Polymorphisms in NFkB1 and NFkBIA Genes Modulate the Risk of Developing Prostate Cancer among Han Chinese

Background: Nuclear factor kappa B (NF-kB) pathway proteins play an important role in modulating inflammation and other carcinogenic processes. Polymorphisms within NF-kB pathway genes may influence cancer risk. This study aimed to examine the association between NFkB1-9-4 ATTG ins-del, NFkBIA 3’ UTR A→G, -826CT and -881AG polymorphisms and prostate cancer risk among Chinese.

Material/Methods: The polymorphisms were genotyped via PCR-RFLP technique on 936 prostate cancer patients and 936 population-based healthy controls. Logistic regression model was used to measure the risk association present.

Results: With the exception of NFkBIA 3’ UTR polymorphism, the heterozygous and mutant genotypes of the other polymorphisms were significantly associated with prostate cancer risk. For NFkB1 polymorphism, a decreased risk was observed, with adjusted OR: 0.69; 95% CI: 0.44, 0.98; P=0.01 (heterozygous) and adjusted OR: 0.60; 95% CI: 0.37, 0.91; P=0.02 (mutant). NFkBIA -826CT and -881AG polymorphisms were in complete linkage disequilibrium and shared the same risk association, with adjusted OR: 1.34; 95% CI: 1.09, 1.62; P=0.02 (heterozygous) and adjusted OR: 2.83; 95% CI: 1.79, 4.50; P=0.01 (mutants). Interestingly, the impact of the NFkB1 polymorphism was not present in nonsmokers and younger (<60 years) subjects (P<0.05).

Conclusions: In conclusion, polymorphisms in NFkB1 and NFkBIA genes may modulate the risk of developing prostate cancer among Chinese.

MeSH Keywords: Asian Continental Ancestry Group • Association • Genetic Association Studies • Genotype • Polymorphism, Genetic

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/893471
Background

Prostate cancer is a common type of cancer in men, with an estimated annual incidence of 238,590 cases [1]. Although most of the prostate cancer cases happen in the developed countries, recent years have witnessed the rapid increase in prostate cancer incidence in developing populations, including China [2]. Prostate cancer is also one of the leading causes of cancer-related mortality, accounting for 10% of all male cancer-related deaths, in both Western and Asian populations [3]. Considering the significance of the disease, there is a pressing need for identifying risk factors of prostate cancer, so that the disease can be detected at an early stage, which facilitates prostate cancer treatment and management.

The mechanism of prostate carcinogenesis is extremely complicated. However, it has been generally accepted that the etiology of prostate cancer is influenced by several factors such as smoking habits, age, ethnicity, and, importantly, genetic factors [4–6]. It has been shown previously that the latter could contribute to up to 42% of prostate cancer risk [6]. Candidate gene association study (CGAS) is an established method for identifying genetic variants associated with a disease [7]. The approach focuses only on genes that could be of relevance to the mechanism of the disease of interest [7,8]. The specific polymorphisms that could either affect gene expression or the protein product of the chosen candidate genes were then selected. Subsequently, the frequencies of the polymorphic genotypes were compared between subjects with and without the disease, and statistical models were used to determine risk association [7,8].

Many studies on prostate carcinogenesis have focused on the role of genetic factors that mediate inflammatory response [9–12], as inflammation has been extensively linked to the etiology of prostate cancer, although other mechanisms such as cell adhesion also play an important role [13,14]. Given the important role of inflammation in carcinogenesis, the present candidate gene association study focused on genes relevant to inflammation. Among all the proteins involved in inflammatory response, the nuclear factor-kappa B (NF-κB) pathway proteins, notably p50 (NF-κB1) and its critical inhibitor, IκBα, appeared to have the most important function. NF-κB activation can lead to the synthesis of several enzymes, including inducible nitric oxide synthase (iNOS) and cyclooxygenase-2, which catalyze the release of reactive oxygen species that damages adjacent cells. The NF-κB pathway has also been shown to regulate the production of many pro-inflammatory cytokines, such as TGF-β, TNF-α, IL-6, and IL-8, which further supports the role of NF-κB in inflammation. Inflammation causes various genomic lesions to the cells, which facilitates cancer development. Additionally, improper activation of NF-κB1 can cause enhanced cell proliferation and evasion of apoptosis, which are 2 of the cancer hallmarks. Therefore, under normal conditions, NF-κB1 is bound to IκBα inhibitor when its expression is not needed.

NF-κB1 and IκBα are encoded by NFKB1 and NFKBIA genes, respectively. The impact of polymorphisms within the NFKB1 and NFKBIA genes on cancer risk has been extensively investigated in a number of cancers [15–21], but surprisingly few studies have been performed on prostate cancer [22,23]. The risk modulation effects of the polymorphisms were inconsistent in different studies, which could be because of the small sample sizes recruited and the ethnic background differences of subjects in different studies. The present study aimed to examine the association of NFKB19-4 ATTG ins→del, NFKBIA 3' untranslated region (UTR) A→G, -826CT and -881AG polymorphisms with risk of developing prostate cancer among Chinese in a large sample size.

Material and Methods

Subjects

Between September 2008 and June 2014, 936 newly diagnosed, histopathologically confirmed sporadic prostate cancer patients were randomly recruited from the First Affiliated Hospital of Bengbu Medical College, Bengbu Third People’s Hospital, the Second Affiliated Hospital of Bengbu Medical College, the People’s Liberation Army 123rd Hospital China and Bengbu First People’s Hospital. Based on medical records, patients who had family history of prostate cancer and those who suffered from malignancies other than prostate cancer were excluded. Healthy males (N=936) were randomly recruited from the general population as controls. Controls were matched with patients by age (+5 years). The age of the patients recruited ranged from 50 to 76 years old, with a mean of 62.0±6.89 years. The controls’ ages ranged from 48 to 73 years old, with a mean of 61.4±7.11. Based on our previous preliminary findings (data not shown), the peak age of prostate cancer incidence in our population occurred at 60 years old. Therefore, subjects below 60 years old were categorized as “young”, while those 60 years or above were categorized as “old” in our analysis. 442 of the patients and 389 of the controls were smokers, while the rest were nonsmokers. Information on the subjects’ age was collected based on the medical registration form (which was in turn based on the official identity card of the People’s Republic of China). On the other hand, information on the smoking habits was obtained from an interview with subjects on recruitment. All subjects were self-described ethnic Han Chinese. They were asked to sign a written informed consent before donating 3 ml blood for genetic analysis. The study was approved by the Medical Ethics Board (MEB) of Bengbu Medical College (approval no. BMC/1/IMO/2008.0415504).

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License
Genotyping of polymorphisms

DNA was extracted from blood samples by using the TIANGEN DNA Purification Kit. All polymorphisms were genotyped by previously described PCR-RFLP technique (NFKB1, [15]; NFKBIA 3’ UTR [21], NFKBIA -826CT, and -881AG [19]), with the researchers blinded to the case/control status of the samples. For NFKB1 polymorphism, the PCR primers were TGGGCACAAGTCGTTTATGA (forward) and CTGGAGCCGGTAGGGAAG (reverse), while the restriction enzyme used was PflMI (Van91I). For NFKBIA 3’ UTR polymorphism, the PCR primers were GGCTGAAAGAACATGGACTTG (forward) and GTACACCATTTACAGGAGGG (reverse), while HaeIII was used for the digesting the PCR products. NFKBIA -826CT and -881AG polymorphisms were amplified simultaneously by using GGTCCTTAAGGTCCAATCG (forward) and GTTGTGGATACCTTGCACTA (reverse). NFKBIA -826CT polymorphism was digested by using Bfai, and NFKBIA -881AG polymorphism was digested by using TspRI. Approximately 10% of randomly selected samples were sequenced to confirm the genotypes.

Statistical analysis

All analyses were performed by using SPSS version 17.0. Continuous variables were assessed by using the independent samples t-test and are presented as mean ±SD. Categorical variables were tested by using the chi-square test. The Hardy-Weinberg equilibrium was analyzed by using a goodness-of-fit chi-square test. An unconditional logistic regression model was performed to calculate the odds ratios (ORs) and their corresponding 95% confidence intervals (95% CI) to assess the association of the polymorphisms with prostate cancer risk. The overall ORs were also adjusted to age and smoking status of the subjects. A p-value <0.05 was statistically significant.

Results

Frequency of NFKB1 and NFKBIA genotypes

The frequency of NFKB1 and NFKBIA genotypes in the 936 patients and 936 controls are shown in Table 1. Significant differences in

| SNP/genotype | Patient | Control | P value | HWE P value (case + control)* |
|--------------|---------|---------|---------|------------------------------|
| NFKB1        |         |         |         |                              |
| Ins/ins      | 63      | 38      | 0.03**  | 0.94                         |
| Ins/Del      | 339     | 331     |         |                              |
| Del/del      | 534     | 567     |         |                              |
| NFKBIA 3’ UTR|         |         | 0.58    | 0.55                         |
| AA           | 173     | 165     |         |                              |
| AG           | 442     | 458     |         |                              |
| GG           | 321     | 313     |         |                              |
| NFKBIA -826CT|         |         | <0.01** | 0.78                         |
| CC           | 508     | 586     |         |                              |
| CT           | 356     | 321     |         |                              |
| TT           | 72      | 29      |         |                              |
| NFKBIA -881AG|         |         | <0.01** | 0.78                         |
| AA           | 508     | 586     |         |                              |
| AG           | 356     | 321     |         |                              |
| GG           | 72      | 29      |         |                              |

* HWE – Hardy-Weinberg Equilibrium; ** significant at P<0.05.
genotype frequency were observed between patients and controls for the \textit{NFKB1} \textit{9-4 ATTG} polymorphism (\(P=0.03\)), \textit{NFKBIA} \textit{-826CT} polymorphism (\(P<0.01\)), and \textit{NFKBIA} \textit{-881AG} polymorphism (\(P<0.01\)). The frequency for \textit{NFKBIA} \textit{-826CT} and \textit{-881AG} polymorphisms was identical, indicating the presence of linkage disequilibrium (LD). In addition, the genotype frequency for all the polymorphisms followed Hardy-Weinberg Equilibrium (Table 1).

### Association of the SNPs and prostate cancer risk

Table 2 showed the association of the SNPs and prostate cancer risk. Significant association was found for \textit{NFKB1} \textit{9-4 ATTG}, \textit{NFKBIA} \textit{-826CT} and \textit{-881AG} polymorphisms, but not the \textit{NFKBIA} 3’ UTR polymorphism. For \textit{NFKB1} \textit{9-4 ATTG} polymorphism, the ins/del genotype and del/del genotype were associated with a lower prostate cancer risk, with adjusted OR: 0.69; 95% CI: 0.44–0.98; \(P=0.01**\) and adjusted OR: 0.60 (0.37–0.91); \(P=0.02**\) respectively. For \textit{NFKBIA} \textit{-826CT} and \textit{-881AG} polymorphisms, a higher prostate cancer risk association was observed. Since both polymorphisms were in LD, they had an identical risk value, with adjusted OR: 1.34; 95% CI: 1.09–1.62; \(P=0.02**\) for heterozygotes and adjusted OR: 2.83; 95% CI: 1.79, 4.50; \(P=0.01**\) for mutants.

### Combined effect of \textit{NFKB1} and \textit{NFKBIA} polymorphisms

Significant polymorphisms were evaluated for their combined effect on prostate cancer risk. The results are shown in Table 3. With the exceptions of \textit{NFKB1} \textit{9-4 ATTG} ins/ins + \textit{NFKBIA} mutant and \textit{NFKB1} \textit{9-4 ATTG} ins/del + \textit{NFKBIA} mutant combinatorial genotypes, all other genotypes were significantly associated with a lower prostate cancer risk (OR<1.00) (Table 3).

### Association of the SNPs and prostate cancer risk in people with different smoking status

The association above was analyzed separately for smokers and nonsmokers. The result is shown in Table 4. For smokers, similar to the overall findings described above, significant association with prostate cancer risk was observed for \textit{NFKB1} \textit{9-4 ATTG}, \textit{NFKBIA} \textit{-826CT}, and \textit{-881AG} polymorphisms. However, for nonsmokers, the association of \textit{NFKB1} polymorphism was absent, and only \textit{NFKBIA} \textit{-826CT} and \textit{-881AG} polymorphisms showed risk association.

For smokers, the del/del genotype of \textit{NFKB1} \textit{9-4 ATTG} polymorphism was associated with a lower prostate cancer risk, with

| SNP/Genotype | Patient | Control | Odds ratio (95% CI)* | \(P\) value |
|-------------|---------|---------|---------------------|------------|
| \textit{NFKB1} Ins/ins | 63 | 38 | – | – |
| Ins/Del | 339 | 331 | 0.69 (0.44–0.98) | 0.01** |
| Del/del | 534 | 567 | 0.60 (0.37–0.91) | 0.02** |
| \textit{NFKBIA} 3’ UTR AA | 173 | 165 | – | – |
| AG | 442 | 458 | 0.90 (0.69–1.22) | 0.61 |
| GG | 321 | 313 | 0.94 (0.68–1.32) | 0.84 |
| \textit{NFKBIA} -826CT CC | 508 | 586 | – | – |
| CT | 356 | 321 | 1.34 (1.09–1.62) | 0.02** |
| TT | 72 | 29 | 2.83 (1.79–4.50) | 0.01** |
| \textit{NFKBIA} -881AG AA | 508 | 586 | – | – |
| AG | 356 | 321 | 1.34 (1.09–1.62) | 0.02** |
| GG | 72 | 29 | 2.83 (1.79–4.50) | 0.01** |

* Adjusted to age and smoking status; ** significant at \(P<0.05\).
Table 3. Combined effect of the SNPs and prostate cancer risk.

| SNP/genotype* | Patient | Control | Odds ratio (95% CI) | P value |
|---------------|---------|---------|---------------------|---------|
| NFKB1 + NFKBIA |         |         |                     |         |
| Ins/ins + WT  | 35      | 16      | –                   | –       |
| Ins/Del + WT  | 186     | 186     | 0.46 (0.24–0.85)    | 0.01**  |
| Del/del + WT  | 285     | 306     | 0.42 (0.23–0.79)    | 0.01**  |
| Ins/ins + Het | 22      | 28      | 0.36 (0.16–0.81)    | 0.01**  |
| Ins/Del + Het | 127     | 119     | 0.49 (0.26–0.93)    | 0.03**  |
| Del/del + Het | 207     | 219     | 0.43 (0.23–0.80)    | 0.01**  |
| Ins/ins + Mut | 6       | 4       | 0.69 (0.17–2.77)    | 0.60    |
| Ins/Del + Mut | 26      | 26      | 0.46 (0.20–1.02)    | 0.06    |
| Del/del + Mut | 40      | 42      | 0.44 (0.21–0.91)    | 0.03**  |

* WT – CC for -826 polymorphism, AA for -881 polymorphism; Het – CT for -826 polymorphism, AG for -881 polymorphism; Mut – TT for -826 polymorphism, GG for -881 polymorphism; ** significant at P<0.05.

OR: 0.42; 95% CI: 0.22, 0.80; P<0.01, while the mutant genotypes of the NFKB1A -826CT and -881AG polymorphisms were associated with a higher prostate cancer risk, with OR: 2.78; 95% CI: 1.46, 5.31; P<0.01. For nonsmokers, NFKB1A -826CT and -881AG polymorphisms showed a higher risk association for prostate cancer in heterozygotes and mutants, with OR: 1.38; 95% CI: 1.07, 1.78; P=0.01 for heterozygotes and OR: 3.24; 95% CI: 1.75, 6.02; P<0.01 for mutants.

Association of the SNPs and prostate cancer risk in older and younger subjects

We also analyzed the association based on the age of the subjects (old vs. young). Table 5 shows the association of the SNPs and prostate cancer risk in older and younger subjects. The finding based on age was similar to the finding based on the smoking status above, such that for older subjects, an association was observed for NFKB1A -4 ATTG, NFKBIAA -826CT, and -881AG polymorphisms, but for younger subjects the NFKB1A-4 ATTG association was lost.

For older subjects, the del/del genotype of NFKBIA-4 ATTG polymorphism was associated with a lower prostate cancer risk, with OR: 0.57; 95% CI: 0.32, 0.99; P=0.05. In contrast, NFKBIAA -826CT and -881AG polymorphisms showed a higher risk association for prostate cancer in heterozygotes and mutants, with OR: 1.33; 95% CI: 1.02, 1.72; P=0.03 for heterozygotes and OR: 2.98; 95% CI: 1.67, 5.33; P<0.01 for mutants. However, in younger subjects, only the mutant genotypes of NFKB1A -826CT and -881AG polymorphisms were associated with prostate cancer risk, with OR: 2.69; 95% CI: 1.34, 5.42; P=0.01.

Discussion

NF-κB pathway proteins play an important role in modulating inflammation and other related cellular processes. Among the many NF-κB pathway proteins, the most abundant p50 (NF-xB1) has been thought to have the most important function in these cellular processes [24–26]. Regulation of p50 (NF-xB1) by its natural inhibitor, IκBα, is necessary for the former to carry out its cellular functions. The p50 (NF-xB1) and IκBα proteins were encoded by NFKB1 and NFKBIA genes, respectively. Polymorphisms within these genes may alter their protein products in several ways, which in turn lead to differential cancer risk. Many studies have been performed to investigate the association of NFKBIA-4 ATTG ins→del, NFKBIAA 3’ UTR A→G, -826CT and -881AG polymorphisms and risk of various cancers [15-21]. However, surprisingly little research was performed on prostate cancers. Only 2 previous studies reported on the association of NFKBIA-4 ATTG ins→del polymorphism and prostate cancer risk [22,23], and no study has focused on NFKBIA polymorphisms. Moreover, the results from the 2 NFKB1A reports contradicted one another. It is generally accepted that polymorphisms present different risk in people with different ethnic background, and that small sample sizes may cause misleading interpretation of results. These factors motivated us to perform the present study in a large sample size among Han Chinese. In this study, we excluded patients who had family history of prostate cancer because familial cancers are usually (but not always) caused by mutations in high-penetrance genes, which are rare in the general population. The present study aimed to identify low-penetrance susceptibility alleles, which, despite having modest risk modification effect, are very common in the general population. Patients
Table 4. Association of the SNPs and prostate cancer risk in people with different smoking status.

| Smoking status | SNP/genotype | Patient | Control | Odds ratio (95% CI) | P value |
|----------------|--------------|---------|---------|---------------------|---------|
| Smokers        |              |         |         |                     |         |
|                | NFKB1        |         |         |                     |         |
|                | Ins/ins      | 31      | 14      | –                   | –       |
|                | Ins/Del      | 155     | 130     | 0.54 (0.27–1.06)    | 0.07    |
|                | Del/del      | 226     | 245     | 0.42 (0.22–0.80)    | <0.01*  |
|                | NFKBIA 3’ UTR|         |         |                     |         |
|                | AA           | 68      | 77      | –                   | –       |
|                | AG           | 207     | 184     | 1.27 (0.89–1.87)    | 0.21    |
|                | GG           | 137     | 128     | 1.21 (0.81–1.82)    | 0.35    |
|                | NFKBIA -826CT|         |         |                     |         |
|                | CC           | 213     | 237     | –                   | –       |
|                | CT           | 164     | 138     | 1.32 (0.99–1.77)    | 0.06    |
|                | TT           | 35      | 14      | 2.78 (1.46–5.31)    | <0.01*  |
|                | NFKBIA -881AG|         |         |                     |         |
|                | AA           | 213     | 237     | –                   | –       |
|                | AG           | 164     | 138     | 1.32 (0.99–1.77)    | 0.06    |
|                | GG           | 35      | 14      | 2.78 (1.46–5.31)    | <0.01*  |
| Nonsmokers     |              |         |         |                     |         |
|                | NFKB1        |         |         |                     |         |
|                | Ins/ins      | 32      | 24      | –                   | –       |
|                | Ins/Del      | 184     | 201     | 0.69 (0.40–1.21)    | 0.19    |
|                | Del/del      | 308     | 322     | 0.72 (0.41–1.25)    | 0.24    |
|                | NFKBIA 3’ UTR|         |         |                     |         |
|                | AA           | 105     | 88      | –                   | –       |
|                | AG           | 235     | 274     | 0.72 (0.52–1.01)    | 0.05    |
|                | GG           | 184     | 185     | 0.83 (0.59–1.18)    | 0.31    |
|                | NFKBIA -826CT|         |         |                     |         |
|                | CC           | 295     | 388     | –                   | –       |
|                | CT           | 192     | 183     | 1.38 (1.07–1.78)    | 0.01*   |
|                | TT           | 37      | 15      | 3.24 (1.75–6.02)    | <0.01*  |
|                | NFKBIA -881AG|         |         |                     |         |
|                | AA           | 295     | 388     | –                   | –       |
|                | AG           | 192     | 183     | 1.38 (1.07–1.78)    | 0.01*   |
|                | GG           | 37      | 15      | 3.24 (1.75–6.02)    | <0.01*  |

* Significant at P<0.05.
### Table 5. Association of the SNPs and prostate cancer risk in older and younger subjects.

| Smoking status | SNP/genotype     | Patient | Control | Odds ratio (95% CI) | P value |
|---------------|------------------|---------|---------|---------------------|---------|
| Old           | NFKB1 Ins/ins    | 36      | 21      | –                   | –       |
|               | NFKB1 Ins/Del    | 186     | 184     | 0.59 (0.33–1.05)    | 0.07    |
|               | NFKB1 Del/del    | 302     | 311     | 0.57 (0.32–0.99)    | 0.05*   |
|               | NFKBIA 3’ UTR AA | 100     | 88      | –                   | –       |
|               | NFKBIA 3’ UTR AG | 224     | 256     | 0.77 (0.55–1.08)    | 0.13    |
|               | NFKBIA 3’ UTR GG | 180     | 172     | 0.92 (0.65–1.31)    | 0.65    |
|               | NFKBIA -826CT CC | 288     | 332     | –                   | –       |
|               | NFKBIA -826CT CT | 192     | 167     | 1.33 (1.02–1.72)    | 0.03*   |
|               | NFKBIA -826CT TT | 44      | 17      | 2.98 (1.67–5.33)    | <0.01*  |
|               | NFKBIA -881AG AA | 220     | 254     | –                   | –       |
|               | NFKBIA -881AG AG | 164     | 154     | 1.23 (0.93–1.63)    | 0.15    |
|               | NFKBIA -881AG GG | 28      | 12      | 2.69 (1.34–5.42)    | 0.01*   |
| Young         | NFKB1 Ins/ins    | 25      | 17      | –                   | –       |
|               | NFKB1 Ins/Del    | 155     | 147     | 0.72 (0.37–1.38)    | 0.32    |
|               | NFKB1 Del/del    | 232     | 256     | 0.62 (0.32–1.17)    | 0.14    |
|               | NFKBIA 3’ UTR AA | 73      | 77      | –                   | –       |
|               | NFKBIA 3’ UTR AG | 198     | 202     | 1.03 (0.71–1.50)    | 0.86    |
|               | NFKBIA 3’ UTR GG | 141     | 141     | 1.05 (0.71–1.57)    | 0.79    |
|               | NFKBIA -826CT CC | 220     | 254     | –                   | –       |
|               | NFKBIA -826CT CT | 164     | 154     | 1.23 (0.93–1.63)    | 0.15    |
|               | NFKBIA -826CT TT | 28      | 12      | 2.69 (1.34–5.42)    | 0.01*   |
|               | NFKBIA -881AG AA | 220     | 254     | –                   | –       |
|               | NFKBIA -881AG AG | 164     | 154     | 1.23 (0.93–1.63)    | 0.15    |
|               | NFKBIA -881AG GG | 28      | 12      | 2.69 (1.34–5.42)    | 0.01*   |

* Significant at P<0.05.
who had malignancies other than prostate cancer were also excluded to prevent misidentification of susceptibility alleles associated with other cancers.

It has been well-established that many inflammatory-related cytokines, such as TGF-β, TNF-α, IL-6, and IL-8, mediate inflammation in the prostate through the NF-κB pathway [22,27–30]. Therefore, genes in the NF-κB pathway, notably NFKB1 and NFKBIA, potentially play a role in prostate carcinogenesis. We showed that NFKB1-9-4 ATTG ins→del, and NFKBIA -826CT and -881AG polymorphisms were associated with prostate cancer risk. We postulate that the polymorphisms modulate prostate cancer risk in our subjects by altering the transcription of the genes, since the polymorphisms were all located in the promoter region. For NFKB1-9-4 ATTG polymorphism, the del allele was associated with a reduced prostate cancer risk. In vitro functional assay has shown that the del allele could decrease the level of transcription by approximately 2-fold [31]. This in turn reduces the protein translation, resulting in a lower production of p50 (NF-κB1) (Figure 1). Since p50 (NF-κB1) plays a role in inflammation, subjects who had less of the protein (i.e., those who carried the del allele) had a reduced level of inflammation. Considering that inflammation is positively linked to prostate carcinogenesis [9–12], subjects having the del allele were associated with a decreased prostate cancer risk, which explains our observation in this study. On the other hand, for the NFKBIA polymorphisms, we showed that the mutant allele was associated with an increased prostate cancer risk. It has been shown previously that the mutant allele can retard the transcription of the gene [32]. This retardation could lead to aberrant NFKB1 expression, which results in an increased prostate cancer risk, as observed in our study (Figure 1).

We also found that all combinative genotypes of NFKB1-9-4 ATTG ins→del and NFKBIA -881/-826 polymorphisms were significantly associated with prostate cancer risk, except the NFKB1-9-4 ATTG ins/ins + NFKBIA mutant and NFKB1-9-4 ATTG ins/del + NFKBIA mutant genotypes. The lack of significance of these 2 combinative genotypes could be caused by the small sample size included. It is interesting to note that all other combinative genotypes led to a reduced prostate cancer risk, which is in accordance to the effect of NFKB1-9-4 ATTG polymorphism but not the NFKBIA polymorphisms. This suggests that the effect of NFKB1-9-4 ATTG polymorphism was stronger than that of the NFKBIA polymorphisms. This observation was not unexpected, since the product of NFKB1 plays a direct role in mediating inflammation, whereas that of NFKBIA plays an indirect role by regulating NFKB1 expression.

There were several strengths and limitations in this study. The greatest strength was the large sample size. The combined sample size of the only 2 available studies on NF-κB pathway gene polymorphism was 487 prostate cancer patients and 513 controls [22,23]. The present study alone involved 936 prostate cancer patients and 936 controls, which had a much higher statistical power than the other 2 studies combined. Another strength of this study was that we analyzed not only the overall effect of the polymorphism on prostate cancer risk, but also the effect in smokers vs. nonsmokers and older vs. younger subjects. Both smoking habits and age have been established as strong risk factors for prostate cancer [4,5]. Therefore, separate analysis of the effect of the polymorphisms on subjects with different smoking habits and age was important to control the potential confounding factors. However, our study also had a limitation. We analyzed only a few polymorphisms in the NF-κB pathway. There are several other polymorphisms that could potentially modulate prostate cancer risk. However, only 4 were included in our study because we anticipated the significance of these 4 polymorphisms based on our literature review.

**Conclusions**

The del allele of the NFKB1-9-4 ATTG polymorphism was associated with a decreased prostate cancer risk, while the mutant allele of NFKBIA -826CT and -881AG polymorphisms was associated with an increased prostate cancer risk. There have been very few studies investigating the association of NF-κB pathway gene polymorphism and prostate cancer risk. Therefore, this study adds valuable information to the currently available literature on the role of these polymorphisms on prostate cancer risk.

**Figure 1.** Potential mechanism by which NFKB1 and NFKBIA polymorphisms mediate risk of prostate cancer.
Acknowledgements

We thank the staff of the Medical Record Departments of Bengbu Medical College, Bengbu Third People's Hospital, the People's Liberation Army 123rd Hospital China and Bengbu First People's Hospital for sorting patients’ medical records for our use.

References:

1. Siegel R, Naishadham D, Jemal A: Cancer statistics, 2013. Cancer J Clin, 2013; 63(1): 11–30
2. McCracken M, Olsen M, Chen MS Jr et al: Cancer incidence, mortality, and associated risk factors among Asian Americans of Chinese, Filipino, Vietnamese, Korean, and Japanese ethnicities. Cancer J Clin, 2007; 57(4): 190–205
3. Ferlay J, Soerjomataram I, Ervik M et al: GLOBOCAN 2012 v1.0, cancer incidence and mortality worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer, 2013. Available from: http://globocan.iarc.fr
4. Murphy AB, Akereyeni F, Nyame YA et al: Smoking and prostate cancer in a multi-ethnic cohort. Prostate, 2013; 73(14): 1518–28
5. Leitzmann MF, Rohrmann S: Risk factors for the onset of prostatic cancer: age, location, and behavioral correlates. Clin Epidemiol, 2012; 4: 1–11
6. Lichtenstein P, Holm NV, Verkasalo PK et al: Environmental and heritable factors in the causation of cancer – analyses of cohorts of twins from Denmark, Sweden, and Finland. N Engl J Med, 2000; 343(2): 78–85
7. Patnala R, Clements J, Batra J: Candidate gene association studies: a comprehensive guide to useful in silico tools. BMC Genetics, 2013; 14: 39
8. Kwon JM, Goate AM: The candidate gene approach. Alcohol Res Health, 2000; 24(3): 164–68
9. Kryvenko ON, Jankowski M, Chitale DA et al: Inflammation and preneoplastic lesions in benign prostate as risk factors for prostate cancer. Mod Pathol, 2012; 25(7): 1023–32
10. Sfnos KS, Isacs WB, De Marzo AM: Infections and inflammation in prostate cancer. Am J Clin Exp Urol, 2013; 1(3): 1–11
11. Sfnos KS, Hempel HA, De Marzo AM: The role of inflammation in prostate cancer. Adv Exp Med Biol, 2014; 816: 153–61
12. Sfnos KS, De Marzo AM: Prostate cancer and inflammation: the evidence. Histopathology, 2012; 40(1): 199–215
13. Wang L, Xie PG, Lin YL et al: Aberrant methylation of PCDH10 predicts worse biochemical recurrence-free survival in patients with prostate cancer after radical prostatectomy. Med Sci Monit, 2014; 20: 1360–68
14. Lin YL, Xie PG, Wang L, Ma JG: Aberrant methylation of protocadherin 17 and its clinical significance in patients with prostate cancer after radical prostatectomy. Med Sci Monit, 2014; 20: 1376–82
15. Mohd Suzairi MS, Tan SC, Ahmad Alzat AA et al: The functional -94 insertion/deletion ATG polymorphism in the promoter region of NFKB1 gene increases the risk of sporadic colorectal cancer. Cancer Epidemiol, 2013; 37(5): 634–38
16. Shiels MS, Engels EA, Shi J et al: Genetic variation in innate immunity and inflammation pathways associated with lung cancer risk. Cancer, 2012; 118(22): 5630–36
17. Cheng CW, Su JL, Lin CW et al: Effects of NFKB1 and NFKBIA gene polymorphisms on hepatocellular carcinoma susceptibility and clinicopathological features. PLoS One, 2013; 8(2): 1–5
18. Curran JE, Weinstein SR, Griffiths LR: Polymorphic variants of NFKB1 and its inhibitory protein NFKBIA, and their involvement in sporadic breast cancer. Cancer Lett, 2002; 188(1–2): 103–7
19. Tan SC, Suzairi MS, Alzat AA et al: Gender-specific association of NFKBIA promoter polymorphisms with the risk of sporadic colorectal cancer. Med Oncol, 2013; 30(4): 693
20. White KL, Vierkant RA, Phelan CM et al: Polymorphisms in NF-kappaB inhibitors and risk of epithelial ovarian cancer. BMC Cancer, 2009; 9: 170
21. Shafi'i MSM, Shahpudin SM, Mustapha MA et al: The genetic variation A>G at 3' UTR of nuclear factor kappa B 1 A (NFKB1A) influences susceptibility of sporadic colorectal cancer in Malaysian population. Int Med J, 2012; 19(2): 98–101
22. Zhang P, Wei Q, Li X et al: A functional insertion/deletion polymorphism in the promoter region of the NFKB1 gene increases susceptibility for prostate cancer. Cancer Genet Cytoengen, 2009; 191(2): 73–77
23. Kopp TI, Frilis S, Christensen J et al: Polymorphisms in genes related to inflammation, NSAID use, and the risk of prostate cancer among Danish men. Cancer Genet, 2013; 206(7–8): 266–71
24. Oecklinghaus A, Ghosh S: The NF-kappaB family of transcription factors and its regulation. Cold Spring Harb Perspect Biol, 2009; 1(4): a000034
25. Taal WP, Firestein GS: NF-kappaB: a key role in inflammatory diseases. J Clin Invest, 2001; 107(1): 7–11
26. Naugler WE, Karin M: NF-kappaB and cancer: identifying targets and mechanisms. Curr Opin Genet Dev, 2008; 18(1): 19–26
27. Lu S, Dong Z: Characterization of TGF-beta-regulated interleukin-8 expression in human prostate cancer cells. Prostate, 2006; 66: 996–1004
28. Wise GI, Marella VK, Talluri G, Shirazian D: Cytokine variations in patients with hormone treated prostate cancer. J Urol, 2000; 164: 722–25
29. Nakashima J, Tachibana M, Horiguchi Y et al: Serum interleukin 6 as a prognostic factor in patients with prostate cancer. Clin Cancer Res, 2000; 6: 2702–6
30. Shariat SF, Andrews B, Kattan MW et al: Plasma levels of interleukin-6 and its soluble receptor are associated with prostate cancer progression and metastasis. Urology, 2001; 58: 1008–15
31. Karban AS, Okazaki T, Panhuysen CI et al: Functional annotation of a novel NFKB1 promoter polymorphism that increases risk for ulcerative colitis. Hum Mol Genet, 2004; 13(1): 35–45
32. Abdallah A, Sato H, Grutters JC et al: Inhibitor kappa B-alpha (IkappaB-alpha) promoter polymorphisms in UK and Dutch sarcoïdosis. Genes Immun, 2003; 4(6): 450–54

Statement

This study was not supported by any grant; rather, it was supported by the private funds of all the authors.