

RESEARCH ARTICLE

Distribution and Haplotype Associations of XPD Lys751Gln, XRCC1 Arg280His and XRCC1 Arg399Gln Polymorphisms with Nasopharyngeal Carcinoma in the Malaysian Population

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Abstract

Background: DNA repair pathways play a crucial role in maintaining the human genome. Previous studies associated DNA repair gene polymorphisms (XPD Lys751Gln, XRCC1 Arg280His and XRCC1 Arg399Gln) with nasopharyngeal carcinoma. These non-synonymous polymorphisms may alter DNA repair capacity and thus increase or decrease susceptibility. The present study aimed to determine the genotype distribution of XPD codon 751, XRCC1 codon 280 and codon 399 polymorphisms and haplotype associations among NPC cases and controls in the Malaysian population. **Materials and Methods:** We selected 157 NPC cases and 136 controls from two hospitals in Kuala Lumpur, Malaysia for this study. The polymorphisms studied were genotyped by PCR-RFLP assay and allele and genotype frequencies, haplotype and linkage disequilibrium were determined using SNPstat software. **Results:** For the XPD Lys751Gln polymorphism, the frequency of the Lys allele was higher in cases than in controls (94.5% versus 85.0%). For the XRCC1 Arg280His polymorphism, the frequency of Arg allele was 90.0% and 89.0% in cases and controls, respectively and for XRCC1 Arg399Gln the frequency of the Arg allele was 72.0% and 72.8% in cases and controls respectively. All three polymorphisms were in linkage disequilibrium. The odds ratio from haplotype analysis for these three polymorphisms and their association with NPC was 1.93 (95% CI: 0.90-4.16) for haplotype CGC vs AGC allele combinations. The global haplotype association with NPC gave a p-value of 0.054. **Conclusions:** Our study provides an estimate of allele and genotype frequencies of XRCC1 Arg280His, XRCC1 Arg399Gln and XPD Lys751Gln polymorphisms in the Malaysian population and showed no association with nasopharyngeal cancer.

Keywords: Single nucleotide polymorphism - haplotype - linkage disequilibrium - DNA repair genes - Malaysia

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Introduction

Numerous genetic association studies have recently focused on genetic polymorphisms and cancer risk. These studies attempted to identify the effects of candidate genes in cell-cycle control, carcinogen metabolism and DNA repair. Because of their crucial role in preserving human genome integrity, certain SNPs in these genes that alter the expression and functional properties of the corresponding proteins support the hypothesis of SNPs as genetic risk factors (Hall et al., 2009).

Polymorphisms in DNA repair genes such as Xeroderma pigmentosum group D (XPD) and X-ray cross-complementing group 1 (XRCC1) genes alter the DNA repair capacity by modifying the biological responses to exogenous and endogenous DNA insults, at both cellular and tissue levels, and hence the susceptibility in developing cancer or age-related diseases (Ladiges, 2006;

Manuguerra et al., 2006; Sterpone and Cozzi, 2010).

XPD gene is located in chromosome 19q13.3 (Benhamou and Sarasin, 2002). This gene encodes a protein that participates in at least three crucial cellular mechanisms: a) repair of damaged DNA in nucleotide excision repair (NER) pathway, b) general transcription and c) cell cycle regulation through its interaction with cyclin-activating kinase (CAK), a pivotal activator of cyclin dependent kinases (CDKs) (Cameron et al., 2010; Compe and Egly, 2012). XRCC1 gene located on chromosome 19q 13.2 encodes a protein that acts as a scaffold protein through its interaction with poly (ADP-ribose) polymerase (PARP), DNA polymerase β and DNA ligase III α in base excision repair (BER) pathway (Ladiges, 2006).

Many studies have reported possible links between XPD codon 751, XRCC1 codon 280 and codon 399 polymorphisms with other cancers and nasopharyngeal

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carcinoma (NPC) in previous studies.

Exposure to carcinogens from dietary and other environmental factors could promote formation of DNA adducts that could damage DNA and lead to genomic instability. Hence, DNA repair pathways play an important role in eliminating these DNA adducts and maintaining genomic stability (Manuguerra et al., 2006).

A few studies have associated SNPs in DNA repair genes and risk of NPC (Cho et al., 2003; Cao et al., 2006; Yang et al., 2007; Laantri et al., 2011) which is a tumour arising from epithelial cells that line the surface of nasopharynx (Brennan, 2006). A meta-analysis study conducted by Huang et al, 2011 to evaluate the effects of XRCC1 variants on NPC risk found an association between Arg399Gln polymorphism and risk of NPC. However, no association was found between Arg280His polymorphisms and risk of NPC. NPC has a distinctive ethnic and geographical distribution with incidences of age standardized rates (ASR) is below 1 per 100,000 person-years (Sun et al., 2011). However, NPC was noted to be frequent in certain populations (Jemal et al., 2011). NPC cancer rates in Malaysia, Indonesia, and Singapore have been reported to be the three highest national incidence rates worldwide (Parkin et al., 2005; Jemal et al., 2011). In Malaysia, NPC is among the top ten cancers among males, especially among the Chinese (14.4 per 100,000 population). According to Malaysia Cancer Statistics 2007, the overall incidence rate of NPC in Malaysia was 6.4 per 100,000 population in males and 2.3 per 100,000 population in females (Ariffin and Saleha, 2011). Epidemiological studies suggest that the risk factors of NPC include dietary factors, Epstein-Barr virus (EBV) infection, genetic susceptibility and environmental factors (Yuan et al., 2002; Thompson and Kurzrock, 2004; Gallicchio et al., 2006; Ng et al., 2009; Hashim et al., 2012; Sun et al., 2012; Murthy et al., 2013).

We conducted a molecular epidemiological study to determine the genotype distribution of XPD codon 751, XRCC1 codon 280 and codon 399 polymorphisms and their haplotype association among NPC cases and controls in the Malaysian population. We also sought to determine if there was linkage disequilibrium (LD). In association mapping, LD plays a fundamental role in identifying candidate genes for a particular disease (Maniatis, 2007). This method could provide high-resolution information to narrow down a chromosomal region of interest and refine the location of the disease's candidate gene (Morton, 2005). To our knowledge, no such studies have been conducted here in Malaysia before, which has a distinctively unique ethnic composition of varying ethnic backgrounds, comprising of Malays, Indians and Chinese.

Materials and Methods

Subjects recruited

One hundred and fifty seven NPC cases and 136 controls were recruited in a hospital-based case-control study. Only cases that were histologically confirmed and controls that have no history of any cancer were recruited. Written informed consent was obtained from all subjects. This study was approved by the Medical Research Ethics

Committees of the Ministry of Health, Malaysia and Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Malaysia.

PCR-RFLP assay

Genomic DNA was extracted from 2.0 mL peripheral blood samples using QIAamp® DNA Mini and Blood Mini kit (Qiagen, USA). Polymorphisms of XRCC1 - Arg280His at exon9(rs25489, position 48748252bp), Arg399Gln at exon10 (rs25487, position 48747566bp) and XPD Lys751Gln exon23 (rs13181, position 45854919bp) were detected using a modified polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) assay. The sequences of primers used to identify the polymorphisms of XRCC1 at codon 280 and codon 399 were derived from Cho et al. (2003) and those of XPD codon 751 were from Duell et al. (2000).

For Lys751Gln polymorphism, a final volume of 25 μ l of PCR reaction mixture which contained ~ 100 ng of DNA isolated from whole blood (quantified with nanophotometer), 5 μ l of 5X PCR buffer, 2.0 μ l of MgCl₂ (25mM), 0.5 μ l of the mixture of dNTP (10 mM), 0.5 μ l of forward and reverse primers (10 μ M), 0.625 U (5 U/ μ l) of GoTaq® DNA polymerase and nuclease free-water (Promega, USA) was prepared. Initial denaturation was at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 40 s, annealing at 56.2°C for 30 s, extension at 72°C for 30 s and final extension at 72°C for 5 min. The PCR products were subjected to restriction digestion for 1 hour at 37°C by MboII followed by 15 minutes at 65°C. All digestion products were resolved on 3.0% (w/v) agarose gel. For Lys/Lys genotype, two bands were observed at 102 bp and 82 bp, for Lys/Gln genotype, three bands were observed at 184 bp, 102 bp and 82 bp and a single band at 184 bp for Gln/Gln genotype.

In genotyping for Arg280His polymorphism, a final volume of 25 μ l of PCR reaction mixture which contained ~ 100 ng of DNA isolated from whole blood, 5 μ l of 5X PCR buffer, 1.5 μ l of MgCl₂ (25 mM), 0.125 μ l of the mixture of dNTP (10mM), 0.25 μ l of forward and reverse primers (10 μ M), 0.625 U (5 U/ μ l) of GoTaq® DNA polymerase and nuclease free-water (Promega, USA) was prepared. Initial denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 40 s, annealing at 54.9°C for 30 s, extension at 72°C for 40 s and final extension at 72°C for 10 min. The PCR products were subjected to restriction digestion overnight at 37°C by RsaI for codon 280. All digestion products were resolved on 3.0% (w/v) agarose gel. For Arg/Arg genotype, two bands were observed at 126 bp and 62 bp, for Arg/His genotype, three bands were observed at 188 bp, 126 bp and 62 bp and a single band at 188 bp for His/His genotype.

For Arg399Gln polymorphism, final volume of 25 μ l of PCR reaction mixture which contained ~100 ng of DNA isolated from whole blood, 5 μ l of 5X PCR buffer, 2.0 μ l of MgCl₂ (25 mM), 0.5 μ l of the mixture of dNTP (10mM), 0.5 μ l of forward and reverse primers (10 μ M), 0.75 U (5 U/ μ l) of GoTaq® DNA polymerase and nuclease free-water (Promega, USA) was prepared. The PCR cycling parameters were: initial denaturation at 94°C for 4 min followed by 35 cycles of denaturation

at 94°C for 40 s, annealing at 61°C for 30 s, extension at 72°C for 40 s and final extension at 72°C for 10 min. The PCR products were subjected to restriction digestion for 8 hours at 37°C by MspI for codon 399. All digestion products were resolved on 3.0% (w/v) agarose gel. For Arg/Arg genotype, two bands were observed at 115 bp and 34 bp, for Arg/Gln genotype, three bands were observed at 149 bp, 115 bp and 34 bp and a single band at 149 bp for Gln/Gln genotype.

Ten percent of all the samples for each polymorphism were subjected to DNA sequencing to verify the PCR-RFLP results. For data validation, 10% of each polymorphism was repeated by PCR-RFLP assay.

Statistical analysis

Allele frequencies and genotype frequencies were calculated by using SNPstat software (Sole et al., 2006). Hardy-Weinberg equilibrium (HWE) was tested using goodness-of-fit chi-square test to compare the observed and expected genotype frequencies among cases and controls. Linkage disequilibrium (LD) of the three loci, haplotypes and their frequencies and associations with NPC were determined by using the SNPstat software (Sole et al., 2006).

Results

Table 1 represents allele frequencies and genotype frequencies for all three polymorphisms. The genotype distribution for all three polymorphisms were in accordance with Hardy-Weinberg equilibrium (HWE) for cases and controls, respectively. For XPD Lys751Gln polymorphism, higher allele frequencies for Lys were observed in cases compared to controls (94.46% vs 85.0%); frequency of Gln allele in cases was 6.54% and 15.0% in controls. For XRCC1 Arg280His polymorphism, the frequency of Arg allele was 90.0% in cases and 89.0% in controls while the frequency of His allele was 10.0%

Table 1. Allele Frequencies and Genotype Frequencies for All Three Polymorphisms

Polymorphisms		Cases n=157 (%)	Controls n=136 (%)	p-value ^a
Lys751Gln				
Genotypes	Lys/Lys	133 (87.5)	100 (73.5)	0.21
	Lys/Gln	18 (11.8)	32 (23.5)	
	Gln/Gln	1 (0.7)	4 (3.0)	
Alleles	Lys	286 (94.5)	232 (85.0)	
	Gln	20 (6.5)	40 (15.0)	
Arg280His				
Genotypes	Arg/Arg	125 (80.0)	109 (80.1)	0.54
	Arg/His	30 (19.0)	24 (17.7)	
	His/His	1 (1.0)	3 (2.2)	
Alleles	Arg	284 (90.0)	242 (89.0)	
	His	32 (10.0)	30 (11.0)	
Arg399Gln				
Genotypes	Arg/Arg	82 (54.0)	73 (53.7)	0.24
	Arg/Gln	56 (37.0)	52 (38.2)	
	Gln/Gln	15 (1.0)	11 (8.1)	
Alleles	Arg	220 (72.0)	198 (72.8)	
	Gln	86 (28.0)	74 (27.2)	

^aHardy-Weinberg equilibrium

Table 2. Linkage Disequilibrium between All Three Polymorphisms (D' value).

	Lys751Gln	Arg280His	Arg399Gln
Lys751Gln	-	0.6014	0.0742
Arg280His	-	-	0.8966
Arg399Gln	-	-	-

Table 3. Haplotype Analysis for All Three Polymorphisms and Association with NPC

Lys751Gln/ Arg280His/ Arg399Gln	Case (2N=314)	Control (2N=272)	OR (95% CI)	p-value
AGC	180	154	1	-
AGT	84	60	0.90 (0.60-1.35)	0.62
AAC	30	29	1.13 (0.62-2.07)	0.69
CGC	14	26	1.93 (0.90-4.16)	0.094
CGT	4	1	3.68 (0.63-21.4)	0.15

Global haplotype association p-value=0.054

in cases whereas in controls it was 11.0%. For XRCC1 Arg399Gln gene, the frequency of Arg allele in cases was 72.0% whereas in controls it was 72.8% while the frequency of Gln allele in cases was 28.0% and 27.2% in controls. Table 2 exhibits the linkage disequilibrium between all three polymorphisms. Table 3 represent results from five possible haplotypes formed from the three polymorphisms. The odds ratio from haplotype analysis for these three polymorphisms and their association with NPC was 1.93 (95% CI: 0.90-4.16) for haplotype CGC vs AGC allele combinations. Global haplotype analysis for all three polymorphisms studied and their associations with NPC was very close to the statistically significant level (p=0.054).

Discussion

For XPD Lys751Gln polymorphism, the wild-type allele is lysine (Lys) at codon 751 and variant allele is glutamine (Gln). This study result is consistent with previous study by Yang et al. (2007) that reported higher allele frequencies for Lys in cases compared to controls (92.0% vs 87.0%) in Sichuan population (Yang et al., 2007). The frequency of Gln allele in cases was 8.0% and 13.0% in controls.

For XRCC1 Arg280His polymorphism, the wild-type allele is arginine (Arg) at codon 280 and the variant allele is histidine (His). Allele frequency for Arg and His observed in this study are consistent with previous NPC studies. A study conducted in North Africa reported that Arg allele frequency in cases was 88.0% and in controls it was 89.0% while His allele frequency in cases was 12.0% and 11.0% in controls (Laantri et al., 2011). Cho et al. (2003) reported that among Taiwanese in China, Arg allele frequency in cases was 91.0% and 88.0% in controls while His allele frequency was 9.0% in cases and 12.0% in controls (Cho et al., 2003).

For XRCC1 Arg399Gln polymorphism, the wild-type allele is arginine (Arg) at codon 399 and the variant allele is glutamine (Gln). Allele frequency for Arg and Gln observed in this study are consistent with previous NPC

studies. A study by Cho et al. (2003) observed XRCC1 Arg399Gln polymorphism and risk of NPC among Taiwanese in China. Arg allele frequency in cases was 0.71 whereas in controls was 0.73. Gln allele frequency in cases was 0.29 and in controls was 0.27. In the Cantonese population, Arg allele frequency in NPC patients was 75.0% whereas in controls it was 74.0%. Gln allele frequency in cases was 25.0% and 26.0% in controls (Cao et al., 2006). A similar study conducted in North Africa reported that Arg allele frequency in cases was 72.0% and 75.6% in controls while the Gln allele frequency in cases was 28.0% and 24.4% in controls (Laantri et al., 2011). However, in the study of Li et al. (2013) a significant difference was found between cases and controls.

The genotype distribution for all three polymorphisms was in accordance with Hardy-Weinberg equilibrium (HWE) for cases and controls, respectively. This implicitly accepts that all the assumptions of HWE are approximately met. Hardy-Weinberg model is a null model, which predicts based on a simplified situation where no biological processes are acting and genotype frequencies are a result of random combination (Hamilton, 2009). Ryckman and Williams (2008) stated that HWE principles are commonly applied to detect disease susceptibility loci in genetic association studies. HWE model is used in population genetics field to describe the distribution of genotypes in a population in a steady-state (a binomial distribution) thus various genotype frequencies could be determined by using gene frequencies found in the gene pool of specific populations. This may narrow down certain genotypes that are unique to a particular population. Consequently, this may give a clue to identify candidate gene for NPC carcinogenesis.

Strong LD was observed between Arg399Gln and Arg280His polymorphisms and moderate LD exists between Lys751Gln and Arg280His polymorphisms in our study. Linkage disequilibrium (LD) across one or a few gene regions has been observed in many other studies. However, LD is very variable within and among loci and populations (Gabriel et al., 2002). The presence of LD between all three polymorphisms studied among Malaysian population may differ in LD patterns in different populations. This may give a clue to identify location of the NPC's candidate gene as this cancer is known to have a distinctive ethnic and geographical distribution.

Haplotype analysis results from this study suggest the chromosome 19q13.2-13.3 region may be associated with susceptibility to NPC (OR=1.93 (95% CI: 0.90-4.16) for haplotype CGC vs AGC allele combinations); XPD and XRCC1 genes are located at chromosome 19q13.2-13.3, and thus the combined XPD and XRCC1 genotypes may be significant with regard to specific haplotypes in contributing to increased disease risk (Mohrenweiser et al., 1989). This region has been associated with several other cancers including breast, lung and ovarian cancers (Mohrenweiser et al., 1989; Nexu et al., 2003; Yin et al., 2008).

In conclusion, our study provides an estimate of allele and genotype frequencies of XRCC1 Arg280His, XRCC1 Arg399Gln and XPD Lys751Gln polymorphisms in the Malaysian population. We observed higher

allele frequencies for Lys allele for XPD Lys751Gln polymorphism in cases compared to controls. All three polymorphisms studied were in linkage disequilibrium. The odds ratio from haplotype analysis for haplotypes CGC vs AGC allele combinations and their association with NPC was 1.93 (95% CI: 0.90-4.16) indicating the chromosome 19q13.2-13.3 region may be associated with susceptibility to NPC

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