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Reseach Article

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Association of *hOGG1* Ser326Cys, ITGA2 C807T and TNF-A -308G>A Polymorphisms with the Risk of NPC

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Abstract

Background: Nasopharyngeal carcinoma (NPC) is a rare form of cancer. NPC is the 4th most common cancer in Malaysia and the incidence rate for Malaysian Chinese is exceptionally high compared to other races. NPC is considered as a relatively radiosensitive tumor and patients diagnosed at early stages tend to survive longer compared to those who with advanced disease. Given that early symptoms of NPC are non-specific, and that the nasopharynx is relatively inaccessible, less invasive screening methods such as biomarker screening might be the key to improve NPC survival and management.

Methodology: A matched case-control study was conducted to investigate the effect of *hOGG1* Ser326Cys, ITGA2 C807T and TNF-α -308G>A polymorphisms on the risk of nasopharyngeal carcinoma and all-cause survival. *hOGG1* gene encodes for a DNA glycosylase, a protein that is involved in DNA repair. ITGA2 is the alpha subunit of the transmembrane receptor integrin and is mainly responsible for cell-cell and cell-extracellular matrix interaction. TNF-α is a cytokine that is released by immune cells during inflammation. Restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) was used to process DNA genotyping studies involving all aforementioned gene polymorphisms. Conditional logistic regression was used for the analysis of NPC risk on gene polymorphisms, controlling for cigarette smoking, salted fish and alcohol consumption.

Results: Conditional logistic regression showed that NPC cases were more likely to ever consume salted fish during childhood compared to controls (OR=1.80, 95% CI=1.32-2.46, p<0.01). Individuals with previous smoking history were also at higher risk of NPC (OR=1.96, 95% CI=1.37-2.81, p<0.01). No significant difference was found between NPC cases and controls for alcohol consumption. No significant association was observed between *hOGG1* Ser326Cys, ITGA2 C807T, TNF-α -308G>A polymorphisms with NPC risk.

Conclusion: None of the aforementioned polymorphisms showed significant association in increasing NPC risk individually.

Keywords: *hOGG1* Ser326Cys; ITGA2 C807T; TNF-A -308G; NPC; Polymorphism

Introduction

Nasopharyngeal carcinoma (NPC) is a rare cancer commonly develops around the lateral wall of nasopharynx. The NPC annual frequency is less than 1 per 100,000 populations in most parts of the world [1]. But there are exceptions where Chinese living in Guangdong province of Mainland China and South-East Asia as well as natives from Arctic region (Alaska and Greenland) experience a much higher NPC risk compared to the rest of the world [2]. NPC is the 4th most common cancer in Malaysia [3]. Given the increasing number of NPC cases per year and the fact that many cases are diagnosed at an advanced stage [4], it is important to find ways of ensuring early diagnosis and prompt treatment. This is a challenge as the nasopharynx is not easily visualized and accessed. Finding potential biomarkers for NPC screening is one of those ways in which a susceptible population could be identified early, which will then help physicians in early detection and treatment of NPC.

Several environmental factors have been shown to be associated with increased risk of NPC. EBV infection [5], consumption of salted fish at an early age [6,7]. Constant occupational exposure to wood dust [8] and long-term cigarette smoking [9]. are examples of risk factors implicated in NPC carcinogenesis. In addition, normal cellular metabolic processes are also capable of producing hydroxyl radicals that can cause oxidative damage to DNA [10]. The role of reactive oxygen species (ROS) has been implicated in different stages of tumorigenesis [11]. One of the most common mutagenic byproduct resulting from oxidative damage is 8-oxo-7,8-dihydroguanine (8-oxoG), which is a G:C to T:A transversion causing agent [12]. Human 8-oxoguanine DNA glycosylase 1 (*hOGG1*) is the primary enzyme responsible for excision of 8-oxoG through base excision repair (BER) [13]. Short-patch BER removes 8-oxoG through the action of DNA glycosylase and AP lyase followed by the re-synthesis of DNA by DNA polymerase β [14].

As it is implicated above, *hOGG1* protein is the initiator of BER because of its ability to identify the damaged base. It is thus possible that the presence and functionality of *hOGG1* protein will directly affect the level of BER activity. The work of Kohno et al. [15] supported such postulation by showing that the ability of *hOGG1* protein to suppress

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spontaneous mutagenesis varied among polymorphic genotypes in an *E. coli* strain. In the study, a polymorphism in exon 7 of *hOGG1* gene resulted in a serine to cysteine amino acid change. *hOGG1*-Ser^{326} (wild type) ability to suppress mutagenesis is stronger than *hOGG1*-Cys^{326} [15]. Several studies on association of *hOGG1* Ser326Cys polymorphism with various cancers have substantiated these findings by demonstrating that *hOGG1*-Cys326 conferred higher risk of cancer [16-19]. For NPC, the findings showed inconsistent results. Cho et al. [20] have demonstrated that polymorphism of *hOGG1* gene (Ser326Cys) is associated with altered risk of NPC (OR=1.6, 95% CI=1.0-2.6). Laantri et al. [21] on the other hand showed that polymorphism of *hOGG1* gene (Ser326Cys) was not associated with NPC risk (OR=1.22, 95% CI=0.77-1.90) [21].

Apart from DNA repair, transmembrane receptor integrins are also important in carcinogenesis. These transmembrane glycoproteins are mainly responsible for mediating cell-cell and cell-extra cellular matrix (ECM) interactions [22]. Integrins are heterodimeric and consist of 2 transmembrane glycoproteins (α and β) that are non-covalently bound together [23]. Integrins are involved in almost every aspect of carcinogenesis, from cell differentiation, cell proliferation, and angiogenesis to metastasis [24-26]. Integrin α2 is a collagen receptor that is mainly expressed on platelets and epithelial cells [27]. Integrin α2β1 was found to facilitate integrin-mediated attachment to collagen type I during the metastasis of breast cancer cells to bone [28]. The binding of collagen I to α2β1 integrin was also shown to promote the malignant phenotype of pancreatic ductal adenocarcinoma [29]. There is indication that ITGA2 C807T polymorphism, a silent nucleotide change in position 807 (TTC/TTT, rs1126643), might be associated with increased susceptibility to cancer. This hypothesis is supported by the findings of significant associations between ITGA2 C807T with increased risk of colorectal and breast carcinoma [30,31].

Chronic or recurrent inflammation is an endogenous process in human body that was observed to have a causative role in promotion and progression of human tumours [32]. Numerous mediators released during dysregulated chronic inflammation have been linked to induce cell growth and invasion at the same time promoting mutagenesis and angiogenesis [33]. Tumor necrosis factor alpha (TNF-α) is one of the earliest cytokine produced in inflammatory process [34]. TNF-α is the key mediator for inflammation and is secreted mainly by macrophages [35]. TNF-α protein was observed to be an endogenous tumor promoter *in vivo* [36]. Significant associations were found between the variant -308A allele and susceptibility to malignant tumors [32,35,37-39] Although ITGA2 C80T and TNF-α -308G>A polymorphisms are not extensively studied in relation to NPC, variant genotypes of both polymorphisms have been shown to carry increased risk for a number of cancers. Together with the evidence indicating the possible roles for both genes in carcinogenesis, ITGA2 C807T and TNF-α-308G>A could potentially serve as screening targets in the early detection of NPC.

No study to date, has explored the association of *hOGG1* Ser326Cys, ITGA2 C807T and TNF-α-308G>A polymorphism with NPC risk in Malaysia. In the present study, we describe results from a case-control study conducted in Malaysia (300 cases and 562 controls) investigating role of *hOGG1* Ser326Cys polymorphism and NPC susceptibility.

Materials and Methods

Ethics statement and study design

This study was approved by the Medical Research Ethics Committee of the Ministry of Health, Malaysia (Appendix) and funded by the Science Fund, Ministry of Science, Technology and Innovation (MOSTI; Project code: 04-11-08-625FR).

Study population

The design of a matched case-control study was selected in the present study because the incidence of NPC varies substantially between different subjects in terms of age, gender and ethnicity. The cases in the present study were NPC patients who were admitted to the public hospitals while matching controls were recruited from the same hospitals where the cases have been recruited. NPC patients were recruited from the Departments of Radiotherapy and Oncology while controls were from the general medical clinics and wards. Inclusion criteria for cases include those who were histologically confirmed NPC patients diagnosed from year 2009 onwards in two public hospitals and above 18 years of age. Individuals without prior history of cancers were recruited as controls. All controls were matched to the cases by age (± 3 years), gender and ethnicity. Personal information on demographic factors, smoking status, alcohol and salted fish consumption were collected at recruitment. Smoking status and alcohol consumption were divided into 2 categories: Never/ever smoked and never/ever consumed alcohol. For salted fish consumption, classification used was never/ever consumed salted fish during childhood. Written informed consent was obtained from eligible cases and controls prior to the enrollment into the study. We assumed the exposure rate of TNF-α-308G>A polymorphism in controls at 18% [40] and estimated that this polymorphism could increase NPC risk by 100%. Using the formula adopted by Schlesselman [41] on matched case-control study, with two-sided alpha level of 0.05, 229 matched pairs was needed to attain a power of 90% to detect a 100% increase in NPC risk in the proportion of patients exposed with TNF-α-308G>A polymorphism. 300 histologically confirmed NPC cases and 562 controls were available for analysis at the end of the study.

DNA extraction and genotyping

2 ml of venous blood was obtained from every research subject. Fresh blood was immediately placed into an EDTA-coated vacutainer. Filled EDTA tube was stored on ice and transferred back to laboratory in the university to be processed. DNA was extracted from the blood using QIAamp® DNA mini kit (QIAGEN, Venlo, Netherlands) and immediately stored in minus 20°C freezer until further use. *hOGG1* Ser326Cys, ITGA2 C807T and TNF-α-308G>A polymorphisms were assessed by using RFLP-PCR (Restriction Fragment Length Polymorphism). Forward and reverse primers used for the PCR were listed in Table 1.

For *hOGG1* Ser326Cys polymorphism, the outcome of the PCR was a 302 bp product. The composition of a total 25 µl PCR reaction was 12.5 µl of GoTaq® Green Master Mix (Promega, Wisconsin, USA), 0.5 µl of each primer (from working concentration of 10 µm), 0.5 µl of genomic DNA, and the remaining was topped up with nuclease free water. The PCR thermal profile used was initial denaturation at 95°C for 5 min, 32 cycles of 95°C for 30s, followed by 63°C for 30s and 72°C for 30s, then ended with final extension of 72°C for 5 min. PCR yield from previous PCR was then digested by the restriction enzyme *Fnu4HI* (New England Biolabs, Ipswich, USA). After the digestion, homozygous

Polymorphisms	Forward primer (5'-3')	Reverse primer (5'-3')
hOGG1 Ser326Cys (rs1052133)	CTT CCA CCT CCC AAC ACT GTC AC	GTG CCT GGC CTT TGA GGT AGT C
ITGA2 C807T (rs1126643)	GTG TTT AAC TTG AAC ACA TAT	ACC TTG CAT ATT GAA TTG CTT
TNF-α -308G>A (rs1800629)	AGG CAA TAG GTT TTG AGG GCC AT	ACA CTC CCC ATC CTC CCT GCT C

Table 1: Sequence of forward and reverse primers used in RFLP-PCR.

Ser/Ser showed only a single 302 bp product while homozygous Cys/Cys was fully digested into two different products that were 186 bp and 116 bp in size. Samples were identified as heterozygous Ser/Cys if 3 products of different sizes appeared in the gel.

For ITGA2 C807T polymorphism, 115 bp product was generated at the end of the PCR protocol. The PCR composition used was 25 µl PCR reaction consisting of 12.5 µl of GoTaq® Green Master Mix (Promega, USA), 1.0 µl of each primer (from working concentration of 10 µm), 0.5 µl of genomic DNA, and the remaining component was nuclease free water. The PCR thermal profile adopted was as follows: 95°C for 5 min, 35 cycles each of 95°C for 30 s, followed by 55°C for 30 s and 72°C for 30 s, with final extension of 72°C for 5 min. The resulting PCR products were digested by restriction enzyme *TaqI* (New England Biolabs, Ipswich, US). Homozygous CC was fully digested into 2 products that were 92 bp and 23 bp in size after the excision by the restriction enzyme. For homozygous TT, no digestion occurred and only a single 115 bp product was visible. All 3 products of different sizes were observed for heterozygous CT.

For TNF-α -308G>A polymorphism, 117 bp PCR product spanning the promoter region of TNF-α from -238 to -308 was generated using the respective forward and reverse primers. The PCR composition used was 25 µl PCR reaction consisting of 12.5 µl of GoTaq® Green Master Mix (Promega, USA), 0.5 µl of each primer (from working concentration of 10 µm), 0.5 µl of genomic DNA, and the remaining component was nuclease free water. The PCR thermal profile used was as follows: 95°C for 5 min; 95°C for 30s, 62°C for 30s and 72°C for 30s for 35 cycles; followed by 72°C for 5 min. Restriction enzyme *NcoI* (New England Biolabs, Ipswich, US) was utilized to excise the PCR products from the previous PCR run. Gel image of homozygous G/G showed only 2 products with 95 bp and 22 bp in size. Homozygous A/A was identified when the gel image showed a single product at 117 bp while samples showing all 3 products were identified as heterozygous G/A.

All digested PCR products were visualized under UV light after separation using electrophoresis on 3% ethidium bromide stained agarose gel. For quality control, 10% of the total PCR products were sent for DNA sequencing to confirm the results of RFLP-PCR for every polymorphism.

Statistical analysis

Relative frequencies were used to describe variables studied including socio-demographic and exposure data using SPSS version 21. Deviation from Hardy Weinberg equilibrium (HWE) was tested using Court Lab Calculator on controls [42]. Conditional logistic regression (STATA 10) was used to estimate adjusted odds ratio (ORs) and 95% confidence interval (CI) for NPC risk, controlling for cigarette smoking, alcohol and salted fish consumption during childhood. A *p*-value less than 0.05 was considered as statistically significant.

Results

Characteristics of the study population

300 NPC cases and 562 controls were available for analysis in the present study. Mean age (± SD) for NPC cases and controls were 52.8 (± 10.9) and 53.5 (± 11.0) years respectively while the male to female ratio was roughly 3:1. Majority of the subjects were ethnic Chinese (71.0% NPC cases vs. 70.3% control) while 28.0% of NPC cases and 28.8% of controls were Malays. The socio-demographic characteristics together with the distribution of cigarette smoking, alcohol consumption, salted fish consumption during childhood are shown in Table 2.

Characteristics	Cases (%) N= 300	Control (%) N= 562	χ^2 (p-value)
Age (years)			
Mean (SD)	52.8 (10.9)	53.5 (11.0)	0.28[b]
Gender, N (%)			
Male	232 (77.3)	425 (75.6)	0.32 (0.57)
Female	68 (22.7)	137 (24.4)	
Ethnicity, N (%)			
Chinese	213 (71.0)	395 (70.3)	0.27 (0.97)
Malay	84 (28.0)	162 (28.8)	
Others	3 (1)	5 (0.9)	
Cigarette smoking, N (%)			
Never	146 (48.7)	358 (63.7)	18.21[a] (0.00)
Ever	154 (51.3)	204 (36.3)	
Alcohol consumption, N (%)			
Never	161 (53.7)	366 (65.1)	10.81[a] (0.00)
Ever	139 (46.3)	196 (34.9)	
Salted Fish consumption during childhood, N (%)			
Never	103 (34.3)	270 (48.0)	14.98[a] (0.00)
Ever	197 (65.7)	292 (52.0)	

[a]p≤0.01
[b]p-value from paired t-test

Table 2: Characteristics of the study population.

For *hOGG1* Ser326Cys polymorphism, 15.7% of NPC cases were Ser/Ser carriers, 53.7% of them were with heterozygous Ser/Cys and the remaining 30.6% were having Cys/Cys genotype. The proportion of homozygous Ser/Ser carriers was slightly higher in the controls (19.0%) while 50.2% and 30.8% of the controls were having heterozygous Ser/Cys and homozygous Cys/Cys genotypes, respectively. For ITGA2 C807T polymorphism, 57.0% of NPC cases were carrying homozygous C/C genotype whereas 34.3% and 8.7% of the remaining cases were heterozygous C/T and homozygous T/T carriers, respectively. For the controls, 51.4% carried the C/C genotype while 38.6% and 10.0% of the remaining controls were heterozygous C/T and homozygous T/T carriers, respectively. For TNF-α -308G>A polymorphism, 81.3% of NPC cases were carriers of homozygous G/G genotype while the remaining cases were carriers of the G/A and A/A genotypes (18.7%). For the controls, the percentage of homozygous G/G carriers was slightly higher (84.7%) while 15.3% of the remaining controls were carrying heterozygous G/A and homozygous A/A genotypes.

Chi-square analysis showed that NPC patients were more likely to ever consume salted fish during childhood compared to controls ($\chi2=14.98$, $p<0.01$). Study participants with previous smoking history were more likely to develop NPC ($\chi2=18.21$, $p<0.01$). Study subjects who ever consumed alcohol were at an increased NPC susceptibility as compared to those who never ($\chi2=10.81$, $p<0.01$).

Allelic and genotypic frequencies of *hOGG1* Ser326Cys, ITGA2 C807T and TNF-α -308G>A polymorphisms with the risk of NPC

The distribution of *hOGG1* Ser326Cys, ITGA2 C807T, TNF-α -308G>A and XPD Lys751Gln polymorphisms in controls did not deviate from Hardy-Weinberg equilibrium (Table 3). Conditional logistic regression showed that NPC cases were more likely to ever consume salted fish during childhood compared to controls (OR=1.80, 95% CI=1.32-2.46, $p<0.01$). Individuals with previous smoking history were also at higher risk of NPC (OR=1.96, 95% CI=1.37-2.81, $p<0.01$). No significant difference was found between NPC cases and controls for alcohol consumption. No significant association was observed between *hOGG1* Ser326Cys, ITGA2 C807T, TNF-α -308G>A polymorphisms with NPC risk (Table 4).

Polymorphisms		Controls (%) N= 562	χ^2 value	p-value
hOGG1				
Genotypes	Ser/Ser	107 (19.0)	0.17	0.68
	Ser/Cys	282 (50.2)		
	Cys/Cys	173 (30.8)		
Alleles	Ser	496 (44.1)		
	Cys	628 (55.9)		
ITGA2				
Genotypes	C/C	289 (51.4)	2.56	0.11
	C/T	217 (38.6)		
	T/T	56 (10.0)		
Alleles	C	795 (70.7)		
	T	329 (29.3)		
TNF-α				
Genotypes	G/G	476 (84.7)	0.05	0.82
	G/A	82 (14.6)		
	A/A	4 (0.7)		
Alleles	G	1034 (92.0)		
	A	90 (8.0)		

Table 3: Allelic and genotypic frequencies of hOGG1 Ser326Cys, ITGA2 C807T, TNF-α -308G>A and XPD Lys751Gln polymorphisms (HWE test).

Variables		Cases (%) N= 300	Controls (%) N= 562	Adjusted[a] OR (95% CI)	*p*-value
hOGG1 Genotypes	Ser/Ser	47 (15.7)	107 (19.0)	1.00	
	Ser/Cys	161 (53.7)	282 (50.2)	1.42 (0.95-2.13)	0.09
	Cys/Cys	92 (30.6)	173 (30.8)	1.28 (0.81-2.02)	0.29
ITGA2 Genotypes	C/C	171 (57.0)	289 (51.4)	1.00	
	C/T	103 (34.3)	217 (38.6)	0.78 (0.56-1.08)	0.13
	T/T	26 (8.7)	56 (10.0)	0.74 (0.43-1.26)	0.26
TNF-α Genotypes	G/G	244 (81.3)	476 (84.7)	1.00	
	G/A + A/A	56 (18.7)	86 (15.3)	1.43 (0.97-2.11)	0.07
Smoking status	Never	146 (48.7)	358 (63.7)	1.00	
	Ever	154 (51.3)	204 (36.3)	1.96 (1.37-2.81)	<0.01[b]
Alcohol consumption	Never	161 (53.7)	366 (65.1)	1.00	
	Ever	139 (46.3)	196 (34.9)	1.42 (0.98-2.05)	0.07
Salted Fish consumption during childhood	Never	103 (34.3)	270 (48.0)	1.00	
	Ever	197 (65.7)	292 (52.0)	1.80 (1.32-2.46)	<0.01[b]

[a]Adjusted for age, gender, ethnicity, cigarette smoking, alcohol and salted fish consumption during childhood.
[b]$p \leq 0.05$

Table 4: hOGG1 Ser326Cys, ITGA2 C807T and TNF-α -308G>A polymorphisms and the risk of NPC.

Discussion

Results from the present study did not show any significant relationship between the aforementioned polymorphisms and the odds of developing NPC. We did however show that cigarette smoking and consumption of salted fish at age younger than 10 were associated with increased odds of NPC. The finding from the present study showed that *hOGG1* Ser/Cys and Cys/Cys genotype carriers increased NPC risk by 1.42-fold (0.95-2.13) and 1.28-fold (0.81-2.02), respectively after adjusting for age, gender, ethnicity, cigarette smoking, alcohol and salted fish consumption during childhood. Judging from the results, lower boundary of the confidence interval for Ser/Cys genotype was very close to the 1 while the upper boundary of 2.13 indicated a more than 100% increase in NPC risk. Similar results were observed in Cho et al. [20] study where Ser/Cys genotype was significantly associated with NPC risk (OR=1.8, 95% CI=1.1-2.9). The finding from Xing et al. [42]. also showed that Cys/Cys genotype significantly increased oesophageal cancer risk by 1.9-fold (95% CI=1.3-2.6). The effect sizes of *hOGG1* Ser326Cys polymorphism in both studies are comparable with the finding from the present study. It is thus possible that with an increase in sample size, *hOGG1* Ser326Cys polymorphism might be significantly associated with NPC risk. There is a possibility of a type II error in this part of the analysis probably due to insufficient power.

hOGG1 Ser326cys polymorphism has been shown to be significantly associated with other cancers, namely nasopharynx [20], esophageal [43], lung [44] and prostate cancer [45]. The ability of wildtype *hOGG1* Ser326 to suppress mutagenesis was found to be stronger than the variant *hOGG1* Cys326 [15]. An *in-vitro* study also reported that *hOGG1* Cys326 protein has lower mutation suppressing activity in human cells compared to its Ser326 counterpart [46]. In addition, homozygous Cys/Cys variant showed a nearly 2-fold lower enzymatic reaction compared to the wildtype homozygous Ser/Serv [47]. In a study involving nitric oxide induced stress condition, *hOGG1* Cys326 variant was found to be more susceptible to oxidation compared to the Ser326 wildtype [48]. Increased level of 8-oxoG, which is the primary target of *hOGG1* DNA glycosylase was observed in lung [49] and oesophageal cancer [50]. Observation for a period of time has shown spontaneous development of lung adenoma/carcinoma in 18-month-old OGG1-KO mice was associated with the increased accumulation of 8-oxoG [51]. A positive correlation between *hOGG1* mRNA expression and urinary excretion of 8-oxoG levels has been reported [52]. The evidence suggested that *hOGG1* mRNA expression could serve as a biomarker of exposure to oxidative damage [52].

In the present study, study subjects who were G/A+A/A carriers showed a 1.43-fold (95% CI=0.97-2.11) increase in NPC risk compared to those having G/G genotype. The effect size of the polymorphism found in the current study is comparable with findings from several meta-analyses. For cervical cancer, TNF-α GA/AA carriers were found to significantly increase cancer risk by 1.25-fold (95% CI=1.01-1.54) [53]. Similar result has been observed in gastric cancer where subjects having GA/AA genotypes were associated with a 35% increase in cancer risk (95%=1.14-1.60) [54,55] Ma et al. has shown that genotypes GA/AA were significantly associated with increased prostate cancer risk (OR=1.53, 95% CI=1.09-2.15). It is biologically plausible that TNF-α -308G>A polymorphism might be clinically associated with NPC risk.

TNF-α is a pro-inflammatory cytokine that plays an essential role in inflammation, which has been implicated in enhancing tumorigenesis [56]. Several pro-tumor properties of TNF-α have been suggested, such as induction of pro-malignant chemokines, matrix metalloproteinases, cell adhesion molecules and angiogenic mediators [57]. Mocellin et al. [58] have shown that dysregulation and overproduction of TNF-α were associated with cancer promotion and progression. The finding that TNF-α suppression increased resistance to skin carcinogenesis in mouse has substantiated the aforementioned observation [59]. The critical role of TNF-α in tumor promotion was further established as significant suppression of hyperplastic tumours has been observed in TNF-α knock-out mice compared to those having TNF-α wildtype (G/G) [60]. In addition, the activation of NF-κB by TNF-α was found to

inhibit carcinogen-induced cytotoxicity, which resulted in the increase of mutated cells that were susceptible to malignant transformation [61]. TNF-α was also reported to promote cancer development through inducing DNA instability by increasing the production of ROS in the cells [62].

The finding of the present study suggests a potential causative role of *hOGG1* Ser326Cys polymorphism in the initiation of NPC. In addition, the effect of aberrant TNF-α expression arising from the -308 G>A polymorphism could have contributed to increased NPC risk. As NPC is considered a relatively radiosensitive tumor, patients diagnosed and treated in the early stages tend to survive longer compared to those with advanced disease [63]. The 5-year disease-free survival and overall survival for NPC patients diagnosed in the early stages who received radiotherapy were 84.7% and 84.2% respectively [63]. Early detection is therefore important due to the fact that symptoms related to NPC in the early stages are usually non-specific. The development of an accurate NPC screening tool such as screening for biomarkers is important in contributing to the early detection of the disease [64]. The predictive effect of *hOGG1* Ser326Cys and TNF-α -308 G>A polymorphisms on NPC risk should be validated in future studies. If significant, the aforementioned polymorphisms could potentially serve as diagnostic markers in future to develop customized approaches in screening modalities for high risk populations susceptible to NPC.

Conclusion

Overall, the evidence from the present study suggests potential causative role of *hOGG1* Ser326Cys and TNF-α -308 G>A polymorphisms in the initiation of NPC despite none of the aforementioned polymorphisms showed significant association. Evidence from previous studies has supported the hypothesis that variation in BER activity due to *hOGG1* Ser326Cys polymorphism might give rise to the accumulation of 8-oxoG and subsequently confer increased risk of NPC and other cancers. The effect of aberrant TNF-α expression arising from the -308 G>A polymorphism on tumor promotion could have contributed to the significant association between the -308G>A polymorphism with increased NPC risk.

References

1. Chou J, Lin YC, Kim J, You L, Xu Z, et al. (2008) Nasopharyngeal carcinoma – Review of the molecular mechanisms of tumorigenesis. Head Neck 30: 946-963.
2. Yu MC, Yuan JM (2002) Epidemiology of nasopharyngeal carcinoma. Semin Cancer Biol 12:421-429.
3. Zainal Ariffin O, Nor Saleha IT (2011) NCR Report 2007. Malaysia: National Cancer Registry.
4. Lee AW, Foo W, Law SC, Poon YF, Sze WM, et al. (1997) Nasopharyngeal carcinoma: presenting symptoms and duration before diagnosis. Hong Kong Med J 3: 355-361.
5. Raab-Traub N (2002) Epstein-Barr virus in the pathogenesis of NPC. Semin Cancer Biol 12: 431-441.
6. Ning JP, Yu MC, Wang QS, Henderson BE (1990) Consumption of salted fish and other risk factors for nasopharyngeal carcinoma (NPC) in Tianjin, a low-risk region for NPC in the People's Republic of China. J Natl Cancer Inst 82: 291-296.
7. Yu MC, Ho JHC, Lai SH, Henderson BE (1986) Cantonese-style salted fish as a cause of nasopharyngeal carcinoma: Report of a case-control study in Hong Kong. Cancer Res 46: 956-961.
8. Hildesheim A, Dosemeci M, Chan CC, Chen CJ, Cheng YJ, et al. (2001) Occupational exposure to wood, formaldehyde, and solvents and risk of nasopharyngeal carcinoma. Cancer Epidemiol Biomarkers Prev 10: 1145-1153.
9. Chow WH, Mclaughlin JK, Nam JM, Blot WJ (1993) Tobacco use and nasopharyngeal carcinoma in a cohort of U.S. veterans. Int. J. Cancer 55: 538-540.
10. Demple B, Harrison L (1994) Repair of oxidative damage to DNA: enzymology and biology. Annu Rev Biochem 63: 915-948.
11. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB (2010) Oxidative stress, inflammation and cancer: How are they linked? Free Radic Biol Med 49: 1603-1616.
12. Cheng KC, Cahill DS, Kasai H, Nishimura S, Loeb LA (1992) 8-hydroxyguanine, an abundant form of oxidative DNA damage, causes G-T and A-C substitutions. J Biol Chem 267: 166-172.
13. Nash HM, Lu R, Lane WS, Verdine GL (1997) The critical active-site amine of the human 8-oxoguanine DNA glycosylase, hOGG1: direct identification, ablation and chemical reconstitution. Chem Biol 4: 693-702.
14. Boiteux S, Radicella JP (2000) The human OGG1 gene: structure, functions, and its implication in the process of carcinogenesis. Arch Biochem Biophys 377: 1-8.
15. Kohno T, Shinmura K, Tosaka M, Tani M, Kim SR, et al. (1998) Genetic polymorphisms and alternative splicing of the hOGG1 gene, that is involved in the repair of 8-hydroxyguanine in damaged DNA. Oncogene 16: 3219-3225.
16. Yuan T, Wei J, Luo J, Liu M, Deng S, et al. (2012) Polymorphisms of base-excision repair genes hOGG1 326Cys and XRCC1 280His increase hepatocellular carcinoma risk. Dig Dis Sci 57: 2451-2457.
17. Xie H, Xia Kai, Rong H, Chen X (2013) Genetic polymorphism in hOGG1 is associated with triple-negative breast cancer risk in Chinese Han women. The Breast 22: 707-712.
18. Ni M, Qiu J, He W, Wang X (2012) The functional Ser326Cys polymorphism in hOGG1 is associated with gastric cancer risk: evidence from 1180 cases and 2444 controls. Eur J Gastroenterol Hepatol 24: 683-687.
19. Elahi A, Zheng Z, Park J, Eyring K, McCaffrey T, et al. (2002) The human OGG1 DNA repair enzyme and its association with orolaryngeal cancer risk. Carcinogenesis 23: 1229-1234.
20. Cho EY, Hildesheim A, Chen CJ, Hsu MM, Chen IH, et al. (2003) Nasopharyngeal carcinoma and genetic polymorphisms of DNA repair enzymes XRCC1 and hOGG1. Cancer Epidemiol Biomarkers Prev 12: 1100-1104.
21. Laantri N, Jalbout M, Khyatti M, Ayoub WB, Dahmoul S, et al. (2011) XRCC1 and hOGG1 genes and risk of nasopharyngeal carcinoma in North African countries. Mol Carcinog 50: 732-737.
22. Yu X, Miyamoto S, Mekada E (2000) Integrin alpha 2 beta 1-dependent EGF receptor activation at cell-cell contact sites. J Cell Sci 113: 2139-2147.
23. Campbell ID, Humphries MJ (2011) Integrin structure, activation, and interactions. Cold Spring Harb Perspect Biol 3: 1-2.
24. Abram CL, Lowell CA (2007) Convergence of immunoreceptor and integrin signaling. Immunol Rev 218: 29-44.
25. Evans R, Patzak I, Svensson L, De Filippo K, Jones K, et al. (2009) Integrins in immunity. J Cell Sci 122: 215-225.
26. Rathinam R, Alahari SK (2010) Important role of integrins in the cancer biology. Cancer Metastasis Rev 29: 223-237.
27. Ding W, Fan XL, Xu X, Huang JZ, Xu SH, et al. (2015) Epigenetic silencing of ITGA2 by MiR-373 promotes cell migration in breast cancer. PLoS One 10: e0135128.
28. Lundstrom A, Holmbom J, Lindqvist C, Nordstrom T (1998) The role of alpha2 beta1 and alpha3 beta1 integrin receptors in the initial anchoring of MDA-MB-231 human breast cancer cells to cortical bone matrix. Biochem Biophys Res Commun 250: 735-740.
29. Armstrong T, Packham G, Murphy LB, Bateman AC, Conti JA, et al. (2014) Type I collagen promotes the malignant phenotype of pancreatic ductal adenocarcinoma. Clin Cancer Res 10: 7427-7437.
30. Gerger A, Hofmann G, Langsenlehner U, Renner W, Weitzer W, et al. (2009) Integrin alpha-2 and beta-3 gene polymorphisms and colorectal cancer risk. Int J Colorectal Dis 24: 159-163.
31. Langsenlehner U, Renner W, Yazdani-Biuki B, Eder T, Wascher TC, et al. (2006) Integrin alpha-2 and beta-3 gene polymorphisms and breast cancer risk. Breast Cancer Res Treat 97: 67-72.

32. Saenz-Lopez P, Carretero R, Cozar JM, Romero JM, Canton J, et al. (2008) Genetic polymorphisms of RANTES, IL1-A, MCP-1 and TNF-A genes in patients with prostate cancer. BMC Cancer 8: 382.

33. Coussens LM, Werb Z (2002) Inflammation and cancer. Nature 420: 860-867.

34. Warzocha K, Ribeiro P, Bienvenu J, Roy P, Charlot C, et al. (1998) Genetic polymorphisms in the tumor necrosis factor locus influence non-Hodgkin's lymphoma outcome. Blood 91: 3574-3581.

35. Feng H, Kuai JH, Zhang MY, Wang GC, Shi YJ, et al. (2014) Tumor necrosis factor-alpha gene -308G > A polymorphism alters the risk of hepatocellular carcinoma in a Han Chinese population. Diagn Pathol 9: 199.

36. Komori A, Yatsunami J, Suganuma M, Okabe S, Abe S, et al. (1993) Tumor necrosis factor acts as a tumor promoter in BALB/3T3 cell transformation. Cancer Res 53: 1982-1985.

37. Akkiz H, Bayram S, Bekar A, Ozdil B, Akgollu E, et al. (2009) G-308A TNF-alpha polymorphism is associated with an increased risk of hepatocellular carcinoma in the Turkish population: A case-control study. Cancer Epidemiol 33: 261-264.

38. Ho SY, Wang YJ, Chen HL, Chen CH, Chang CJ, et al. (2004) Increased risk of developing hepatocellular carcinoma associated with carriage of the TNF2 allele of the -308 tumor necrosis factor-alpha promoter gene. Cancer Causes Control 15: 657-663.

39. Oh BR, Sasaki M, Perinchery G, Ryu SB, Park YI, et al. (2000) Frequent genotype changes at -308, and 488 regions of the tumor necrosis factor-alpha (TNF-alpha) gene in patients with prostate cancer. J Urol 163: 1584-1587.

40. Shih CM, Lee YL, Chiou HL, Chen W, Chang GC, et al. (2006) Association of TNF-alpha polymorphism with susceptibility to and severity of non-small cell lung cancer. Lung Cancer 52: 15-20.

41. Schlesselman JJ (1982) Case-control studies: Design, conduct, analysis. Oxford University Press, New York, United States.

42. http://www.tufts.edu/

43. Xing DY, Tan W, Song N, Lin DX (2001) Ser326Cys polymorphism in hOGG1 gene and risk of esophageal cancer in a Chinese population. Int J Cancer 95: 140-143.

44. Sugimura H, Kohno T, Wakai K, Nagura K, Genka K, et al. (1999) hOGG1 Ser326Cys polymorphism and lung cancer susceptibility. Cancer Epidemiol Biomarkers Prev 8: 669-674.

45. Zhou C, Xie LP, Lin YW, Yang K, Mao QQ, et al. (2013) Susceptibility of XPD and hOGG1 genetic variants to prostate cancer. Biomed Rep 1: 679-683.

46. Yamane A, Kohno T, Ito K, Sunaga N, Aoki K, et al. (2004) Differential ability of polymorphic OGG1 proteins to suppress mutagenesis induced by 8-hydroxyguanine in human cell *in vivo*. Carcinogenesis 25: 1689-1694.

47. Bravard A, Vacher M, Moritz E, Vaslin L, Hall J, et al. (2009) Oxidation status of human OGG1-S326C polymorphic variant determines cellular DNA repair capacity. Cancer Res 69: 3642-3649.

48. Moritz E, Pauly K, Bravard A, Hall J, Radicella JP, et al. (2014) hOGG1-Cys326 variant cells are hypersensitive to DNA repair inhibition by nitric oxide. Carcinogenesis 35: 1426-1433.

49. Yano T, Shoji F, Baba H, Koga T, Shiraishi T, et al. (2009) Significance of the urinary 8-OHdG level as an oxidative stress marker in lung cancer patients. Lung Cancer 63: 111-114.

50. Rasanen JV, Sihvo EI, Ahotupa MO, Farkkila MA, Salo JA (2007) The expression of 8-hydroxydeoxyguanosine in oesophageal tissues and tumours. Eur J Surg Oncol 33: 1164-1168.

51. Sakumi K, Tominaga Y, Furuichi M, Xu Z, Tsuzuki T, et al. (2003) OGG1 knockout-associated lung tumorigenesis and its suppression by MTH1 gene disruption. Cancer Res 63: 902-905.

52. Hatt L, Loft S, Risom L, Moller P, Sorensen M, et al. (2008) OGG1 expression and OGG1 Ser326Cys polymorphism and risk of lung cancer in a prospective study. Mutat Res 639: 45-54.

53. Li HH, Zhu H, Liu LS, Huang Y, Guo J, et al. (2015) Tumour necrosis factor-alpha gene polymorphism is associated with metastasis in patients with triple negative breast cancer. Sci Rep 5: 10244.

54. Zhu F, Zhao H, Tian X, Meng X (2014) Association between tumor necrosis factor-alpha rs1800629 polymorphism and risk of gastric cancer: A meta-analysis. Tumour Biol 35: 1799-1803.

55. Ma L, Zhao J, Li T, He Y, Wang J, et al. (2014) Association between tumor necrosis factor-alpha gene polymorphisms and prostate cancer risk: A meta-analysis. Diagn Pathol 9: 74.

56. Szlosarek PW, Balkwill FR (2003) Tumour necrosis factor alpha: A potential target for the therapy of solid tumours. Lancet Oncol 4: 565-573.

57. Szlosarek PW, Grimshaw MJ, Kulbe H, Wilson JL, Wilbanks GD, et al. (2006) Expression and regulation of tumor necrosis factor alpha in normal and malignant ovarian epithelium. Mol Cancer Ther 5: 382-390.

58. Mocellin S, Rossi CR, Pilati P, Nitti D (2005) Tumor necrosis factor, cancer and anticancer therapy. Cytokine Growth Factor Rev 16: 35-53.

59. Scott KA, Moore RJ, Arnott CH, East N, Thompson RG, et al. (2003) An anti-tumor necrosis factor-alpha antibody inhibits the development of experimental skin tumors. Mol Cancer Ther 2: 445-451.

60. Oshima M, Oshima H, Matsunaga A, Taketo MM (2005) Hyperplastic gastric tumors with spasmolytic polypeptide-expressing metaplasia caused by tumor necrosis factor-alpha-dependent inflammation in cyclooxygenase-2/microsomal prostaglandin E synthase-1 transgenic mice. Cancer Res 65: 9147-9151.

61. Yang H, Bocchetta M, Kroczynska B, Elmishad AG, Chen Y, et al. (2006) TNF-alpha inhibits asbestos-induced cytotoxicity via a NF-kappaB-dependent pathway, a possible mechanism for asbestos-induced oncogenesis. Proc Natl Acad Sci USA 103: 10397-10402.

62. Babbar N, Casero RA (2006) Tumor necrosis factor-alpha increases reactive oxygen species by inducing spermine oxidase in human lung epithelial cells: A potential mechanism for inflammation-induced carcinogenesis. Cancer Res 66: 11125-11130.

63. Chang JT, See LC, Liao CT, Chen LH, Leung WM, et al. (1998) Early stage nasopharyngeal carcinoma: radiotherapy dose and time factors in tumor control. Jpn J Clin Oncol 28: 207-213.

64. Tabuchi K, Nakayama M, Nishimura B, Hayashi K, Hara A (2011) Early detection of nasopharyngeal carcinoma. Int J Otolaryngol 2011: 638058.