Genetic Association Between \textit{NFKBIA} -881A">G Polymorphism and Cancer Susceptibility

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Abstract: Several epidemiological studies have focused on the role of nuclear factor-kappa-B inhibitor-alpha (\textit{NFKBIA}) -881 A">G polymorphism in cancer susceptibility. However, the published data have led to contentious results. This study was designed to examine the association between -881 A">G polymorphism and cancer risk.

Comprehensive search of PubMed, Web of science and Embase, identified a total of 5 case-control studies. To assess the association, comparison among all subjects plus subgroup analysis by ethnicity was performed and odds ratio (OR) along with 95% confidence interval (CI) was calculated with the fixed-effect model or the random-effects model dependent on the heterogeneity.

The pooling data consisting of 1965 cancer cases and 2717 cancer-free controls demonstrated no significant association with overall cancer risk. However, the subgroup of Asian populations showed statistical evidence for an increase in risk of cancer (GG vs. AA, OR, 2.14; 95% CI, 1.03–4.46; GG vs. AA, OR, 2.09; 95% CI, 1.01–4.34). This investigation on the association of -881 A">G polymorphism and cancer susceptibility reveals that -881 A">G polymorphism may act as a candidate for cancer development in Asian populations.

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Abbreviations: CI = confidence interval, HWE = Hardy–Weinberg equilibrium, NF-kB = nuclear factor kappabeta, \textit{NFKBIA} = nuclear factor-kappa-B inhibitor-alpha, OR = Odds ratios, SNPs = single nucleotide polymorphisms.

INTRODUCTION

The transcription activator nuclear factor kappa-beta (NF-kB) controls the expression of various genes involved in cancer-related processes including immune and inflammatory response, cell adhesion, proliferation, differentiation, apoptosis, and angiogenesis.\textsuperscript{1,2} NF-kB comprises a multicomponent protein complex as a central mediator of many viral and cellular genes.\textsuperscript{3} It attaches to the cytoplasm of inactive cell lines by forming a complex with IkB inhibitor proteins (IkBo and IkB\beta), which are responsible for nuclear localization of NF-kB in mammalian cells.\textsuperscript{4,5} It has been identified that phosphorylation of the IkB proteins causes rapid ubiquitination and subsequent proteolysis by 26S proteosome. Degradation of IkB proteins initiated by proteosome induces NF-kB dimers, resulting in accumulation of NF-kB in the nucleus and aberrant expression of specific target genes.\textsuperscript{6} Of the IkB family members (IkBo, IkB\beta, IkB\gamma, IkB\delta, IkB\epsilon, IkB\zeta, IkB-R, Bcl-3, p100, and p105) that can be found in cytoplasm and nucleus, all are constitutively expressed except that the typical form IkBo expressed inducibly.\textsuperscript{7,8} IkBo suppresses the activation of inflammation by binding to NF-kB in most normal cell types and plays a major role in circumventing oncogenesis due to its missing inhibitory function in a variety of cancer cells.\textsuperscript{9–13}

Functioning as a candidate tumor suppressor gene involved in controlling the oncogenic potential of NF-kB, nuclear factor-kappa-B inhibitor-alpha (\textit{NFKBIA}) precludes nuclear translocation, DNA binding, and phosphorylation by protein kinase alpha.\textsuperscript{14} The highly polymorphic \textit{NFKBIA} gene at chromosome 14q13 spanning approximately 3.5 kb consists of 6 exons. Single nucleotide polymorphisms (SNPs) in the promoter region of \textit{NFKBIA} are reported to be associated with the initiations of breast cancer, colorectal cancer, Hodgkin lymphoma, multiple myeloma, and melanoma.\textsuperscript{4,15–18} A commonly functional SNP located at GATA binding protein 2 is -881 A">G (rs3138053), which has been frequently implicated in hepatocellular carcinoma, colorectal cancer, ovarian cancer, and oral cancer.\textsuperscript{19–23} Since a single study using a sufficiently large sample size is currently unknown, which constitutes the main limitation of determining the association between -881 A">G polymorphism and cancer susceptibility. To clarify this issue, we performed a meta-analysis plus subgroup analysis from all eligible data associating the occurrence of cancer with -881 A">G polymorphism.

METHODS

Literature Search Strategy

Potentially relevant studies focusing on the association between -881 A">G polymorphism and cancer susceptibility published in English were searched using the databases of PubMed, Web of science and Embase. The key words \textit{NFKBIA}, -881 A">G/rs3138053, polymorphism/polymorphisms/variant/SNP, and cancer were used in separation or in combination for the search strategy. The identification of additional original studies was carried out through screening the citations used in the papers that may have usable data for this meta-analysis. The study was approved by the ethics committee of southwest hospital.

Inclusion and Exclusion Criteria

Eligibility of this studies for the meta-analysis was examined based on the following criteria: first, a case-control study looking at cancer susceptibility in relation to -881 A">G
polymorphism; second, published as a full text detailing genotype data to calculate an odds ratio (OR) and corresponding 95% confidence interval (CI). Abstracts, unpublished reports, and articles written in non-English language were not considered. When the same case series were used in a subsequently published paper, we selected the study with more genotyped subjects.

**Data Extraction**

Two authors independently gathered information from each study, including the first author’s name, year of publication, ethnic origin of the participants (Asian or Caucasian), study design, cancer type, and genotype frequencies for AA, AG, and GG genotypes. For the disputable views on the extracted data, a senior reviewer was invited to resolve the differences.

**Statistical Analysis**

Stata software (version 12.0, Stata Corp LP, College Station, TX) was performed to analyze all statistical data in the meta-analysis. Deviation from Hardy–Weinberg equilibrium (HWE) was checked for the genotype frequencies of controls in each study by using a χ²-test. An OR along with 95% CI was calculated through comparison among all subjects plus subgroup analysis by ethnicity to estimate cancer susceptibility associated with -881 A>G polymorphism. In addition to the Cochran Q-test, $I^2$ statistic by Higgins and Thompson was quantified to examine heterogeneity between studies, $P < 0.10$ or $I^2 > 50\%$ being considered statistically significant. The fixed-effects model using the Mantel–Haenszel method was performed for OR estimate if $P > 0.10$ or $I^2 < 50\%$. Conversely, the random-effects model using the DerSimonian Laird method was used. Sensitivity analysis was performed to detect the stability of the meta-analysis results by removing the studies, one at a time. Symmetrical funnel plots and $P$ values of the Egger test above 0.10 revealed little significant publication bias across studies.

**RESULTS**

**Characteristics of Included Studies**

A total of 27 relevant papers were retrieved from initial screening. Among these, 13 full texts remained for eligibility evaluation, of which 8 publications were finally excluded for focusing on cancer risk and other polymorphisms of NFKB1 gene. Therefore, our final data pooling consisted of 5 case-control studies involving 1965 cancer cases and 2717 cancer-free controls (Figure 1).

**Quantitative Synthesis**

Overall, there were 2 hepatocellular carcinoma studies, 1 prostate cancer study, 1 ovarian cancer study, and 1 oral cancer study. Of the 5 case-control studies, 4 were for the subjects of Asian ancestry and 1 for Caucasian ancestry. Moreover, all publications were in line with HWE except the study by He et al (Table 1).

**TABLE 1. Characteristics of Studies Included in the Meta-Analysis**

| Study | Ethnicity | Study Design | Cancer Type | HWE $P$ Value |
|-------|-----------|--------------|-------------|---------------|
| He    | Asian     | Case-control | Hepatocellular carcinoma | 0.05         |
| White | Caucasian | Case-control | Ovarian cancer    | 0.31         |
| Lin   | Asian     | Case-control | Oral cancer     | 0.80         |
| Cheng | Asian     | Case-control | Hepatocellular carcinoma | 0.80      |
| Tan   | Asian     | Case-control | Colorectal cancer | 0.46         |

HWE = Hardy–Weinberg equilibrium.
In the present study, heterogeneity across studies was evaluated by Q test and the $I^2$ index, where significant between-study heterogeneity was indicated in the allele model (G vs. A, $P$, 0.068; $I^2$, 54.2%) (Table 2). Subgroup analysis by ethnicity and subsequent sensitivity analysis restricted the main source to the study by White and co-workers.\textsuperscript{20} Intriguingly, when removing this study from the total meta-analysis, heterogeneity was lost ($P$, 0.120; $I^2$, 48.5%) and additionally this genetic model was found to be significantly associated with cancer risk (before removal, OR, 1.16; 95% CI, 0.96–1.41; after removal, OR, 1.25; 95% CI, 1.05–1.49).

**FIGURE 2.** Forest plot of the association between *NFKBIA* -881 A>G polymorphism and cancer susceptibility under GG vs. AA genetic model.

**FIGURE 3.** Forest plot of the association between *NFKBIA* -881 A>G polymorphism and cancer susceptibility under GG + GA vs. AA genetic model.
Publication Bias

We performed Begg test and Egger test to diagnose the publication bias of all included studies. The symmetrical funnel plot \( (P, 0.860) \) and Egger test \( (P, 0.521) \) revealed no statistical evidence for significant publication bias in this meta-analysis (for GG + GA vs. AA) (Figure 5).

DISCUSSION

To the best of our knowledge, our study is the available meta-analysis comprehensively investigating for the first time cancer susceptibility associated with -881 A>G polymorphism in the promoter region of the NFKBIA gene. It is believed that a study of predisposition genetic polymorphisms with a large

TABLE 2. Stratified Analysis of the NFKBIA -881A>G (rs3138053) Polymorphism on Cancer Risk

| Genetic Model (No. of Studies) | Sample Size | Test of Association | Heterogeneity |
|-------------------------------|-------------|---------------------|---------------|
|                              | Case | Control | OR (95 % CI) | Analysis Model | P Value | I² (%) |
| All                           | 1965 | 2717 | 1.22 (0.91, 1.65) | Fixed-effects | 0.342 | 10.1 |
| GG vs. AA                     | 1036 | 1681 | 2.14 (1.03, 4.46) | Fixed-effects | 0.700 | 0 |
| GG + GA vs. AA                | 2.22 (1.01, 1.47) | Fixed-effects | 0.180 | 38.6 |
| GG vs. GA + AA                | 2.09 (1.01, 4.34) | Fixed-effects | 0.789 | 0 |
| G vs. A                       | 1.23 (0.96, 1.58) | Random-effects | 0.120 | 48.5 |
| GA vs. AA                     | 1.19 (0.98, 1.44) | Fixed-effects | 0.200 | 35.4 |
| Caucasian                     | 929  | 1036 | 1.09 (0.78, 1.51) | Fixed-effects |          |      |
| GG vs. AA                     | 1.03 (0.88, 1.19) | Fixed-effects |          |      |
| GG + GA vs. AA                | 1.07 (0.78, 1.48) | Fixed-effects |          |      |
| G vs. A                       | 1.03 (0.91, 1.18) | Random-effects |          |      |
| GA vs. AA                     | 1.02 (0.87, 1.21) | Fixed-effects |          |      |
ever, colorectal cancer was observed to be decreased in women carcinogenesis. Due to the central role played in immunological activation stimulates NF-

misleads further replicated studies. Given the importance of -881 A polymorphism in cancer-related pathways and the existence of contentious association with cancer, we examined the correlation by performing a meta-analysis, where we found significantly increased cancer risk in Asian populations.

The finding of our meta-analysis should be interpreted with caution. We observed significant heterogeneity in the allele model and found the major source of the heterogeneity masked the true association between this comparison with risk of cancer. Although no obvious inter-study heterogeneity was indicated in the Q-test and statistic under other genetic models, we cannot exclude the possibility that our findings have been affected by potential heterogeneity across studies. Furthermore, cancer risk was not shown to be related to -881 A>G polymorphism in Caucasian populations. In the current study, only 1 publication based on Caucasians was included, which may have underestimated the association. Then, the susceptibility of -881 A>G polymorphism to certain cancers has been suggested to be different in women and men. Therefore, other confounding factors, such as sex, should be taken into account in future studies if sufficient data are provided.

To sum up, this meta-analysis of the association between -881 A>G polymorphism and cancer susceptibility suggested that -881 A>G polymorphism did not modulate overall cancer risk. However, we found statistical evidence for an increased risk of cancer in Asian populations. Further large sample-sized studies are necessary to provide new insights into the mechanism of cancer development related to -881 A>G polymorphism.

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