi L., Muller-Myhsok B., Mussack T., Wetzke M., Klein W., Epplen J.T., Griga T., Schiemann U., Lohse P., Seiderer J., Schnitzler F., Brand S., Ochsenkuhn T., Folwaczny M. and Folwaczny C. (2006). Role of the NFKB1 -94ins/delATTG promoter polymorphism in IBD and potential interactions with polymorphisms in the CARD15/NOD2, IKBL, and IL-1RN genes. Inflamm. Bowel Dis. 12, 606-611.

Glavac D., Ravnik-Glavac M., O'Brien S.J. and Dean M. (1994). Polymorphisms in the 3' untranslated region of the I kappa B/MAD-3 (NFKBI) gene located on chromosome 14. Hum. Genet. 93, 694-696.

Gylvin T., Bergholdt R., Nerup J. and Pociot F. (2002). Characterization of a nuclear-factor-kappa B (NFkappaB) genetic marker in type 1 diabetes (T1DM) families. Genes Immun. 3, 430-432.

Hegazy D.M., O'Reilly D.A., Yang B.M., Hodgkinson A.D., Millward B.A. and Demaine A.G. (2001). NFkappaB polymorphisms and susceptibility to type 1 diabetes. Genes Immun. 2, 304-308.

Heron E., Deloukas P. and van Loon A.P. (1995). The complete exon-intron structure of the 156-kb human gene NFKB1, which encodes the p105 and p50 proteins of transcription factors NF-kappa B and I kappa B-gamma: implications for NF-kappa B-mediated signal transduction. Genomics 30, 493-505.

Hideshima T., Richardson P., Chauhan D., Palombella V.J., Elliott P.J., Adams J. and Anderson K.C. (2001). The proteasome inhibitor PS-341 inhibits growth, induces apoptosis, and overcomes drug resistance in human multiple myeloma cells. Cancer Res. 61, 3071-3076.

Hilliard B.A., Mason N., Xu L., Sun J., Lamhamedi-Cherradi S.E., Liou H.C., Hunter C. and Chen Y.H. (2002). Critical roles of c-Rel in autoimmune inflammation and helper T cell differentiation. J. Clin. Invest. 110, 843-850.

Hisamatsu T., Suzuki M., Reinecker H.C., Nadeau W.J., McCormick B.A. and Podolsky D.K. (2003). CARD15/NOD2 functions as an antibacterial factor in human intestinal epithelial cells. Gastroenterology 124, 993-1000.

Hunot S., Brugg B., Ricard D., Michel P.P., Muriel M.P., Ruberg M., Faucheux B.A., Agid Y. and Hirsch E.C. (1997). Nuclear translocation of NF-kappaB is increased in dopaminergic neurons of patients with parkinson disease. Proc. Natl. Acad. Sci. USA 94, 7531-7536.

Iscovich J. and Howe G.R. (1998). Cancer incidence patterns (1972-91) among migrants from the Soviet Union to Israel. Cancer Causes Control 9, 29-36.

Jagannath S., Barlogie B., Berenson J., Siegel D., Irwin D., Richardson P.G., Niesvizky R., Alexanian R., Limentani S.A., Alsina M., Adams J., Kauffman M., Esseltine D.L., Schenkein D.P. and Anderson K.C. (2004). A phase 2 study of two doses of bortezomib in relapsed or refractory myeloma. Br. J. Haematol. 127, 165-172.

Jungnickel B., Staratschek-Jox A., Brauninger A., Spieker T., Wolf J., Diehl V., Hansmann M.L., Rajewsky K. and Kuppers R. (2000). Clonal deleterious mutations in the IkappaBalpha gene in the malignant cells in Hodgkin's lymphoma. J. Exp. Med. 191, 395-402.

Karban A.S., Okazaki T., Panhuysen C.I., Gallegos T., Potter J.J., Bailey-Wilson J.E., Silverberg M.S., Duerr R.H., Cho J.H., Gregersen P.K., Wu Y., Achkar J.P., Dassopoulos T., Mezey E., Bayless T.M., Nouvet F.J. and Brant S.R. (2004). Functional annotation of a novel NFKB1 promoter polymorphism that increases risk for ulcerative colitis. Hum. Mol. Genet. 13, 35-45.

Karin M., Yamamoto Y. and Wang Q.M. (2004). The IKK NF-kappa B system: a treasure trove for drug development. Nat. Rev. Drug Discov. 3, 17-26.

Kim L.H., Lee H.S., Kim Y.J., Jung J.H., Kim J.Y., Park B.L. and Shin H.D. (2003). Identification of novel SNPs in the interleukin 6 receptor gene (IL6R). Hum. Mutat. 21, 450-451.

Klein W., Tromm A., Folwaczny C., Hagedorn M., Duerig N., Epplen J.T., Schmiegel W.H. and Griga T. (2004). A polymorphism of the NFKBIA gene is associated with Crohn's disease patients lacking a predisposing allele of the CARD15 gene. Int. J. Colorectal. Dis. 19, 153-156.

Kolesnick R. and Golde D.W. (1994). The sphingomyelin pathway in tumor necrosis factor and interleukin-1 signaling. Cell 77, 325-328.

Kosoy R. and Concannon P. (2005). Functional variants in SUMO4, TAB2, and NFkappaB and the risk of type 1 diabetes. Genes Immun. 6, 231-235.

La Ferla K., Seegert D. and Schreiber S. (2004). Activation of NF-kappaB in intestinal epithelial cells by E. coli strains isolated from the colonic mucosa of IBD patients. Int. J. Colorectal. Dis. 19, 334-342.

Le Beau M.M., Ito C., Cogswell P., Espinosa R. 3rd, Fernald A.A. and Baldwin A.S. Jr (1992). Chromosomal localization of the genes encoding the p50/p105 subunits of NF-kappa B (NFKB2) and the I kappa B/MAD-3 (NFKBI) inhibitor of NF-kappa B to 4q24 and 14q13, respectively. Genomics 14, 529-531.

Lin S.C., Liu C.J., Yeh W.I., Lui M.T., Chang K.W. and Chang C.S. (2006). Functional polymorphism in NFKB1 promoter is related to the risks of oral squamous cell carcinoma occurring on older male areca (betel) chewers. Cancer Lett. 243, 47-54.

Liptay S., Schmid R.M., Perkins N.D., Meltzer P., Altherr M.R., McPherson J.D., Wasmuth J.J. and Nabel G.J. (1992). Related subunits of NF-kappa B map to two distinct loci associated with translocations in leukemia, NFKB1 and NFKB2. Genomics 13, 287-292.

Liu J. and Beller D.I. (2003). Distinct pathways for NF-kappa B regulation are associated with aberrant macrophage IL-12 production in lupus- and diabetes-prone mouse strains. J. Immunol. 170, 4489-4496.

Mackay H., Hedley D., Major P., Townsley C., Mackenzie M., Vincent M., Degendorfer P., Tsao M.S., Nicklee T., Birle D., Wright J., Siu L., Moore M. and Oza A. (2005). A phase II trial with pharmacodynamic endpoints of the proteasome inhibitor bortezomib in patients with metastatic colorectal cancer. Clin. Cancer Res. 11, 5526-5533.

Makarov S.S. (2001). NF-kappa B in rheumatoid arthritis: a pivotal regulator of inflammation, hyperplasia, and tissue destruction. Arthritis Res. 3, 200-206.

Martin J., Perez-Armengol C., Miranda-Filloy J.A., Vilchez J.R., Lopez-Nevot M.A., Garcia-Porrua C. and Gonzalez-Gay M.A. (2006). Lack of association of a functional -94ins/delATTG NFKB1 promoter polymorphism with susceptibility and clinical expression of biopsy-proven giant cell arteritis in northwest Spain. J. Rheumatol. 33, 285-288.

Martinez A., Santiago J.L., de la Calle H., Figueredo M.A., Fernandez-Arquero M., de la Concha E.G. and Urcelay E. (2006). NFkappaB1 gene does not affect type 1 diabetes predisposition in a Spanish population. Tissue Antigens 67, 143-145.

Maskarinec G. and Noh J.J. (2004). The effect of migration on cancer incidence among Japanese in Hawaii. Ethn. Dis. 14, 431-439.

Mathew S., Murty V.V., Dalla-Favera R. and Chaganti R.S. (1993). Chromosomal localization of genes encoding the transcription factors, c-rel, NF-kappa Bp50, NF-kappa Bp65, and lyt-10 by fluorescence in situ hybridization. Oncogene 8, 191-193.

Miagkov A.V., Kovalenko D.V., Brown C.E., Didsbury J.R., Cogswell J.P., Stimpson S.A., Baldwin A.S. and Makarov S.S. (1998). NF-kappaB activation provides the potential link between inflammation and hyperplasia in the arthritic joint. Proc. Natl. Acad. Sci. USA 95, 13859-13864.

Mirza M.M., Fisher S.A., Onnie C., Lewis C.M., Mathew C.G., Sanderson J. and Forbes A. (2005). No association of the NFKB1 promoter polymorphism with ulcerative colitis in a British case control cohort. Gut 54, 1205-1206.

Miterski B., Bohringer S., Klein W., Sindern E., Haupts M., Schimrigk S. and Epplen J.T. (2002). Inhibitors in the NFkappaB cascade comprise prime candidate genes predisposing to multiple sclerosis, especially in selected combinations. Genes Immun. 3, 211-219.

Murata Y., Ishiguro Y., Itoh J., Munakata A. and Yoshida Y. (1995). The role of proinflammatory and immunoregulatory cytokines in the pathogenesis of ulcerative colitis. J. Gastroenterol. 30 Suppl 8, 56-60.

Nadeau W.J., Pistole T.G. and McCormick B.A. (2002). Polymorphonuclear leukocyte migration across model intestinal epithelia enhances Salmonella typhimurium killing via the epithelial derived cytokine, IL-6. Microbes. Infect. 4, 1379-1387.

Neurath M.F., Fuss I., Schurmann G., Pettersson S., Arnold K., Muller-Lobeck H., Strober W., Herfarth C. and Buschenfelde K.H. (1998). Cytokine gene transcription by NF-kappa B family members in patients with inflammatory bowel disease. Ann. NY Acad. Sci. 859, 149-159.

Ni W.F., Tsai C.H., Yang S.F. and Chang Y.C. (2006). Elevated expression of NF-kappaB in oral submucous fibrosis - Evidence for NF-kappaB induction by safrole in human buccal mucosal fibroblasts. Oral Oncol. (in press).

Okamoto T., Ono T., Hori M., Yang J.P., Tetsuka T., Kawabe T. and Sonta S. (1998). Assignment of the IkappaB-beta gene NFKBIB to human chromosome band 19q13.1 by in situ hybridization. Cytogenet. Cell Genet. 82, 105-106.

Oliver J., Gomez-Garcia M., Paco L., Lopez-Nevot M.A., Pinero A., Correro F., Martin L., Brieva J.A., Nieto A. and Martin J. (2005). A functional polymorphism of the NFKB1 promoter is not associated with ulcerative colitis in a Spanish population. Inflamm. Bowel Dis. 11, 576-579.

Olsson L. and Lindblom A. (2003). Family history of colorectal cancer in a Sweden county. Fam. Cancer 2, 87-93.

Orozco G., Sanchez E., Collado M.D., Lopez-Nevot M.A., Paco L., Garcia A., Jimenez-Alonso J. and Martin J. (2005). Analysis of the functional NFKB1 promoter polymorphism in rheumatoid arthritis and systemic lupus erythematosus. Tissue Antigens 65, 183-186.

Ota N., Nakajima T., Shirai Y., and Emi M. (1999). Isolation and radiation hybrid mapping of a highly polymorphic CA repeat sequence at the human nuclear factor kappa-beta subunit 1 (NFKB1) locus. J. Hum. Genet 44, 129-130.

Ouaaz F., Arron J., Zheng Y., Choi Y. and Beg A.A. (2002). Dendritic cell development and survival require distinct NF-kappaB subunits. Immunity 16, 257-270.

Parker K.M., Ma M.H., Manyak S., Altamirano C.V., Tang Y.M., Frantzen M., Mikail A., Roussos E., Sjak-Shie N., Vescio R.A. and Berenson J.R. (2002). Identification of polymorphisms of the IkappaBalpha gene associated with an increased risk of multiple myeloma. Cancer Genet. Cytogenet.137, 43-48.

Parkin D.M. (2004). International variation. Oncogene 23, 6329-6340.

Przedborski S. and Ischiropoulos H. (2005). Reactive oxygen and nitrogen species: weapons of neuronal destruction in models of Parkinson's disease. Antioxid. Redox. Signal. 7, 685-693.

Richardson P.G. (2003). Bortezomib: a novel therapy approved for multiple myeloma. Clin. Adv. Hematol. Oncol. 1, 596-600.

Rogler G., Brand K., Vogl D., Page S., Hofmeister R., Andus T., Knuechel R., Baeuerle P.A., Scholmerich J. and Gross V. (1998). Nuclear factor kappaB is activated in macrophages and epithelial cells of inflamed intestinal mucosa. Gastroenterology 115, 357-369.

Rueda B., Lopez-Nevot M.A., Ruiz M.P., Ortega E., Maldonado J., Lopez M. and Martin J. (2004). CA microsatellite polymorphism of the nuclear factor kappa B1 gene in celiac disease. Eur. J. Immunogenet. 31, 129-132.

Rueda B., Nunez C., Lopez-Nevot M.A., Paz Ruiz M., Urcelay E., De la Concha E.G. and Martin J. (2006). Functional polymorphism of the NFKB1 gene promoter is not relevant in predisposition to celiac disease. Scand. J. Gastroenterol. 41, 420-423.

Sospedra M. and Martin R. (2005). Immunology of multiple sclerosis. Annu. Rev. Immunol. 23, 683-747.

Sud N., Sharma R., Ray R., Chattopadhyay T. and Ralhan R. (2004). Differential expression of beta mannosidase in human esophageal cancer. Int. J. Cancer 112, 905-907.

Tilakaratne W.M., Klinikowski M.F., Saku T., Peters T.J. and Warnakulasuriya S. (2006). Oral submucous fibrosis: review on aetiology and pathogenesis. Oral. Oncol. 42, 561-568.

Tomczak M.F., Erdman S.E., Poutahidis T., Rogers A.B., Holcombe H., Plank B., Fox J.G. and Horwitz B.H. (2003). NF-kappa B is required within the innate immune system to inhibit microflora-induced colitis and expression of IL-12 p40. J. Immunol. 171, 1484-1492.

Wintermeyer P., Riess O., Schols L., Przuntek H., Miterski B., Epplen J. T. and Kruger R. (2002). Mutation analysis and association studies of nuclear factor-kappaB1 in sporadic Parkinson's disease patients. J. Neural. Transm. 109, 1181-1188.

Wong H.K., Kammer G.M., Dennis G. and Tsokos G.C. (1999). Abnormal NF-kappa B activity in T lymphocytes from patients with systemic lupus erythematosus is associated with decreased p65-RelA protein expression. J. Immunol. 163, 1682-1689.

Yamamoto Y. and Gaynor R.B. (2001). Role of the NF-kappaB pathway in the pathogenesis of human disease states. Curr. Mol. Med. 1, 287-296.

Ziegler-Heitbrock H.W., Wedel A., Schraut W., Strobel M., Wendelgass P., Sternsdorf T., Bauerle P.A., Haas J.G. and Riethmuller G. (1994). Tolerance to lipopolysaccharide involves mobilization of nuclear factor kappa B with predominance of p50 homodimers. J. Biol. Chem. 269, 17001-17004.

Accepted May 7, 2007