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# Genetic polymorphism of *NFKB1* and *NFKBIA* genes and liver cancer risk: a nested case–control study in Shanghai, China

Jing Gao,[1,2] Hong-Li Xu,[2] Shan Gao,[3] Wei Zhang,[2] Yu-Ting Tan,[2] Nat Rothman,[4] Mark Purdue,[4] Yu-Tang Gao,[2] Wei Zheng,[5] Xiao-Ou Shu,[5] Yong-Bing Xiang[1,2]

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For numbered affiliations see end of article.

**Correspondence to**
Professor Y-B Xiang;
ybxiang@shsci.org

## ABSTRACT

**Objectives:** Genetic variations of nuclear factor-κB (NF-κB) signalling pathway were found to be associated with inflammatory diseases and several malignancies. However, little is known about NF-κB pathway gene polymorphisms and susceptibility of liver cancer. The aim of this study was to investigate whether genetic variants of *NFKB1* and *NFKBIA* were associated with risk of liver cancer in a Chinese population.

**Design:** The study was designed as a nested case–control study within two prospective cohorts (the Shanghai Women's Health Study, SWHS, 1996–2000 and the Shanghai Men's Health Study, SMHS, 2002–2006).

**Settings:** This population-based study was conducted in urban Shanghai, China.

**Participants:** A total of 217 incident liver cancer cases diagnosed through 31 December 2009 and 427 healthy controls matched by sex, age at baseline (±2 years) and date (±30 days) of sample collection were included in the study.

**Primary and secondary outcome measures:** Genetic polymorphisms of *NFKB1* and *NFKBIA* were determined blindly by TaqMan single-nucleotide polymorphism (SNP) genotyping assay. OR and its 95% CIs were estimated by an unconditional logistic regression model to measure the association between selected SNPs and the risk of liver cancer.

**Results:** After adjusted for potential confounding factors, rs28362491 ins/del or del/del genotypes were associated with higher risk of liver cancer with an adjusted OR 1.54 (95% CI 1.04 to 2.28). rs230496 AG and GG genotypes were also noted with higher risk of liver cancer with an adjusted OR 1.53 (95% CI 1.03 to 2.26). Haplotype analysis indicated that carriers of the *NFKB1* GA and AA (rs230525-rs230530) haplotypes had higher risk of liver cancer under an additive model. No association was observed between *NFKBIA* variants and risk of live cancer.

**Conclusions:** Our results suggest that genetic variants of *NFKB1* influence liver cancer susceptibility in Chinese population, although replication in other studies is needed.

## Strengths and limitations of this study

- This study was the first population-based study to evaluate the polymorphic variants of Nuclear Factor-κB and risk of liver cancer.
- Only incident cases from two prospective cohorts were included in the study which ruled out the possibility of recall and selection bias.
- The limitations of the study include relatively small sample size, unmeasured hepatitis B virus (HBV) infection, hepatitis C virus (HCV) infection and aflatoxin exposure. However, we did take into consideration the participants' history of hepatitis and liver cirrhosis, although the presence of HCV infection and aflatoxin is very low in the study population.

## INTRODUCTION

Liver cancer is a common disorder worldwide which ranks the fifth and seventh most common cancer among men and women. It was estimated that more than 80% of liver cancers occur in developing countries and about 54% occur in China.[1] Among the main risk factors for liver cancer, chronic infections of hepatitis B virus (HBV) and hepatitis C virus (HCV) are the most important in humans, accounting for more than 70% of liver cancer cases worldwide.[2–4] Liver cirrhosis, heavy alcohol consumption, exposure to aflatoxin and diabetes also account for part of liver cancer occurrence.[2–4]

Chronic inflammation has been widely accepted to play an important role in hepatocarcinogenesis. Most of the known risk factors of liver cancer such as HBV, HCV infection and alcohol drinking can cause persistent inflammatory reaction of the liver and promote cancer development.[5 6] However, the molecular and cellular mechanisms linking inflammation and liver cancer remain unclear. Recent findings have

suggested that nuclear factor-κB (NF-κB) may play a crucial role in bridging the actions of growth factors and chronic inflammation to hepatic oncogenesis.[7–10]

NF-κB, a collection of dimeric transcription factors, was originally identified as a nuclear factor bound to the enhancer of the immunoglobulin κ-light chain gene[11] specific to B cells and presents in all cell types.[12] It is a major transcription regulator of the immune response, cell adhesion, differentiation, proliferation and apoptosis.[13] NF-κB dimers are formed by seven distinct proteins: NF-κB1 (p105 and p50), NF-κB2 (p100 and p52), RelA (p65), RelB and c-Rel, of which NF-κB p50/RelA is the most common dimer form.[9] In the resting cell, most NF-κB dimers are inactivated in the cytoplasm by binding to specific inhibitors: IκB family, of which IκBα is the most common one. In the classical activation pathway, IκB is phosphorylated and degraded by IκB kinase complex, and then NF-κB dimers are released and translocate to the nucleus where they coordinate the transcriptional activation of target genes.[14] Several genetic variations of NF-κB signalling pathway have been reported to be associated with cancer risks such as breast,[15] prostate,[16] stomach,[17] colorectum[18] and mouth.[19] However, little is known about the role of genetic polymorphisms of NF-κB genes and susceptibility of liver cancer.

In a population-based case–control study nested in two prospective cohorts of the Shanghai Women's (SWHS) and Men's Health Studies (SMHS), we investigated the relationships between genetic variants of *NFKB1* and *NFKBIA*, two key genes involved in classical signalling pathway of NF-κB, and the risk of liver cancer among Chinese men and women.

## MATERIALS AND METHODS

### Study population

Participants of this study came from the SWHS and SMHS. The design and methods used in these two studies have been described in detail elsewhere.[20–23] Briefly, the SWHS enrolled 74 941 women aged 40–74 years between 1 March 1997 and 31 May 2000, with a response rate of 92.7%. SMHS enrolled 61 491 men aged 40–74 years without history of cancer at recruitment from 1 April 2002 to 30 June 2006, with a response rate of 74.1%. Both studies were approved by the relevant Institutional Review Boards for human research in China and the USA, and a written informed consent was obtained from all participants.

In-person interview was conducted by trained interviewers using a structured questionnaire at baseline to obtain information on demographics, lifestyle, dietary habits, medical history and other characteristics. Anthropometric measurements, including current weight, height and circumferences of the waist and hips, were also measured. Of the eligible participants, 56 831 (75.8%) of the SWHS and 46 332 (75.3%) of the SMHS provided a 10 mL blood sample at baseline. The samples were drawn into an EDTA Vacutainer tube and then kept in a portable styrofoam box with ice packs (at approximately 0–4°C) and processed within 6 h for long-term storage at −70°C. A biospecimen collection form was completed for each participant at the time of sample procurement which included the date and time of collection, time of last meal, and date of last menstruation, intake of selected foods, smoking, as well as use of any medications over the previous 24 h and during the previous week.

### Cohort follow-up and outcome ascertainment

Both cohorts were followed for occurrence of cancer and other chronic diseases by active in-person surveys conducted every 2–3 years as well as annual record linkage to the databases of the population-based Shanghai Cancer Registry, Shanghai Vital Statistics Registry and Shanghai Resident Registry. For the SWHS, four rounds of in-person follow-ups were completed, and the response rates for the first (2000–2002), second (2002–2004), third (2004–2007) and fourth (2008–2011) follow-up surveys were 99.8%, 98.7%, 96.7% and 92%, respectively. For the SMHS, two rounds of follow-up surveys were completed. The response rates for the first (2004–2008) and second (2008–2011) follow-up surveys were 97.6% and 93.6%, respectively. For cohort members who developed liver cancer during the follow-up, medical charts were reviewed by a panel of oncologists to verify the diagnosis. Liver cancer data through 31 December 2009 was used for the present study.

Included in this nested case–control study are 217 incident liver cancer cases and 427 matched controls who had donated blood sample. Liver cancer cases were defined as having an International Classification of Disease, Ninth Revision (ICD-9), codes of 155.0 (primary malignant neoplasms), 155.1 (malignant neoplasms of the intrahepatic bile ducts) or 155.2 (unspecified malignant neoplasms of the liver).[24] Two control subjects were randomly selected from the cohorts who donated a blood sample at baseline and matched to each case for sex, age at baseline (±2 years) and date (±30 days) of sample collection. All controls were free of any cancer at the time of cancer diagnosis for the corresponding case.

### Genotyping

Single-nucleotide polymorphisms (SNPs) were selected based on tag SNP and their putative functional significance. Tagging SNPs were selected by searching the Han Chinese data from the HapMap project.[25] The following criteria were used to identify tagging SNPs: (1) SNPs located in the genes or within the 5 kb flanking region, (2) a minor allele frequency ≥0.05 and (3) other unselected SNPs could be captured by one of the tagging SNPs with a linkage disequilibrium of $r^2 \geq 0.90$. A total of eight SNPs were selected for genotyping which were rs28362491, rs230530, rs230525, rs230496 for *NFKB1* and rs3138053, rs3138055, rs2273650, rs696 for *NFKBIA*

Table 1 Descriptions of genetic polymorphisms of *NFKB1* and *NFKBIA* genes under investigation

| Gene | Assay ID | Sequence | Location |
|---|---|---|---|
| *NFKB1* | rs28362491 | CTCCGTGCTGCCTGCGTTCCCCGACC[-/ATTG] ATTGGGCCCGGCAGGCGCTTCCTGG | 5′-near gene |
| | rs230530 | TTTTTAGCACCAAACATCTTAATTT[A/G]CATTCAAATAAATGAGAACCACCAT | Intron |
| | rs230525 | TACGGGAAAAGTGATTCTTGTTTAC[A/G]GAGCCCTCTTTCACAGTTTCATGTT | Intron |
| | rs230496 | TGTCTGGATTTGCTTGAGACAGCCC[A/G]GTTTGCCCCTGACCTAATTGTTTAT | Intron |
| *NFKBIA* | rs3138053 | ATTCGTTTATGCTATCTGACCTACA[C/T]TGTGCTCCCGCAGAAAAAGGATCGT | 5′-near gene |
| | rs3138055 | AATCAACGGGATGACAGAATGACAA[C/T]GGAGAGGTCTCCAACCACAGGCCAA | 3′-near gene |
| | rs2273650 | AACAATACATTATGTACACCATTTA[C/T]AGGAGGGTAACACAAACCTTGACAG | 3′-UTR |
| | rs696 | CCTACCACAATAAGACGTTTTGGGC[C/T]AGGCAGTGTGCAGTGTGGATATAAG | 3′-UTR |

UTR, untranslated region.

(table 1). Genomic DNA was extracted from buffy coat using Promega DNA Extraction Kit according to the manufacturer's instructions (Promega Corporation, Madison, Wisconsin, USA). Genotyping were performed by the TaqMan assay, using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, California, USA), in 384-well format, with dual fluorescent reporter probes VIC and FAM. rs28362491 was genotyped using custom-designed probes and primers. The primer sequences were: 5′-GCCTCCGTGC TGCCT-3′ (forward primer), 3′-AGGGAAGCCCCCAGG AA-5′ (reverse primer). The probe sequences were: 5′-TTCCCCGACCATTGG-3′ (del), 5′-CCGACCATTGAT TGG-3′ (ins). Other SNPs were genotyped using predesigned assays (Applied Biosystems). The quality and potential misclassification of the genotyping results were assessed by evaluating 5% of duplicate DNA samples that were randomly selected from the whole samples. Their replicates were 100% concordant. All serum samples were tested blindly and were identified only by a unique identification number blinded with case–control status.

### Statistical analysis

Subjects with both survey data and genotyping results were included in the final analysis. Means and percentages of selected characteristics for cases and controls were calculated. The distributions of selected characteristics were compared between cases and controls by either Student t test (continuous variables) or $\chi^2$ test (categorical variables). OR and its 95% CI were estimated by unconditional logistic regression model to measure the association between selected SNPs and the risk of primary liver cancer. In the multivariable analysis, potential confounding factors were adjusted for, which included age (continuous variable); education level (four categories: elementary school or less, middle school, high school and college or above); history of hepatitis (yes or no); family history of liver cancer (yes or no); and history of other chronic liver diseases or cirrhosis (yes or no). Statistical analyses were carried out using the SAS software package (V.9.2; SAS Institute, Cary, North Carolina). Tests for trend were performed by entering categorical variables as continuous variables in the regression model. All p values were calculated by two-sided tests and were considered statistically significant if p value was less than 0.05.

Hardy-Weinberg equilibrium and linkage disequilibrium were accessed with HaploView V.4.0.[26] Associations between haplotypes and the risk of liver cancer were evaluated with HAPSTAT V.3.0 using the most common haplotype as the referent category, assuming an additive model.[27]

## RESULTS

Selected baseline characteristics of study participants were presented in table 2. The average ages of cases and controls were 59.61 and 59.47, respectively. Compared with controls, liver cancer cases were more likely to have a lower education level, a history of hepatitis, a family history of liver cancer in first degree relatives and history of chronic liver diseases or cirrhosis. Besides, men with liver cancer were more probable to have lower body mass index and be non-regular exercisers compared with controls, although the difference was at borderline significance. Whereas in women, cases were more likely to have a history of type 2 diabetes than controls. No differences were observed in family income, smoking, drinking habits, waist-to-hip ratio and family history of other cancers between the two groups.

The associations of *NFKB1* SNPs with liver cancer risk were summarised in table 3. The genotypes of rs28362491, rs230530 and rs230525 showed no deviation from Hardy-Weinberg equilibrium in controls except for rs230496. After adjusted for potential confounding factors, rs28362491 ins/del or del/del genotypes were associated with higher risk of liver cancer with an OR 1.54 (95% CI 1.04 to 2.28). rs230496 AG and GG genotypes were also noted with higher risk of liver cancer with an adjusted OR 1.53 (95% CI 1.03 to 2.26). Carriers of rs230525 AG or GG genotypes had about 30% increased risk of liver cancer, but the risk was insignificant. No association was found between rs230530 and liver cancer risk.

Table 4 presents the distribution of *NFKBIA* SNPs in cases and controls. The genotypes of rs3138055, rs696

**Table 2** Distribution of selected characteristics in the study cases and controls*

| Characteristics | All participants | | | Male | | | Female | | |
|---|---|---|---|---|---|---|---|---|---|
| | Cases (n=217) | Controls (n=427) | p Value | Cases (n=131) | Controls (n=262) | p Value | Cases (n=86) | Controls (n=165) | p Value |
| Age at interview, mean±SD | 59.61±9.56 | 59.47±9.55 | 0.853 | 60.05±9.93 | 59.86±9.95 | 0.858 | 58.95±8.98 | 58.85±8.87 | 0.928 |
| Education level (%) | | | | | | | | | |
| Elementary school or less | 63 (29.30) | 115 (27.00) | – | 18 (13.95) | 35 (13.41) | – | 45 (52.33) | 80 (48.48) | – |
| Middle school | 69 (32.09) | 148 (34.74) | – | 54 (41.86) | 104 (39.85) | – | 15 (17.44) | 44 (26.67) | – |
| High school | 62 (28.84) | 91 (21.36) | – | 41 (31.78) | 61 (23.37) | – | 21 (24.42) | 30 (18.18) | – |
| College or above | 21 (9.77) | 72 (16.90) | 0.031 | 16 (12.40) | 61 (23.37) | 0.053 | 5 (5.81) | 11 (6.67) | 0.341 |
| Family income (%)† | | | | | | | | | |
| Low | 50 (23.04) | 90 (21.13) | – | 17 (12.98) | 37 (14.12) | – | 33 (38.37) | 53 (32.32) | – |
| Medium | 112 (51.61) | 208 (48.83) | – | 76 (58.02) | 130 (49.62) | – | 36 (41.86) | 78 (47.56) | – |
| High | 55 (25.35) | 128 (30.05) | 0.454 | 38 (29.01) | 95 (36.26) | 0.271 | 17 (19.77) | 33 (20.12) | 0.606 |
| Ever smoked (%) | 93 (42.86) | 173 (40.52) | 0.569 | 90 (68.70) | 163 (62.21) | 0.206 | 3 (3.49) | 10 (6.06) | 0.384 |
| Ever drank alcohol (%) | 45 (20.74) | 98 (22.95) | 0.523 | 42 (32.06) | 97 (37.02) | 0.333 | 3 (3.49) | 1 (0.61) | 0.084 |
| Body mass index, kg/m$^2$, mean±SD | 23.79±3.65 | 24.16±3.31 | 0.198 | 23.16±3.25 | 23.77±2.89 | 0.06 | 24.75±4.02 | 24.78±3.80 | 0.961 |
| WHR, mean±SD | 0.87±0.07 | 0.87±0.07 | 0.936 | 0.90±0.06 | 0.90±0.06 | 0.379 | 0.82±0.05 | 0.83±0.06 | 0.261 |
| Regular physical activity (%) | 94 (43.32) | 207 (48.48) | 0.215 | 49(37.40) | 124 (47.33) | 0.062 | 45 (52.33) | 83 (50.30) | 0.761 |
| physical activity, MET-h/week | 81.58±47.12 | 83.71±43.59 | 0.570 | 66.86±40.33 | 68.00±34.61 | 0.78 | 104.00±48.0909 | 108.60±44.83 | 0.450 |
| History of hepatitis (%) | 74 (34.10) | 25 (5.85) | <0.001 | 57 (43.51) | 16 (6.11) | <0.001 | 17 (19.77) | 9 (9.45) | <0.001 |
| Family history of cancer (%) | 69 (31.80) | 116 (27.17) | 0.220 | 41 (31.30) | 70 (26.72) | 0.342 | 28 (32.56) | 46 (27.88) | 0.441 |
| Family history of liver cancer (%) | 28 (12.90) | 18 (4.22) | <0.001 | 20 (15.27) | 10 (3.82) | <0.001 | 8 (9.30) | 8 (4.85) | 0.171 |
| History of type 2 diabetes (%) | 25 (11.52) | 35 (8.20) | 0.171 | 14 (10.69) | 25 (9.54) | 0.72 | 11 (12.79) | 10 (6.06) | 0.068 |
| History of chronic liver disease or cirrhosis (%) | 35 (16.13) | 11 (2.58) | <0.001 | 26 (19.85) | 10 (3.82) | <0.001 | 9 (10.47) | 1 (0.61) | <0.001 |

*Missing data were excluded from the analysis.
†Family income level (low income for <¥5000/year in the SWHS and <¥12 000/year in the SMHS; medium income for ¥5000 to <¥10 000/year in the SWHS and ¥12 000 to <¥24 000/year in the SMHS; and high income for >¥10 000/year in the SWHS and >¥24 000 /year in the SMHS).
SMHS, Shanghai Men's Health Study; SWHS, Shanghai Women's Health Study.

Table 3 *NFKB1* genetic polymorphisms with the risk of primary liver cancer

| SNPs | Cases | Controls | p Value for $\chi^2$ | OR* | 95% CI | OR† | 95% CI | OR‡ | 95% CI |
|---|---|---|---|---|---|---|---|---|---|
| rs28362491 | | | | | | | | | |
| ins/ins | 68 | 171 | – | 1.00 | – | 1.00 | – | 1.00 | – |
| ins/del | 102 | 160 | – | 1.60 | 1.10 to 2.33 | 1.60 | 1.10 to 2.33 | 1.71 | 1.13 to 2.60 |
| del/del | 40 | 79 | 0.047 | 1.27 | 0.79 to 2.05 | 1.27 | 0.79 to 2.04 | 1.21 | 0.71 to 2.05 |
| *p value for trend* | – | – | – | 0.144 | – | 0.146 | – | 0.233 | – |
| ins/del or del/del | 142 | 239 | 0.023 | 1.50 | 1.05 to 2.12 | 1.49 | 1.05 to 2.12 | 1.54 | 1.04 to 2.28 |
| rs230496 | | | | | | | | | |
| AA | 64 | 164 | – | 1.00 | – | 1.00 | – | 1.00 | – |
| AG | 101 | 169 | – | 1.53 | 1.05 to 2.24 | 1.53 | 1.05 to 2.24 | 1.68 | 1.10 to 2.58 |
| GG | 47 | 91 | 0.087 | 1.33 | 0.84 to 2.09 | 1.32 | 0.84 to 2.09 | 1.25 | 0.75 to 2.09 |
| *p value for trend* | – | – | – | 0.141 | – | 0.143 | – | 0.235 | – |
| AG or GG | 148 | 260 | 0.041 | 1.46 | 1.03 to 2.08 | 1.46 | 1.03 to 2.08 | 1.53 | 1.03 to 2.26 |
| rs230525 | | | | | | | | | |
| AA | 79 | 186 | – | 1.00 | – | 1.00 | – | 1.00 | – |
| AG | 102 | 175 | – | 1.38 | 0.96 to 1.97 | 1.38 | 0.96 to 1.98 | 1.46 | 0.98 to 2.18 |
| GG | 32 | 63 | 0.224 | 1.20 | 0.73 to 1.98 | 1.20 | 0.73 to 1.97 | 1.11 | 0.63 to 1.94 |
| *p value for trend* | – | – | – | 0.236 | – | 0.236 | – | 0.347 | – |
| AG or GG | 134 | 238 | 0.100 | 1.33 | 0.95 to 1.87 | 1.33 | 0.95 to 1.87 | 1.36 | 0.94 to 1.99 |
| rs230530 | | | | | | | | | |
| AA | 64 | 114 | – | 1.00 | – | 1.00 | – | 1.00 | – |
| AG | 99 | 175 | – | 1.01 | 0.68 to 1.49 | 1.01 | 0.68 to 1.49 | 1.05 | 0.68 to 1.62 |
| GG | 48 | 129 | 0.102 | 0.66 | 0.42 to 1.04 | 0.66 | 0.42 to 1.04 | 0.67 | 0.40 to 1.12 |
| *p value for trend* | – | – | – | 0.079 | – | 0.078 | – | 0.132 | – |
| AG or GG | 147 | 304 | 0.423 | 0.86 | 0.60 to 1.24 | 0.86 | 0.60 to 1.24 | 0.89 | 0.59 to 1.34 |

*Adjusted for age.
†Adjusted for age and sex.
‡Adjusted for age, sex, education level, family history of liver cancer, history of hepatitis and chronic liver diseases or cirrhosis.
SNP, single-nucleotide polymorphism.

and rs2273650 showed no deviation from Hardy-Weinberg equilibrium in controls but for rs3138053. Generally, all the four SNPs showed no relationship with liver cancer.

We further analysed the haplotypes of these SNPs with risk of liver cancer (table 5). For *NFKB1* gene, two SNPs (rs230525 and rs230530) demonstrated strong linkage disequilibrium ($D'$=1.0, $r^2$=0.59). Compared with men carrying rs230525-rs230530 AG haplotype, those with GA or AA haplotypes were at a significant increased risk of liver cancer with adjusted ORs 1.46 (95% CI 1.05 to 2.03) and 1.81 (95% CI 1.15 to 2.86), respectively. For *NFKBIA*, rs3138053 and rs2273650 were in linkage disequilibrium ($D'$=0.97, $r^2$=0.31), but none of the haplotypes was significantly associated with liver cancer.

## DISCUSSION

In this nested case–control study, we found that the variants of rs28362491 and rs230496 of *NFKB1* gene might be associated with risk of primary liver cancer. After adjusting for possible confounders, rs28362491 deletion allelle and rs230496 AG or GG genotype were found to increase the risk of liver cancer. In addition, haplotype analysis indicated that carriers of the *NFKB1* GA and AA (rs230525-rs230530) haplotypes had higher risk of liver cancer under the additive model, although this association was only observed in men. These findings suggested that variants of NF-κB signalling pathway may play a role in liver cancer susceptibility.

*NFKB1* gene was mapped on chromosome 4q23-q24 and composed of 24 exons.[28] This gene encodes p105 which is a non-DNA binding protein. As an inactive precursor, it is activated to p50, a DNA binding protein by proteasome-mediated degradation. Several genetic polymorphisms were defined in *NFKB1*, and researches have been focused on a common polymorphism of -94 del/ins (rs28362491) in the promoter region. Recent studies show that genetic polymorphism of rs28362491 was associated with a number of cancer risks including sporadic breast,[15] prostate,[16] gastric,[17] colorectal[18] and oral cancers,[19] but little is known about its relationship with liver cancer. He *et al* conducted a case–control study of 202 hepatocellular carcinoma (HCC) cases of HBV carrier and 404 healthy controls without HBV infection.[29] Results showed that after adjusting for age and gender, -94 ins/del and ins/ins genotypes might increase the risk of HCC, with ORs 1.60 (95% CI 1.01 to 2.53) and 3.01 (95% CI 1.87 to 4.85), respectively. A report from Taiwan also found ins allele more prevalent in patients with HCC (OR=2.23, 95% CI 1.32 to 3.77).[30] In our study, we found that ins/del and del/del

Table 4 *NFKBIA* genetic polymorphisms with the risk of primary liver cancer

| SNPs | Cases | Controls | p Value | OR* | 95% CI | OR† | 95% CI | OR‡ | 95% CI |
|---|---|---|---|---|---|---|---|---|---|
| rs3138053 | | | | | | | | | |
| AA | 173 | 336 | – | 1.00 | – | 1.00 | – | 1.00 | – |
| AG | 21 | 48 | – | 0.85 | 0.49 to 1.47 | 0.84 | 0.48 to 1.45 | 0.97 | 0.54 to 1.74 |
| GG | 19 | 40 | 0.823 | 0.92 | 0.52 to 1.64 | 0.94 | 0.52 to 1.68 | 0.98 | 0.51 to 1.88 |
| *p value for trend* | – | – | – | 0.638 | – | 0.653 | – | 0.920 | – |
| AG or GG | 40 | 88 | 0.556 | 0.88 | 0.58 to 1.34 | 0.88 | 0.58 to 1.34 | 0.97 | 0.61 to 1.54 |
| rs3138055 | | | | | | | | | |
| CC | 62 | 128 | – | 1.00 | – | 1.00 | – | 1.00 | – |
| CT | 109 | 215 | – | 1.05 | 0.72 to 1.54 | 1.05 | 0.72 to 1.54 | 1.22 | 0.79 to 1.87 |
| TT | 42 | 81 | 0.956 | 1.07 | 0.66 to 1.73 | 1.07 | 0.66 to 1.73 | 1.33 | 0.78 to 2.27 |
| *p value for trend* | – | – | – | 0.772 | – | 0.771 | – | 0.276 | – |
| CT or TT | 151 | 296 | 0.778 | 1.06 | 0.74 to 1.51 | 1.06 | 0.74 to 1.52 | 1.25 | 0.83 to 1.88 |
| rs696 | | | | | | | | | |
| CC | 65 | 149 | – | 1.00 | – | 1.00 | – | 1.00 | – |
| CT | 115 | 196 | – | 1.35 | 0.93 to 1.96 | 1.35 | 0.93 to 1.96 | 1.47 | 0.97 to 2.23 |
| TT | 33 | 76 | 0.210 | 0.99 | 0.60 to 1.64 | 0.99 | 0.60 to 1.64 | 1.17 | 0.67 to 2.03 |
| *p value for trend* | – | – | – | 0.694 | – | 0.695 | – | 0.360 | – |
| CT or TT | 148 | 272 | 0.218 | 1.25 | 0.88 to 1.78 | 1.25 | 0.88 to 1.78 | 1.38 | 0.93 to 2.06 |
| rs2273650 | | | | | | | | | |
| CC | 108 | 215 | – | 1.00 | – | 1.00 | – | 1.00 | – |
| CT | 84 | 173 | – | 0.97 | 0.68 to 1.37 | 0.97 | 0.68 to 1.37 | 0.86 | 0.58 to 1.26 |
| TT | 20 | 37 | 0.938 | 1.08 | 0.60 to 1.95 | 1.07 | 0.59 to 1.94 | 0.89 | 0.45 to 1.73 |
| *p value for trend* | – | – | – | 0.937 | – | 0.945 | – | 0.493 | – |
| CT or TT | 104 | 210 | 0.933 | 0.99 | 0.71 to 1.38 | 0.99 | 0.71 to 1.37 | 0.86 | 0.60 to 1.24 |

*Adjusted for age.
†Adjusted for age and sex.
‡Adjusted for age, sex, education level, family history of liver cancer, history of hepatitis and chronic liver diseases or cirrhosis.
SNP, single-nucleotide polymorphism.

genotypes were more prevalent in liver cancer cases than controls. It was observed that the association of rs28362491 polymorphism with cancer susceptibility varied with cancer site and study populations. Ins allele was reported to increase the risk of oral cancer,[31] melanoma,[32] prostate cancer,[16] gastric cancer,[17] nasopharyngeal carcinoma[33] and cervical cancer.[34] Two studies in European population found that del allele might increase the risk of colorectal cancer,[35 36] while in Chinese population, none or even reverse association was obtained.[35 37] The difference of polymorphisms may probably result from interactions or combined effects with non-genetic risk factors. Well-designed studies with larger sample size are needed to validate these findings.

To the best of our knowledge, this is the first report on the variants of rs230496, rs230525 and rs230530 with liver cancer susceptibility. A study in European American descent found rs230530 polymorphism associated with alcohol dependence, and the evidence came primarily from those individuals who met the criteria for alcoholism earlier.[38] As alcohol is one of the major risk factors of liver cancer, rs230530 might play a role in alcohol associated liver cancer. Unfortunately, subject to the limitation of relatively small sample size, we were not able to explore this issue. In addition, although the functions of intronic SNPs are still obscure, studies have indicated that they can affect the secondary structure of either local DNA or RNA, thereby regulating gene expression.[39 40]

*NFKBIA* gene, which encodes IκBα, the inhibitor of *NFKB1*, was mapped to 14q13 with six exons spanning approximately 3.5 kb.[41] As a major component of the IκB family, the dysfunction or down regulation of IκBα will lead to over activation of NF-κB. Epidemiological studies on *NFKBIA* were relatively rare. A 2758G/A polymorphysim (rs696) in 3′-untranslated region might regulate the expression of IκBα and thus affect the activation of NF-κB. Sun and colleagues found that frequency of AG genotype was increased in Chinese patients ≥50 years of age (OR=3.06, 95% CI 1.55 to 6.02) with colorectal cancer.[42] Another study on breast cancer fails to obtain a significant association.[15] There was no previous report on rs696 and risk of liver cancer.

Of the four SNPs of *NFKBIA* gene evaluated, we did not observe a significant association. In previous studies, the rs3138053 variant was found to be associated with HCC in a Chinese mainland population[29] but not Taiwanese.[30]

There are several strengths of our study. This study was based on two well-designed prospective cohort studies. To the best of our knowledge, it was the first population-based study to evaluate the polymorphic variants of NF-κB and risk of liver cancer. All study participants were ethnic Chinese and residents of Shanghai with similar genetic backgrounds, which minimised the

Table 5 ORs and 95% CIs for liver cancer in relation to *NFKB1/NFKBIA* haplotypes

| | All participants* | | | Female† | | | Male† | | |
|---|---|---|---|---|---|---|---|---|---|
| | Cases (%) | Controls (%) | OR (95% CI) | Cases (%) | Controls (%) | OR (95% CI) | Cases (%) | Controls (%) | OR (95% CI) |
| *NFKB1* (rs230525-rs230530) | n=215 | n=425 | – | n=86 | n=165 | – | n=129 | n=260 | – |
| AG | 46.49 | 52.01 | ref | 52.14 | 49.39 | ref | 42.69 | 53.53 | ref |
| GA | 38.97 | 35.50 | 1.23 (0.95 to 1.58) | 36.47 | 36.59 | 0.94 (0.63 to 1.41) | 40.55 | 34.88 | 1.46 (1.05 to 2.03) |
| AA | 14.54 | 12.49 | 1.30 (0.91 to 1.86) | 11.39 | 14.02 | 0.77 (0.42 to 1.39) | 16.76 | 11.59 | 1.81 (1.15 to 2.86) |
| *NFKBIA* (rs3138055-rs2273650) | | | | | | | | | |
| TC | 45.00 | 43.94 | ref | 48.24 | 42.31 | ref | 43.15 | 45.14 | ref |
| CT | 29.05 | 28.58 | 1.00 (0.75 to 1.31) | 31.30 | 31.21 | 0.88 (0.57 to 1.35) | 27.79 | 27.12 | 1.07 (0.75 to 1.55) |
| CC | 25.65 | 27.00 | 0.93 (0.70 to 1.24) | 20.47 | 26.48 | 0.68 (0.42 to 1.10) | 28.50 | 26.95 | 1.11 (0.77 to 1.60) |

*Adjusted for age and sex.
†Adjusted for age.

potential confounding due to ethnics. Only incident cases were included which ruled out the possibility of recall and selection bias. Liver cancer cases were carefully verified with multiple approaches which minimised the disease misclassification. Also, we controlled potential confounding variables in the analysis. The limitations of our study should also be noted. First, we focused on only two genes involved in the canonical pathway of NF-κB, other regulatory genes in the NF-κB signalling pathway may also contribute to the pathogenesis of liver cancer. Second, we did not test for HBV infection, HCV infection or aflatoxin exposure, so we cannot rule out possible confounding due to that although the presence of HCV infection and aflatoxin is very low in the study population,[43] but we did take into consideration the participants' history of hepatitis and liver cirrhosis. Finally, owing to the relatively small sample size, the frequencies of some homozygous variants were low in subgroups and therefore reduced the statistical power and limited us from evaluating the joint effects in stratified analysis. Replication in other studies is needed.

In summary, in this nested case–control study, we provided additional evidence for the role of NF-κB SNPs and haplotypes in the aetiology of liver cancer. Studies in larger, varied polulations are warranted to confirm these findings. Furthermore, functional studies are required in order to explore the underlying mechanisms.

**Author affiliations**
[1]State Key Laboratory of Oncogene and Related Genes, Shanghai Cancer Institute, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China
[2]Department of Epidemiology, Shanghai Cancer Institute, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China
[3]Department of Infection Management, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China
[4]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, Maryland, USA
[5]Division of Epidemiology, Department of Medicine, Vanderbilt Epidemiology Center, Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, Tennessee, USA

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