

RESEARCH ARTICLE

Association of DNA Base-excision Repair XRCC1, OGG1 and APE1 Gene Polymorphisms with Nasopharyngeal Carcinoma Susceptibility in a Chinese Population

Qing Li^{1*}, Jian-Min Wang², Yu Peng¹, Shi-Heng Zhang¹, Tao Ren¹, Hao Luo¹, Yi Cheng¹, Dong Wang^{1*}

Abstract

Background: Numerous carcinogens and reactive oxygen species (ROS) may cause DNA damage including oxidative base lesions that lead to risk of nasopharyngeal carcinoma. Genetic susceptibility has been reported to play a key role in the development of this disease. The base excision repair (BER) pathway can effectively remove oxidative lesions, maintaining genomic stability and normal expression, with X-ray repair crosscomplementing1 (XRCC1), 8-oxoguanine glycosylase-1 (OGG1) and apurinic/apyrimidinic endonuclease 1 (APE1) playing important roles. **Aims:** To analyze polymorphisms of DNA BER genes (OGG1, XRCC1 and APE1) and explore their associations, and the combined effects of these variants, with risk of nasopharyngeal carcinoma. **Materials and Methods:** We detected SNPs of XRCC1 (Arg399Gln), OGG1 (Ser326Cys), APE1 (Asp148Glu and -141T/G) using the polymerase chain reaction (PCR) with peripheral blood samples from 231 patients with NPC and 300 healthy people, furtherly analyzing their relations with the risk of NPC in multivariate logistic regression models. **Results:** After adjustment for sex and age, individuals with the XRCC1 399Gln/Gln (OR=1.96; 95% CI:1.02-3.78; $p=0.04$) and Arg/Gln (OR=1.87; 95% CI:1.29-2.71; $p=0.001$) genotype variants demonstrated a significantly increased risk of nasopharyngeal carcinoma compared with those having the wild-type Arg/Arg genotype. APE1-141G/G was associated with a significantly reduced risk of NPC (OR=0.40;95% CI:0.18–0.89) in the smoking group. The OR calculated for the combination of XRCC1 399Gln and APE1 148Gln, two homozygous variants, was significantly additive for all cases (OR=2.09; 95% CI: 1.27-3.47; $p=0.004$). **Conclusion:** This is the first study to focus on the association between DNA base-excision repair genes (XRCC1, OGG1 and APE1) polymorphism and NPC risk. The XRCC1 Arg399Gln variant genotype is associated with an increased risk of NPC. APE1-141G/G may decrease risk of NPC in current smokers. The combined effects of polymorphisms within BER genes of XRCC1 399Gln and APE1 148Gln may contribute to a high risk of nasopharyngeal carcinoma.

Keywords: Base excision repair - single nucleotide polymorphisms (SNP) - nasopharyngeal carcinoma (NPC)

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Introduction

Nasopharyngeal carcinoma (NPC) is a malignant tumor arising in the epithelial lining of the nasopharynx. Etiological factors include Epstein-Barr virus infection, tobacco smoking, and consumption of salted fish (Yuan et al., 2000; Yuan et al., 2000; Lin et al., 1986). The incidence of nasopharyngeal carcinoma (NPC) varies substantially worldwide, with an endemic pocket in Southern China, North Africa, and parts of the Mediterranean basin (Fachiroh et al., 2012). Since the nasopharynx is anatomically hidden, and disease onset linked with unspecific clinical symptoms, NPC generally presents at a late stage (Lee et al., 2003; Ji et al., 2007). Numerous carcinogens, and Reactive oxygen species (ROS) may

cause DNA damage including oxidative base lesions that contribute to the risk of nasopharyngeal carcinoma.

Genetic polymorphisms in individuals have recently been implicated to account for some of the observed differences in nasopharyngeal carcinoma (NPC) susceptibility. Recent genetic association studies on cancer risk have focused on identifying effects of single nucleotide polymorphisms (SNPs) in candidate genes, among which DNA repair genes are increasingly studied because of their critical roles in maintaining genome integrity (Hoeijmakers, 2001; Wood et al., 2001).

BER is initiated by recognition and excision of damaged base by the specific DNA glycosidase. Mammalian cells contain a series of different genes (each with a specialized-function), of which X-ray repair cross-

¹Cancer Center, Daping Hospital and Research Institute of Surgery, Third Military Medical University, ²The 6th Department of Research Institute of Surgery, Daping Hospital and Research Institute of Surgery, Third Military Medical University, Chongqing, China *For correspondence: dongwang64@hotmail.com

complementing-1 (XRCC1), 8-oxoguanine glycosylase-1 (OGG1), and apurinic/apyrimidinic endonuclease 1 (APE1) genes are three key enzymes in this repair pathway (Pampel, 2003; Hung et al., 2005; Robertson et al., 2009). OGG1 initiates the highly conservative BER pathway by releasing the modified base, especially the 8-oxo-G, resulting in an apurinic/apyrimidinic site. The abasic site is then cleaved by APE1, leaving a 5'-deoxyribose phosphate residue. This residue is removed by the AP-lyase activity of DNA polymerase β , which then also inserts a correct nucleotide. Finally, DNA ligase III seals the repaired DNA strand. XRCC1 interacts with a complex of DNA repair proteins including poly (ADP-ribose) polymerase, DNA ligase III and DNA polymerase β , and coordinates the gap-sealing process in the short-patch BER (Petermann et al., 2006; Maynard et al., 2009).

Numerous studies have reported the association SNPs of DNA repair genes with the risk of NPC in the population of China. Most studies involving relation between XRCC1, OGG1 and APE1 polymorphisms and cancer risk focused on cancers of the bladder, breast, pancreatic, esophagus and cervix uteri. However, their combined analysis was rarely reported (Hu et al., 2002; Smith et al., 2003; Li et al., 2011; Nakao et al., 2012). In this paper, we investigated the association of DNA BER genes XRCC1, OGG1 and APE1 polymorphisms with the risk of NPC in a hospital-based study of 231 patients and 300 cancer-free control participants.

Materials and Methods

Study Subjects

The study group consisted of 231 patients with incident nasopharyngeal carcinoma and 300 cancer-free control participants who were frequency-matched by age, gender, smoking status, and family history. All subjects were from the Chinese Han population. The patients were consecutively enrolled from January 2008 to December 2012 in Daping Hospital, Third Military Medical University (Chongqing, China) without restrictions of age, gender, histology and stage. All patients were first-visit outpatients at Daping Hospital and were newly diagnosed based on the pathological examination. At recruitment, informed consent was obtained from each subject and each participant was then interviewed to solicit detailed information on demographic characteristics and lifetime history of tobacco use. Overall, 240 eligible cases and 315 eligible controls agreed to further risk factor interviews administered by a trained nurse-interviewer, with the final study consisting of 231 cases (96.2% of eligible) and 300 controls (95.2% of eligible). Exclusion criteria included reported previous cancer history and radiotherapy or chemotherapy for unknown conditions. These patients accounted for almost all ($\geq 95\%$) subjects with nasopharyngeal carcinoma admitted into NPC clinics of Daping Hospital during the same period. The remaining patients were excluded due to lack of information on smoking and the suitable blood specimens. A comparable group of cancer-free control participants was randomly sampled from subjects participating in health examinations conducted in communities of Chongqing during the same

period. Controls with a history of cancer were excluded. These control participants were frequency matched to the patients by age (± 2 years), gender. The study was approved by the ethics committee of Daping Hospital and informed consent was obtained from each participant.

The studies used a structured questionnaire that was completed for each subject by well-trained interviewers to collect information. The questionnaire included demographic characteristics (such as age, gender, family history of cancer), lifestyle factors (such as number of cigarettes smoked) and medical care history. Family history of cancer was defined as any self-reported cancer in first-degree relatives including parents, siblings, or children. For smoking status, a person who was then smoking at least one cigarette/day for >1 year was regarded as a current smoker; otherwise, persons were considered non-smokers. Former smokers were defined as those who had abstained from smoking for >1 year.

Blood Sample extraction

Venous blood samples of all participants were collected in EDTAK2 anticoagulation tubes from an antecubital vein. Genomic DNA was extracted from peripheral whole blood by using standard kit (the E.Z.N.A. SE Blood DNA kit, Omega, Bio-tek, 2009, USA) for subsequent PCR assay.

SNPs Selection and Genotyping

According to the literature, we selected the common, nonsynonymous SNPs of the BER genes: XRCC1 (rs25487; Arg399Gln; G/A; in exon 10), OGG1 (rs1052133; Ser326Cys; C/G; in exon 7) and APE1 (rs1130409; Asp148Glu; T/G; in exon 5) and promoter polymorphism of APE1: APE1 (rs1760944; 141T/G; in the promoter region). Four SNPs were genotyped in all study samples. Genetic polymorphisms were analyzed using PCR-CTPP (PCR with confronting two-pair primers) method as described earlier (Hamajima, 2001). Primer pairs and product lengths were designed for each allele and the allele was distinguished based on the SNPs in Base-excision Repair Genes. All four primers were added into the same tube. The primers for the OGG1 Ser326Cys polymorphism were F1: 5'-CAG CCC AGA CCC AGT GGA CTC -3', R1: 5'-TGG CTC CTG AGC ATG GCG GG-3' FOR C allele size of PCR products (252bp); F2: 5'-CAG TGC CGA CCT GCG CCA ATG-3', R2: 5'-GGT AGT CAC AGG GAG GCC CC-3' FOR G allele size of PCR products (194bp). The XRCC1 Arg399Gln polymorphism primers were F1: 5'-TCC CTG CGC CGC TGC AGT TTC T-3', R1: 5'-TGG CGT GTG AGG CCT TAC CTC C-3' FOR G allele size of PCR products (447bp); F2: 5'-TCG GCG GCT GCC CTC CCA-3', R2: 5'-AGC CCT CTG TGA CCT CCC AGG C-3' FOR A allele size of PCR products (222bp). The APE1 Asp148Glu polymorphism primers were F1: 5'-CCT ACG GCA TAG GTG AGA CC-3', R1: 5'-TCC TGA TCA TGC TCC TCC-3' FOR G allele size of PCR products (167bp); F2: 5'-TCT GTT TCA TTT CTA TAG GCG AT-3', R2: 5'-GTC AAT TTC TTC ATG TGC CA-3' FOR T allele size of PCR products (236bp). The APE1-141T/G primers were F1: 5'-CTA ACT GCC AGG GAC

Table 1. Distribution of Demographic Characteristics of NPC Case and Control Participants

Variables	Cases (n = 231)	Controls (n = 300)	P
Age (%)			0.91
Mean age	49.18	50.96	
<50years	119(51.52%)	156(52.00%)	
≥50years	112(48.49%)	144(48.00%)	
Gender (%)			0.91
Male	163(70.56%)	213(71.00%)	
Female	68(29.44%)	87(29.00%)	
Smoking (%)			0.22
Never	90(38.96%)	137(45.67%)	
Former	44(19.05%)	39(13.00%)	
Current	97(59.05%)	124(41.33%)	
Family history of cancer (%)			0.11
No	203(85.04%)	276(98.33%)	
Yes	28(14.96%)	24(1.67%)	
Histological type (%)			
Anaplastic carcinoma	18(7.79%)		
Poorly differentiated carcinoma	133(57.58%)		
Higher differentiation carcinoma	80(34.63%)		

Table 2. Observed and Expected Genotypic Frequencies of Each SNPs in the Control Group

Genes	Observed n (%)	Expected n (%)	P(HWE)
OGG1Ser326Cys			
Ser/Ser	42(14.00%)	43.7(14.7%)	0.68
Ser/Cys	145(48.33%)	141.6(47.5%)	
Cys/Cys	113(37.67%)	114.7(37.8%)	
APE1 Asp148Glu			
Asp/ Asp	116(38.67%)	118.4(39.5%)	0.55
Asp/ Glu	145(48.33%)	140.2(46.7%)	
Glu/ Glu	39(13.00%)	41.4(13.8%)	
APE1 -141T/G			
TT	94(31.33%)	91.3(30.4%)	0.53
TG	143(47.67%)	148.4(49.5%)	
GG	63(21.00%)	60.3(20.1%)	
XRCC1 Arg399Gln			
Arg/Arg	166(55.3%)	165.8(47.7%)	0.94
Arg/Gln	114(38.0%)	114.5(42.7%)	
Gln/Gln	20(6.7%)	19.7(9.6%)	

HWE, Hardy-Weinberg equilibrium

GCC GA-3', R1:5'-ACA CTG ACT TAA GAT TCT AAC TA-3' FOR T allele size of PCR products (136bp); F2:5'-ACT GTT TTT TTC CCT CTT GCA CAG-3' R2:5'-TGA GCA AAA GAG CAA CCC CG-3' FOR G allele size of PCR products (335bp). They were designed based on the GenBank reference sequence.

PCR amplification was performed in a 25- μ l mixture using glass capillaries. PCR mixture contained Go tag MIX(2*): 12.5 μ l, Primer:1 μ l, Dd H₂O: 6.5 μ l, DNA:2 μ l. Reaction conditions included XRCC1: 94°C-5 min, (94°C-1min, 66°C-1min, 72°C-45S) 30cycle, 72°C-10 min. OGG1: 95°C-10min, (95°C-1min, 64°C-1min, 72°C-1min) 30 cycle, 72°C-5 min. APE1: 95°C-5min, (95°C-1min, 60°C-1min, 72°C-1min) 32 cycle, 72°C-10 min. APE1Promoter: 95°C-5min, (95°C-30s, 58°C-45s, 72°C-30s) 35 cycle, 72°C-10 min. PCR products were analyzed by agarose gel electrophoresis. Genotype results were regularly confirmed by randomly selecting 5% of the samples to directly measure DNA sequencing. The results were reproducible with no discrepancy in genotyping.

Statistical analysis

Statistical analyses were performed with SPSS (v.16.0 for Windows). Differences in demographic variables, smoking habits, and family history of cancer between case and control participants were compared by using chi-square test. Each polymorphism was tested for deviation from Hardy-Weinberg equilibrium by comparing the observed and expected genotype frequencies using the chi-square test. Odds ratios (ORs) were calculated and given with 95% confidence intervals (95% CI) by unconditional logistic regression analysis with adjustment for age, gender, smoking status, and family history of cancer. A multivariate logistic regression analysis including polymorphisms of OGG1, XRCC1 and APE1 gene as the exposure variables and nasopharyngeal carcinoma as the dependent variable was performed. The level of significance at $p < 0.05$ was considered for all statistical analyses.

Results

Study Subjects

A total of 231 NPC cases and 300 controls were recruited for the present study. Selected demographic characteristics of study subjects are summarized in Table 1. The smokers may have an increased risk of nasopharyngeal carcinoma (NPC) compared with those non-smokers (OR=1.22; 95%CI: 0.97-1.53 $p=0.09$). However, there was no significant statistical difference according to age, gender distribution, smoking status and family history of cancer between cases and controls.

Genotype Distribution and Hardy-Weinberg Equilibrium

The distribution of OGG1 (Ser326Cys), XRCC1 (Arg399Gln) and APE1 (141T/G; Asp148Glu) genotypes and allele frequencies in control participants are shown in Table 2. All genotype frequencies in the control population were in agreement with those predicted under HWE ($p > 0.05$).

Single Genotype Distribution and Nasopharyngeal Carcinoma (NPC) Risk

Table 3 depicts the genotype and the allele distributions for the DNA repair gene polymorphisms that were studied in nasopharyngeal carcinoma cases and controls. Individuals with XRCC1 399Gln/Gln (OR=1.96; 95%CI: 1.02-3.78; $p=0.04$) and Arg/Gln (OR=1.87; 95% CI: 1.29-2.71; $p=0.001$) genotype were at a significantly increased risk of nasopharyngeal carcinoma (NPC) compared with those wild-type of the Arg/Arg genotype. Further chi-square test analyses revealed that observably boost nasopharyngeal carcinoma risk was associated with the XRCC1 399Gln allele, compared with the Arg allele (OR=1.55; 95% CI: 1.19-2.02; $p=0.001$). The variant allele of OGG1 326Cys and APE1 148Glu showed a deleterious effect with an OR of 1.04 and 1.62, respectively. Slightly depressed ORs were obtained for individuals homozygous for the variant alleles of APE1-141G/G (OR=0.61, 95%CI: 0.36-1.05, $p=0.07$), indicating that this allele may decrease nasopharyngeal carcinoma (NPC) risk, but there is no significant difference. Also, no statistically significant

Table 3. Distribution of Genotypes and Odds Ratios (OR) Determined for All NPC Cases and Controls

Genes	Cases n(%)	Controls n(%)	Association OR(95%CI) ^a	P value
OGG1Ser326Cys				
Genotype				
Ser/Ser	33(14.29%)	42(14.00%)	1	
Ser/Cys	106(45.89%)	145(48.67%)	0.92(0.54~1.57)	0.68
Cys/Cys	92(39.82%)	113(37.67%)	1.12(0.61~1.77)	0.78
Allele				
Ser	172(37.23%)	229(38.33%)		
Cys	290(62.77%)	371(61.83%)	1.04(0.81-1.34)	0.76
XRCC1Gln399Arg				
Genotype				
Arg/Arg	92(39.8%)	166(47.33%)	1	
Arg/Gln	117(50.7%)	114(43.67%)	1.87(1.29~2.71)	0.001*
Gln/Gln	22(9.5%)	20(9.00%)	1.96(1.02~3.78)	0.04*
Allele				
Arg	301(65.2%)	446(74.3%)		
Gln	161(34.8%)	154(25.7%)	1.55(1.19-2.02)	0.001*
APE1 pro-141T/G				
Genotype				
TT	71(30.74%)	94(31.33%)	1	
TG	126(54.11%)	143(47.67%)	1.05(0.70~1.56)	0.83
GG	34(15.15%)	63(21.00%)	0.61(0.36~1.05)	0.07
Allele				
T	268(57.79%)	331(55.17%)		
G	194(42.21%)	269(44.83%)	0.90(0.70-1.15)	0.35
APE1Asp148Glu				
Genotype				
Asp/Asp	81(35.06%)	116(38.67%)	1	
Asp/Glu	108(46.75%)	145(48.33%)	1.04(0.70~1.53)	0.86
Glu/Glu	42(18.19%)	39(13.00%)	1.62(0.94~2.79)	0.09
Allele				
Asp	270(58.44%)	377(62.83%)		
Glu	192(41.56%)	223(37.17%)	1.20(0.94-1.54)	0.15

^aAdjusted for age, gender, smoking status, and family history of cancer; **p* < 0.05

differences were found in genotype or allele distributions differences of OGG1 Ser326Cys and APE1 Asp148Glu between cases and controls.

Smoking is a major cause of a variety of malignancies including cancers of the larynx, oral cavity and pharynx, esophagus, bladder, and lung. Numerous studies have consistently shown that cigarette smoke may play an important role as an environmental etiological factor in the development of NPC in China (Zhu et al., 1995; Yuan et al., 2000). Cigarette smoke contains a myriad of genotoxic agents and carcinogens such as nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). XRCC1 399Gln/Gln genotype carriers reportedly have a decreased capacity for repair of NNK-induced sister chromatid exchange (Wei et al., 1996; Lei et al., 2002). By analyzing the interaction of the polymorphisms with the confounding factor of smoking in a logistic regression model, we tested for a possible difference in the effect of each polymorphism in nonsmokers or current smokers. For the APE1-141T/G polymorphism, using the homozygous TT genotype as the reference group, we observed a pronounced protective effect among current smokers with the GG genotype (OR=0.40; 95% CI, 0.18-0.89; *p*=0.02), but no such protective effect was found in nonsmokers. The XRCC1 399Arg/Gln showed a harmful effect with an OR=1.64, there is no significant difference. However, no association between the other three polymorphisms and the risk of developing NPC in either nonsmokers or current-smokers was observed in this study (Table 4).

Table 4. Distribution of Genotypes and ORs for NPC Stratified by Smoking Habit

	Non-smokers			Current-Smokes		
	Case/Control	OR(95%CI) ^a	P(value)	Case/Control	OR(95%CI) ^a	P(value)
OGG1Ser326Cys						
Ser/Ser	9/16	1.00 (reference)		18/22	1.00 (reference)	
Ser/Cys	48/63	1.35(0.55-3.33)	0.51	45/62	0.89(0.43-1.84)	0.75
Cys/Cys	33/58	1.01(0.41-2.54)	0.98	34/40	1.04(0.48-2.25)	0.92
XRCC1Gln399Arg						
Arg/Arg	52/71	1.00(reference)		37/63	1.00 (reference)	
Arg/Gln	35/54	0.89(0.51-1.54)	0.67	48/50	1.64(0.93-2.88)	0.09
Gln/Gln	3/12	0.34(0.09-1.27)	0.1	12/11	1.86(0.75-4.63)	0.18
APE1 pro-141T/G						
TT	26/50	1.00 (reference)		31/30	1.00 (reference)	
TG	49/60	1.57(0.86-2.88)	0.14	51/60	0.82(0.44-1.54)	0.54
GG	15/27	1.07(0.49-2.35)	0.87	14/34	0.40(0.18-0.89)	0.02*
APE1Asp148Glu						
Asp/Asp	25/55	1.00 (reference)		39/53	1.00 (reference)	
Asp/Glu	45/66	1.50(0.82-2.75)	0.19	42/55	1.04(0.58-1.85)	0.9
Glu/Glu	15/18	1.83(0.80-4.22)	0.15	16/16	1.36(0.61-3.05)	0.46

^aAdjusted for age and family history of cancer; **p* < 0.05

Table 5. NPC Risk in Individuals Homozygous for More than One Variant "Deleterious" Allele

Homozygous variant alleles per individual	Case n(%)	Control n (%)	Association OR (95% CI) ^a	<i>p</i>
All cases analyzed	231(100%)	300(100%)		
0	35(15.15%)	67(22.33%)	1.00(reference)	
1	103(44.59%)	148(49.33%)	1.33(0.82-2.15)	0.24
2	93(40.26%)	85(28.34%)	2.09(1.27-3.47)	0.004*

Accumulation of variant alleles that indicated a trend for NPC risk when tested separately:XRCC1 399Gln + APE1 148Gln; a Adjusted for age, gender, smoking status, and family history of cancer; **p* < 0.05

Combination of Variants and NPC Risk

More than one gene variant occurred in a considerable number of individuals, when comparing the incidence of the different polymorphisms in the study population. Therefore, NPC risk was analyzed for those individuals who were homozygous for more than one variant allele by calculating the adjusted ORs for specific combinations. The combination of gene variants was concentrated in individuals who were homozygous for the variant alleles. When compared with heterozygous individuals, these individuals usually exhibited stronger effects of these alleles. All individuals who have no homozygous variant allele for these genes were defined as The reference population. Though the variant allele of OGG1 326Cys, XRCC1 399Gln and APE1 148Glu showed a deleterious effect with an OR of 1.04, 1.55 and 1.62, the OR of OGG1 326Cys is no significant (OR=1.04). So, we analyzed the simultaneous incidence of the other two potential deleterious alleles, i.e, XRCC1 399Gln and APE1 148Gln, which exhibited augment ORs for the single polymorphisms (Table 5). As shown in the table, ORs calculated for this combination, which own two gene variants were significantly added for all cases (OR=2.09; 95% CI: 1.27-3.47; *p*=0.004).

Discussion

Nasopharyngeal carcinoma (NPC), a prevalent tumor in southern China and southeast Asia, is found with the highest incidence rate in head and neck cancers and has an extremely poor prognosis. It is difficult to diagnose

at the early stage because initial signs and presenting symptoms of NPC are often nonspecific and confusing, leading to a delay in treatment (Skinner et al., 1991). In order to identification of new potential susceptibility risk factors for the prevention, early detection and improving the survival rate of NPC is of utmost importance.

An increasing body of evidence suggests that oxidative DNA damage is a driving force for carcinogenesis, aging and other human pathological conditions (Loft et al., 2006; Hatt et al., 2008). As one of the DNA repair pathways, the BER pathway removes various forms of base damage via a number of coordinated sequential reactions that detect and process the damage resulting from reactive oxygen species, hydroxylation, and other cellular processes (Krokan et al., 2000; Hoeijmakers, 2001; Petermann et al., 2006). Therefore, genetic polymorphism in BER genes may influence individual variations in DNA repair capacity, which may be associated with risk of developing lung cancer. In this study we evaluated the relation between sequence variants in three BER genes (XRCC1, OGG1 and APE1) and NPC risks. To the best of our knowledge, this is the first case-control study on the relation among these three BER SNPs and the risk of NPC.

The human XRCC1 gene has 17 exons (with spans~31.9 kb) and is located at chromosome 19q13.2. The XRCC1 gene is an important component of the BER pathway and fixes base damage and DNA single strand breaks caused by ionizing radiation and alkylating agents. A meta-analysis of XRCC1 399Gln genotype showed that which increased NPC risk under the co-dominant model among all subjects (Huang et al., 2011). Even though devoid of any known enzymatic activity, XRCC1 is thought to act as a scaffold protein, play a coordinating role for consecutive stages of the BER system (Ladiges, 2006). The XRCC1 Arg399Gln polymorphism is located within the XRCC1 BRCA1 carboxyl-terminal domain (BRCT I) and is hypothesized to have functional significance because it is located within a well-conserved region and encodes a nonconservative amino acid change. However, studies examining its relation with markers of DNA damage or DNA repair function have yielded mixed results, with some studies showing a positive relation (Duell et al., 2002; Qu and Morimoto, 2005) and others observing no relation with the variants (Palli et al., 2001; Pastorelli et al., 2002; Tuimala et al., 2002; Hu et al., 2005; Leng et al., 2005; Laantri et al., 2011). In present study we found homozygous variants of XRCC1 Arg399Gln had observably associations with NPC risk. Our data clearly showed that the homozygous Gln/Gln and Arg/Gln genotype significantly increase NPC risk compared with the homozygous TT genotype. Further Chi-square test analyses revealed that observably boost nasopharyngeal carcinoma risk was associated with the XRCC1 399Gln allele, compared with the Arg allele (OR=1.55). In accord with our finding, a meta-analysis of XRCC1 Arg399Gln collecting from 22 reports were included with 1644 cases and 1678 controls revealed that an increased NPC risk for Arg399Gln variant (Huang et al., 2011). whereas Yun Cao et al. and En-Yu Cho et al. had reported no significant association was observed between XRCC1 Arg399Gln polymorphism and NPC

(Cho et al., 2003; Yun et al., 2006). Our result is generally consistent with previous findings for lung cancer (Park et al., 2002) and head and neck cancer (Sturgis et al., 1999). However, there are again conflicting data in the literature. For example, this polymorphism has previously been shown to be protective for bladder cancer (Stern et al., 2001), pancreatic adenocarcinoma (Duell et al., 2002), and gastric cardia cancer (Ratnasinghe et al., 2004). The conflicting results may stem from the complexity etiology of cancer with regard to exposure to carcinogens, DNA repair genotypes or other genetic factors, and the small sample size.

The 8-Oxoguanine DNA glycosylase 1 (OGG1) gene, which is a DNA repair gene whose protein product is involved in base excision repair (BER) pathway and that is responsible for the repair of 7,8-dihydro-8-oxoguanine (8-OHG), which is the most important lesion resulting from reactive oxygen species and therefore has been extensively studied in vitro (OGG1 deficient cell lines) and in vivo (knockout mice; Hirano, 2008) (Karahalil et al., 2012) is located at chromosome 3p26.2, a region that frequently shows loss of heterozygosity in several human cancers (Campalans et al., 2005; Tudek, 2007). In large-scale studies, OGG1 Ser326Cys polymorphism has a significant impact on lung cancer risk. OGG1 Ser326Cys polymorphism could be the promising biomarker of orolaryngeal, lung and bladder cancer susceptibility (Park et al., 2002). En-Yu Cho et al. reported OGG1 326Cys showed a detrimental effect of NPC risk (Cho et al., 2003). But others observing show no relation with the variants and NPC (Laantri et al., 2011). In this study, OGG1 326Cys showed a deleterious effect of NPC risk, but consistent with most of the previous studies, we also did not observe any significant association between OGG1 Ser326Cys and NPC.

The APE1 gene consists of five exons and four introns with a 2.21-kb span., which is located at chromosome 14q11.2-q12 and encodes a 317 amino acid protein. It is the essential enzyme in the BER pathway, which is the primary mechanism for the repair of endogenous DNA damage resulting from cellular metabolisms including those resulting from reactive oxygen species, methylation, deamination, and hydroxylation (Hoeijmakers, 2001). In addition to its role in DNA repair, The APE1 is involved in both BER and regulation of gene expression as a redox co-activator of different transcription factors, such as p53 NF- κ B, Myb, HIF-1 α , HLF, PAX and AP-1 (Tell et al., 2005). There are a total of 18 polymorphisms had been reported in APE1, but the most extensively studied polymorphism is a T to G transversion, Asp148Glu (rs3136820, T1349G). This polymorphism has shown that the G allele is associated with an increased mitotic delay after exposure to ionizing radiation (Hu et al., 2001; Xi et al., 2004). The polymorphism of APE1 (Asp148Glu and -141T/G) has been massive reported in lung cancer and breast cancer, but it rarely studied in NPC. In the current study we demonstrated that the APE1 pro-141G/G polymorphism was associated with a decrease nasopharyngeal carcinoma (NPC) risk, but there is no significant difference ($p=0.07$). In addition, we found some stratified variables may influence the NPC risk with

APE1pro -141T/G polymorphism. Using the homozygous TT genotype as the reference group of APE1pro-141T/G polymorphism. Our data showed that variant genotypes were significantly associated with a decreased risk among current smokers with NPC (Table 4). It is suggested that the APE1 promoter polymorphism only had salutary effect on the risk of current-smokers instead of non-smokers. It is possible that the variant protein is associated with increased repair activity and that this increase is influenced by gene environment interaction (Li et al., 2011). However, few report association of APE1 polymorphism with NPC risk. In the present study, compared with those harboring the 148Asp/Asp genotype, that individuals with 148Glu/Glu genotype had a higher slight but no significant increased risk of NPC.

Numerous genes involved in DNA repair exist as multiple genetic variants, which may have additive effects on DNA repair activity and nasopharyngeal carcinoma risk. In present study we analyzed the impact of allele combinations on nasopharyngeal carcinoma risk. There are several studies where the joint effects of more than one variant allele were investigated, mainly in bladder, breast cancer and cervical cancer (Hu et al., 2002; Smith et al., 2003; Shen et al., 2003; Farkasova et al., 2008). We observe associations between DNA Base-excision Repair Genes (XRCC1, OGG1 and APE1) polymorphism and NPC risk. Data obtained from this study suggest a potential gene-gene interaction among the variant alleles of XRCC1 399Gln and APE1 1481Gln, which significantly increased NPC risk. These results suggest that specific gene-gene interactions within one repair pathway are factors affecting nasopharyngeal carcinoma risk. As the functional impact of a single variant is low, the interaction of several variant proteins with slightly increased or reduced functional activity may be necessary to significantly affect DNA repair activity and ultimately to affect cancer risk. Given the great variety of genotype combinations, only a limited number of individuals with a specific genotype combination could be studied in our cohort. The results from our study should therefore be interpreted with caution until our findings are reproduced and/or be confirmed in a larger study.

In summary, This is the first study to focus on the association between DNA Base-excision Repair Genes (XRCC1, OGG1 and APE1) polymorphism and NPC risk. More than one gene variant significantly increased the risk of NPC, but APE1-141G/G may decrease risk of NPC in current smokers. These findings revealed that BER gene polymorphisms may exert a series of effect on the risk of NPC. Because of uncontrolled biases in the selection of participants and the low penetrance of the common SNPs in NPC susceptibility, it is likely that all of these findings were by chance. Therefore, further larger population-based studies including other BER genes are needed in order to confirm our findings as well as to fully examine the possible relationship between DNA repair gene polymorphisms and NPC risk.

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