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The role of *XRCC6/Ku70* in nasopharyngeal carcinoma

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Abstract. The association between *XRCC6/Ku70*, an upstream player in the DNA double-strand break repair system, and the risk of nasopharyngeal carcinoma (NPC) was examined. In this case–control study, 176 NPC patients and 352 cancer-free controls were genotyped, and the associations of *XRCC6* promoter T–991C (rs5751129), promoter G–57C (rs2267437), promoter G–31A (rs132770), and intron 3 (rs132774) polymorphisms with NPC risk were evaluated. NPC tissue samples were also assessed for their *XRCC6* mRNA and protein expression by real-time quantitative reverse transcription PCR and Western blotting, respectively. With regard to the *XRCC6* promoter T–991C, the TC and CC genotypes were associated with a significantly increased risk of NPC compared with wild-type TT genotype (adjusted odds ratio 2.02 and 3.42, 95% confidence interval 1.21–3.32 and 1.28–8.94, $P = 0.0072$ and 0.0165, respectively). The mRNA and protein expression levels for NPC tissues revealed significantly lower *XRCC6* mRNA and protein expression in the NPC samples with TC/CC genotypes compared to those with the TT genotype ($P = 0.0210$ and 0.0164, respectively). These findings suggest that *XRCC6* may play an important role in the carcinogenesis of NPC and could serve as a chemotherapeutic target for personalized medicine and therapy.

Key words: genotype; immunohistochemistry; nasopharyngeal carcinoma; non-homologous end-joining; polymorphism; real-time quantitative reverse transcription; *XRCC6/Ku70*.

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Nasopharyngeal carcinoma (NPC) – cancer originating in the nasopharynx – occurs relatively infrequently in the West (age-standardized incidence rate (ASR) of <1/100,000), but remains a leading tumour among those in southern China (ASR 30–50/100,000), Southeast Asia (ASR 9–12/100,000), and Taiwan (ASR 8.2–8.4/100,000).[1] The geographical pattern of incidence suggests an interaction of complicated environmental and genetic factors. Although the aetiology of NPC remains to be elucidated, those people with an Epstein–Barr virus (EBV) infection,[2] environmental risk factor exposure,[3] risky dietary habits,[4] and risky genotypes with single nucleotide polymorphisms (SNPs), may have a higher susceptibility to NPC.[5]

The integrity of the human genome is controlled by the human DNA repair system, and accumulated mutations or defects are thought to be essential for carcinogenesis.[6] Therefore, it is reasonable to hypothesize that the loss of DNA repair capacity or a decrease in the function via genomic variation might have a significant influence on the carcinogenesis of NPC. In humans, genetic variations affecting non-homologous end-joining (NHEJ), together with those affecting the alternative homologous recombination DNA double-strand break (DSB) repair system, have been postulated to be important contributors to the aetiology of cancer.[7]

In recent years, several proteins involved in the NHEJ pathway have been identified, including ligase IV, XRCC4, XRCC6 (Ku70), XRCC5 (Ku80), DNA-PKcs,

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Artemis, and XLF.[8] Inappropriate NHEJ has been shown to lead to translocations and telomere fusion, which are hallmarks of tumour cells.[9] As for NHEJ, some genetic polymorphisms have been reported to influence DNA repair capacity and confer a predisposition to several types of cancer, including skin,[10] breast,[11–13] bladder,[14,15] lung,[16] and oral cancers.[17,18] However, there is no information regarding NPC and NHEJ gene polymorphisms.

Recent epidemiological studies have investigated the association between *XRCC6* polymorphism and the risk of various types of cancer, including gastric cancer,[19] oral cancer,[17] breast cancer,[20] lung cancer,[16] and renal cell carcinoma.[21] We hypothesized that the different *XRCC6* genotypes, together with their RNA and protein expression, may also contribute to NPC susceptibility.

To test this hypothesis, the present study was designed to investigate the association of *XRCC6* genotypes with the risk of NPC; this was a case–control study involving a population in central Taiwan. In addition, we also investigated the association of the *XRCC6* mRNA and protein expression patterns with NPC risk by real-time polymerase chain reaction (PCR) and Western blotting, respectively, in order to assess the potential functional effect of *XRCC6* genotype on NPC risk. To the best of our knowledge, this is the first study to evaluate the association between the *XRCC6* genotypes and NPC susceptibility and to explore the potential function of XRCC6 in NPC at the same time.

Methods

Study population

One hundred and seventy-six patients diagnosed with NPC were recruited at the general surgery outpatient clinics of the study hospital in Taichung, Taiwan, between 2003 and 2009. All patients participated voluntarily, completed a self-administered questionnaire, and provided peripheral blood samples. The questionnaire included questions on history and frequency of alcohol consumption, betel quid chewing, and smoking habits, and 'ever' was defined as more than twice a week for years. Self-reported alcohol consumption, betel quid chewing, and smoking habits were evaluated and classified as categorical variables.

For each case patient, two age- and gender-matched healthy controls, who had no NPC or other type of cancer, were selected from those attending the hospital for a health examination (age matching was done within less than 5 years of the case patient's first diagnosis). These volunteers attended the hospital for regular health assessments by multidisciplinary team approach with registered health practitioners during the years 2002–2012; most of the volunteers underwent health examinations every 5–6 months. A total of 10,358 participants aged 1–104 years were recruited into this cohort, and those who were cancer-free by the age at diagnosis of the case patient, according to the International Classification of Diseases, Ninth Revision (ICD-9) codes, were chosen. Finally, 352 participants were included for analysis in the present study. For the convenience of the gene–environment interaction analysis, we preferentially selected those with alcoholism, betel quid chewing, and smoking habits when selecting the controls for genotyping and further analysis. Thus, the control group was not a general population control, but rather an alcohol-, betel quid-, and smoking-related control group. The overall agreement rate in this study was more than 85% in collection.

The study was approved by the institutional review board of the medical university hospital and written informed consent was obtained from all participants.

Genotyping protocol

The total genomic DNA of each subject was extracted from peripheral blood leucocytes using a QIAamp Blood Mini Kit (Qiagen, Taipei, Taiwan) and stored as reported previously.[22,23] The primers used for *XRCC6* were as follows: promoter T–991C, forward 5′-AACTCATGGACCCACGGTTGTGA-3′, and reverse 5′-CAACTTAAATACAGGAATGTCTTG-3′; promoter G–57C, forward 5′-AAACTTCAGACCACTCTCTTCT-3′, and reverse 5′-AAGCCGCTGCCGGGTGCCCGA-3′; promoter G–31A, forward 5′-TACAGTCCTGACGTAGAAG-3′, and reverse 5′-AAGCGACCAACTTGGACAGA-3′; intron 3, forward 5′-GTATACTTACTGCATTCTGG-3′, and reverse 5′-CATAAGTGCTCAGTACCTAT-3′. The following cycling conditions were used: one cycle at 94 °C for 5 min; 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s; and a final extension at 72 °C for 10 min.

Restriction fragment length polymorphism (RFLP) conditions

For the *XRCC6* promoter T–991C, the resulting 301-base pair (bp) PCR product was mixed with 2 U *Dpn*II. The restriction site was located at −991, with a T/C polymorphism; the C form PCR products could be digested further, while the T form could not. Two fragments of 101 bp and 200 bp were present if the product was the digestible C form. The reaction was incubated for 2 h at 37 °C. Then, 10 μl of product was loaded into a 3% agarose gel containing ethidium bromide for electrophoresis. The polymorphism was categorized as (1) C/C homozygote (digested), (2) T/T homozygote (undigested), or (3) C/T heterozygote.

For the *XRCC6* promoter G–57C, the resulting 298-bp PCR product was mixed with 2 U *Hae*II. The restriction site was located at −57, with a G/C polymorphism; the G form PCR products could be digested further, while the C form could not. Two fractions of 103 bp and 195 bp were present if the product was the digestible G form. The reaction was incubated for 2 h at 37 °C. Then, 10 μl of product was loaded into a 3% agarose gel containing ethidium bromide for electrophoresis. The polymorphism was categorized as (1) G/G homozygote (digested), (2) C/C homozygote (undigested), or (3) C/G heterozygote.

For the *XRCC6* promoter G–31A, the resulting 226-bp PCR product was mixed with 2 U *Mnl*I. The restriction site was located at −31, with a G/A polymorphism; the A form PCR products could be digested further, while the G form could not. Two fractions of 80 bp and 146 bp were present if the product was the digestible A form. The reaction was incubated for 2 h at 37 °C. Then, 10 μl of product was loaded into a 3% agarose gel containing ethidium bromide for electrophoresis. The polymorphism was categorized as (1) A/A homozygote (digested), (2) G/G homozygote (undigested), or (3) A/G heterozygote.

For the *XRCC6* intron 3, the resulting 160-bp PCR product was mixed with 2 U *Msc*I. The restriction site was located at intron 3, with a GG/GC polymorphism; the GG form PCR products could be digested further, while the GC form could not. Two fractions of 46 bp and 114 bp were present if the product was the digestible GG form. The reaction was incubated for 2 h at 37 °C. Then, 10 μl of product was loaded into a 3% agarose gel containing ethidium bromide for electrophoresis. The polymorphism was categorized as (1) GG/GG homozygote (digested), (2) GC/GC homozygote (undigested), or (3) GG/GC heterozygote.

mRNA *XRCC6* expression pattern

To evaluate the correlation between *XRCC6* mRNA expression and *XRCC6*