Nasopharyngeal Carcinoma and Genetic Polymorphisms of DNA Repair Enzymes XRCC1 and hOGG1


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Abstract

Nitrosamine consumption and polymorphisms in CYP2E1, the product of which is involved in the activation of nitrosamines into reactive intermediates, have been shown to be associated with nasopharyngeal carcinoma (NPC) risk. Given that reactive intermediates created during nitrosamine metabolism are capable of DNA damage, we further hypothesized that differences between individuals in their ability to repair DNA damage might be important in NPC pathogenesis. To evaluate this hypothesis, this study focused on effects of genetic polymorphisms of DNA repair genes hOGG1 and XRCC1 on the development of NPC. We conducted a case-control study to investigate the genotypes of 334 patients with NPC and 283 healthy community controls matched by sex, age, and residence. The PCR-based RFLP assay was used to identify genetic polymorphisms. After adjustment for sex, age, and ethnicity, the odds ratio (OR) of developing NPC for hOGG1 codon 326 genotypes of Ser/Cys and Cys/Cys compared with the Ser/Ser genotype was 1.6 (95% CI, 1.0–2.6). For XRCC1 codon 280 genotypes of Arg/His and His/His compared with the Arg/Arg genotype, the OR was 0.64 (95% CI, 0.43–0.96). Among subjects with putative high-risk genotypes for both hOGG1 and XRCC1, the OR was 3.0 (95% CI, 1.0–8.8). Furthermore, subjects with putative high-risk genotypes for hOGG1, XRCC1, and CYP2E1 had an OR of disease of 25 (95% CI, 3.5–177). Polymorphisms of the DNA repair genes hOGG1 (codon 326) and XRCC1 (codon 280) are associated with an altered risk of NPC. Carriers of multiple putative high-risk genotypes have the highest risk of developing NPC.

Introduction

NPC3 has a striking geographic and ethnic distribution, with particularly high rates observed among southeast Chinese and other individuals of Chinese descent (1, 2). NPC is linked to EBV infection (3–7). In addition to EBV, numerous other environmental and host factors have been shown to be associated with the development of NPC (8–15). In particular, long-term cigarette smoking, consumption of salted fish and foods containing nitrosamine or nitrosamine precursors at an early age, and occupational exposure to wood dust have been shown to be consistently associated with this disease. Host factors previously shown to be associated with NPC development include HLA class I and II alleles (likely involved via their regulation of the immunological response to EBV infection) and CYP2E1 gene polymorphisms (likely involved via its modulation of the activation of environmental procarcinogens, including nitrosamines, into reactive intermediates capable of DNA damage; Refs. 2, 16).

Various cellular metabolic processes result in the formation of hydroxyl radicals that can cause oxidative damage to DNA (17). This damage often results in single base changes that can be reversed by BER mechanisms (18, 19). hOGG1 and XRCC1 are two of the enzymes participating in the BER pathway, the DNA repair system involved in the repair of damage resultant from oxidative stress. hOGG1 can recognize and excise 8-hGua, the major form of oxidative DNA damage induced by reactive free radicals (20, 21). XRCC1 complexes with DNA polymerase β via the NH2-terminus domain and with DNA ligase III via a blue ribbon commission on transportation (BRCT) domain to repair nicks or gaps left in the BER pathway (22, 23). XRCC1 has also been shown to be involved in the detection of single strand breaks between incision and ligation, an effect that likely occurs via poly(ADP-ribose) polymerase-dependent and poly(ADP-ribose) polymerase-independent mechanisms (24–26).

Genetic polymorphisms of DNA repair genes have been reported to determine susceptibility to several cancers, including lung, esophageal, bladder, and nonmelanoma skin cancers (19, 27–31). No studies, to date, have examined the association between genetic polymorphisms in DNA repair genes and NPC. In this study, we describe results from a case-control study (334 NPC cases; 283 community controls) conducted in Taiwan in which polymorphisms in the hOGG1 (codon 326) and XRCC1 (codons 280 and 399) genes are investigated. We were moti-

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The abbreviations used are: NPC, nasopharyngeal carcinoma; hOGG1, human 8-oxoguanine DNA glycosylase; XRCC1, X-ray repair cross-complementing group 1; BER, base excision repair; 8-hGua, 8-hydroxyguanine; OR, odds ratio; CI, confidence interval.
vated to evaluate DNA repair mechanisms by previous results from our case-control study, suggesting that exposure to nitro-
samines and nitrosamine precursors from various sources (diet
and cigarette smoking) is associated with NPC development
and that polymorphisms in the \textit{CYP2E1} gene (a gene respon-
sible for the activation of nitroamines and other procarci-
genins into reactive intermediates capable of inducing DNA
damage) were also associated with disease development (10,
14, 16). We hypothesize that if DNA damage induced via
activation by \textit{CYP2E1} of nitroamines and other procarci-
genins is important in the development of NPC, DNA repair
mechanisms should also play an important role in the devel-
opment of this tumor.

\section*{Materials and Methods}

The methods for case ascertainment and control selection were
described in detail previously (10, 16). In brief, 378 eligible
NPC cases were recruited from July 15, 1991, through Decem-
ber 31, 1994, at two large referral hospitals in Taipei, Taiwan.
For each eligible case subject, we attempted to match one
community control subject by age (5-year groups), sex, and
residence (the same district/township). Ninety-nine percent of
eligible cases ($n = 375$) and 87% of eligible controls ($n = 327$)
agreed to a detailed risk factor interview administered by a
trained nurse-interviewer. Blood specimens were obtained from
367 cases and 321 controls. In this study, 334 cases (88% of
eligibles) and 283 controls (75% of eligibles) were included
because DNA from the remaining subjects was exhausted by
previous testing for other factors. No differences were noted
between the 617 subjects included in the current analysis and
the 71 subjects for whom DNA was unavailable for testing,
with respect to gender, ethnicity, education, and smoking. The
71 untested subjects were slightly older than the 617 subjects
included in our study (mean age = 48.5 \textit{versus} 45.4 years; \textit{P} =
0.04). This study was reviewed and approved by the Institu-
tional Review Boards at the National Cancer Institute and the
National Taiwan University. All participants provided informed
consent.

\textit{hOGG1} genotyping was performed using a PCR-RFLP
 technique. The primers used to identify the polymorphism at
codon 326 of \textit{hOGG1} were as follows: forward, \textit{5'-CTGTC
CAGAAGCCGAAGCAGC-3'} and reverse, \textit{5'-GAACTGCT
CTCCAACG-3'}. A 40-\textmu l reaction mixture containing 29.71 \textmu 
M of double-distilled water, 10\times PCR buffer (4 \textmu l), 1 \textmu M of each
primer (5 \textmu M/\textmu l), 1 \textmu l of the mixture of deoxynucleoside
triphosphates (2.5 \textmu M/\textmu l), 1.2 \textmu l of MgCl$_2$ (50 \textmu M/\textmu l), and 0.45 unit (5 unit/\textmu l) TaqDNA poly-
merase (Amersham Pharmacia Biotech) was used. The
PCR condition was initiated by a 4-min denaturation step at
94°C, followed by 35 cycles at 94°C for 40 s, 55/57°C for 30 s,
72°C for 40 s, and a final step at 72°C for 10 min. The PCR
products were subjected to restriction digestion overnight at
37°C by \textit{RsaI} for codon 280 and by \textit{MspI} for codon 399.
The digestion products were resolved on 2.5% agarose gels. Two
bands at 126 and 62 bp characterize the wild-type \textit{Arg} allele for
codon 280; a single band at 188 bp characterizes the variant
type \textit{His} allele. Two bands at 115 and 34 bp characterize the
wild-type \textit{Arg} allele for codon 399; a single band at 149 bp
characterizes the variant type \textit{Gln} allele.

An 8% masked, random sample ($n = 51$) of subjects was
tested in replicate. Three (6%) masked duplicates had discord-
ant results after genotyping; these discrepancies were resolved
by repeat testing. The statistical analysis of our data were
performed using the SAS statistical software (SAS, Cary, NC).
The ethnic-specific genotype distribution for each of the poly-
morphisms evaluated was compared using Pearson’s \textit{\chi}^2
test (32). Using a goodness-of-fit test, we compared the observed
and expected genotype counts and computed the \textit{\chi}^2
statistic as a measure of the deviation from Hardy-Weinberg equi-
librium (33). Unconditional logistic regression models were used
to estimate the OR and 95\% CI of disease associated with genetic
polymorphisms (32, 34, 35). Unconditional logistic regression
was chosen over conditional logistic regression to avoid losses
of cases and controls without a matched pair. Both unadjusted
OR estimates and OR estimates adjusted for age, gender, and
ethnicity were presented. Additional adjustment for other risk
factors associated with NPC in our population (e.g., cigarette
smoking, family history of NPC, dietary nitrosamine consump-
tion during childhood, HLA alleles, and occupational exposure
to wood dust) did not affect the results (data not shown). Trend
tests were performed by including the categorical variable of
interest as a continuous variable in the logistic variable and
assessing departure of the resultant \beta coefficient from 0.

\section*{Results}

Three-hundred thirty-four cases and 283 controls are in-
cluded in this analysis. The average age of cases and controls
was 45.3 and 45.6, respectively. The gender ratio for both
cases and controls was \~2:1. Ethnically, 81.7\% of cases and
70.9\% of controls were of Fukienese origin; 8.4\% of cases and
6.4\% of controls were of Hakka origin; the remaining
9.9\% of cases and 22.7\% of controls were of Cantonese,
Aboriginal, or other Han origin ($P = 0.001$). A total of
42.2\% of cases and 30.1\% of controls reported less than a
junior high school education; 41.1\% of cases and 51.1\% of
controls reported higher than a senior high school education
($P = 0.04$). Other relevant risk factors reported from this
population include most notably \~25 years of cigarette
smoking (OR, 1.7; 95\% CI, 1.1–2.9) and homozygosity for the
\textit{CYP2E1} \textit{RsaI} \textit{c}2 variant allele (OR, 2.6; 95\% CI, 1.2–
5.7; Refs. 10, 16).

We first investigated whether there was evidence for het-
erogeneity in genotype distributions or allele frequencies by
ethnicity in our study (Table 1). All distributions were in
Hardy-Weinberg equilibrium. No significant differences were
noted for the three polymorphisms examined when individuals
of Fukienese, Hakka, and other ethnic origins were compared
among our community controls. For \textit{XRCC1} codon 280, how-
ever, there was a suggestion that the \textit{His} variant allele fre-
frequency was lower (0.03) among the small group (n = 18) of individuals of Hakka descent compared with Fukienese or other Chinese ethnic groups (0.14 and 0.11, respectively). Because no significant differences were noted between ethnic groups and because the vast majority of individuals in our study (77%) were of Fukienese origin (the only group with sufficiently large numbers) yielded similar results to those reported herein (data not shown).

Next, we examined the association between hOGG1 and XRCC1 polymorphisms and NPC (Table 2). After adjusting for gender, age, and ethnicity, the OR for NPC associated with the Cys/Cys or Ser/Cys genotypes combined compared with the Ser/Ser genotype was 1.6 (95% CI, 1.0–2.6). The adjusted OR for NPC associated with XRCC1 codon 280 genotypes His/His or Arg/His combined compared with the Arg/Arg genotype was 0.64 (95% CI, 0.43–0.96). No significant association was observed between XRCC1 codon 399 polymorphism and NPC.

Table 3 presents results of the analysis that evaluated the joint effect of polymorphisms at hOGG1 codon 326 and XRCC1 codon 280. For simplicity, we considered as the referent group for this analysis carriers of the genotypes found to be at lowest risk of disease (i.e., Ser/Ser for hOGG1 and Arg/His/His for XRCC1). As shown in the table, individuals who carried only one of the two polymorphisms associated with NPC risk (i.e., hOGG1 Ser/Cys-Cys/Cys or XRCC1 Arg/Arg) were at an ~2-fold increased risk of NPC, whereas individuals who carried both putative risk genes had an OR of 3.0 (95% CI, 1.0–8.8).

Because individuals homozygous for an allele of the CYP2E1 gene that is detected by Rsal digestion (c2 allele) were previously found to have an increased risk of NPC in our study (OR, 2.6; 95% CI, 1.2–5.7; Ref. 16), we next examined the joint effect of polymorphisms in the CYP2E1, hOGG1, and XRCC1 genes on NPC risk. A clear dose response of increasing risk with increasing number of putative genes was observed (Ptrend = 0.001). Relative to carriers of none of the three putative high-risk genes, carriers of one putative high-risk gene had an OR of 3.0 (95% CI, 0.78–11.1), carriers of two putative high-risk genes had an OR of 11.1 (95% CI, 2.2–53.9), and carriers of all three putative high-risk genes had an OR of 53.9 (95% CI, 10.8–260.2).

Table 1 Distribution of hOGG1 codon 326, XRCC1 codon 280, and XRCC1 codon 399 in different ethnic groups among 283 community controls

<table>
<thead>
<tr>
<th>Grouping of DNA repair genes</th>
<th>Fukien no. (%)</th>
<th>Hakka no. (%)</th>
<th>Other no. (%)</th>
<th>Hardy-Weinberg equilibrium χ² P</th>
</tr>
</thead>
<tbody>
<tr>
<td>hOGG1 codon 326</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ser/Ser</td>
<td>33 (16.5)</td>
<td>3 (16.7)</td>
<td>10 (15.4)</td>
<td></td>
</tr>
<tr>
<td>Ser/Cys</td>
<td>89 (44.5)</td>
<td>10 (55.6)</td>
<td>30 (46.2)</td>
<td></td>
</tr>
<tr>
<td>Cys/Cys</td>
<td>78 (39.0)</td>
<td>5 (27.8)</td>
<td>25 (38.5)</td>
<td></td>
</tr>
<tr>
<td>Cys allele frequency</td>
<td>0.61</td>
<td>0.56</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>XRCC1 codon 280</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>147 (73.5)</td>
<td>17 (94.4)</td>
<td>51 (78.5)</td>
<td></td>
</tr>
<tr>
<td>Arg/His</td>
<td>51 (25.5)</td>
<td>1 (5.6)</td>
<td>14 (21.5)</td>
<td></td>
</tr>
<tr>
<td>His/His</td>
<td>2 (1.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>His allele frequency</td>
<td>0.14</td>
<td>0.03</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>XRCC1 codon 399</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>109 (54.8)</td>
<td>8 (44.4)</td>
<td>35 (53.9)</td>
<td></td>
</tr>
<tr>
<td>Arg/Gln</td>
<td>74 (37.2)</td>
<td>9 (50.0)</td>
<td>26 (40.0)</td>
<td></td>
</tr>
<tr>
<td>Gln/Gln</td>
<td>16 (8.0)</td>
<td>1 (5.6)</td>
<td>4 (6.2)</td>
<td></td>
</tr>
<tr>
<td>Gln allele frequency</td>
<td>0.27</td>
<td>0.31</td>
<td>0.26</td>
<td></td>
</tr>
</tbody>
</table>

a Other included the aboriginal, Cantonese, and other Han origins.

b The genotype of one subject couldn’t be identified.

Table 2 Association between hOGG1 and XRCC1 genotypes and NPC

<table>
<thead>
<tr>
<th>Grouping the DNA repair genes</th>
<th>NPC case patients No. (%)</th>
<th>Community control subjects No. (%)</th>
<th>Unadjusted OR</th>
<th>Adjusted OR, OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hOGG1 codon 326</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ser/Ser</td>
<td>36 (10.8)</td>
<td>46 (16.3)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Ser/Cys</td>
<td>175 (52.6)</td>
<td>129 (45.6)</td>
<td>1.7b</td>
<td>1.8 (1.1–2.9)</td>
<td>1.6 (1.0–2.6)</td>
</tr>
<tr>
<td>Cys/Cys</td>
<td>122 (36.6)</td>
<td>108 (38.2)</td>
<td>1.4</td>
<td>1.4 (0.86–2.4)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XRCC1 codon 280</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>275 (82.8)</td>
<td>215 (76.0)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Arg/His</td>
<td>55 (16.6)</td>
<td>66 (23.3)</td>
<td>0.65b</td>
<td>0.64 (0.43–0.97)</td>
<td>0.64 (0.43–0.96)</td>
</tr>
<tr>
<td>His/His</td>
<td>2 (0.6)</td>
<td>2 (0.7)</td>
<td>0.78</td>
<td>0.66 (0.09–4.7)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>2</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XRCC1 codon 399</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>174 (52.1)</td>
<td>152 (54.0)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Arg/Gln</td>
<td>128 (38.3)</td>
<td>109 (38.7)</td>
<td>1.0</td>
<td>1.0 (0.74–1.5)</td>
<td>1.0</td>
</tr>
<tr>
<td>Gln/Gln</td>
<td>32 (9.6)</td>
<td>21 (7.5)</td>
<td>1.3</td>
<td>1.3 (0.72–2.4)</td>
<td>1.3 (0.68–2.2)</td>
</tr>
<tr>
<td>Unknown</td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a OR estimates adjusted for age, gender, and ethnicity.
b Indicates unadjusted ORs in which 95% CI excludes 1.0.
risk alleles had an OR of 4.3 (95% CI, 1.2–16.0), and carriers of all three putative high-risk genotypes had an OR of 25 (95% CI, 3.5–177). Adjustment for age, gender, and ethnicity did not materially alter these estimates.

Discussion

Limitations of the present study include the modest sample size that reduced our ability to evaluate gene-gene interactions and the 6% genotyping error rate observed among the 8% random sample selected for blind duplicate testing. Despite these limitations, results from this study support a role of DNA repair enzymes in the etiology of NPC. In our study of 334 patients diagnosed with NPC and 283 health community controls, we observed associations with NPC for polymorphisms in both the hOGG1 and XRCC1 DNA repair genes. For the hOGG1 gene, an OR of 1.6 was observed among individuals with Cys/Cys or Ser/Ser genotypes. For the XRCC1 gene, an OR of 0.64 was observed among individuals with Arg/His or His/His genotypes, whereas no association with disease was noted for polymorphisms at codon 399 of XRCC1. Interestingly, individuals with putative risk genotypes for both hOGG1 (Cys/Cys or Ser/Ser) and XRCC1 (Arg/Arg) were at 3-fold increased risk of NPC. Furthermore, when we evaluated individuals who had both putative DNA repair risk genes and who were also carriers of the c2/r2 allele of CYP2E1 (an allele previously shown to be associated with increased risk of NPC, presumably because of its increased ability to activate nitrosamines into reactive intermediates capable of DNA damage), we observed a 25-fold increased risk of NPC when compared with individuals who were carriers of none of the three putative risk genes. Although intriguing, these gene-gene joint effect findings should be interpreted with caution, given the modest size of the present study to evaluate joint effects.

Our findings are the first to suggest an association between polymorphisms in DNA repair genes and risk of developing NPC. At least one other study has observed an association between the Cys326 form of the hOGG1 gene and risk of other cancers such as esophageal cancer and lung cancer (29, 36). Furthermore, some evidence exists suggesting decreased Cys326 form of this gene (37). However, not all evidence points to functional differences between these two forms of hOGG1. Some studies that have evaluated the possibility that the Cys326 form of hOGG1 has a lower ability to repair OHGua failed to detect such a difference (38–40).

With regard to polymorphisms in the XRCC1 gene, evidence suggests that variability at codon 399 correlates with differences in DNA repair ability (41). However, in our study, we observed no significant association between XRCC1 codon 399 polymorphisms and NPC risk, whereas differences in codon 280 did correlate with disease risk. To our knowledge, no studies have evaluated biological differences in the DNA repair ability of the Arg280 and His280 forms of the XRCC1 gene, and epidemiological studies that have evaluated the association between polymorphisms at this codon of XRCC1 and disease for tumors other than NPC have had conflicting results (28, 30, 42). The results from our study should therefore be interpreted with caution until our findings are reproduced and/or biological support for the observed association is obtained.

In summary, we observe associations between polymorphisms in two DNA repair genes, hOGG1 and XRCC1, and NPC risk. The association was stronger for individuals who carried both putative risk genes (OR, 3) and strongest for the subset of individuals who also were carriers of the high-risk c2/r2 allele of CYP2E1. This is the first study to focus on the association between genetic polymorphisms in DNA repair genes and NPC risk. In the future, polymorphisms in this and other DNA repair genes should be studied to confirm or to refute the involvement of DNA repair mechanisms in the etiology of NPC.

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References

Nasopharyngeal Carcinoma and Genetic Polymorphisms of DNA Repair Enzymes  *XRCC1* and *hOGG1*
