Polymorphism Thr241Met of the XRCC3 Gene and Lack of Association with Colorectal Cancer Susceptibility Risk among Malaysian Population: A Preliminary Report

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ABSTRACT

Background: **The genesis of colorectal cancer (CRC) involves a series of steps in which environmental and/or endogenous carcinogens interact with genetic factors and induce or promote cancer development. Genetic polymorphisms in DNA repair genes may influence individual variation in DNA repair capacity and may be associated with a high risk of developing cancer. Studies on the association between DNA repair gene polymorphisms and CRC appear to be limited and nil from Malaysia.**

Objective: **To examine the polymorphism at codon 241 of the X-Ray Cross Complementing group 3 (XRCC3) in 118 CRC cases and 118 normal controls and to investigate the associated risk of this polymorphism for CRC susceptibility.**

Material and Method: **Peripheral blood from the study subjects were collected in EDTA tubes, genomic DNA extracted and XRCC3 Thr241Met genotyped by using PCR-RFLP technique using *Nla III* restriction enzyme. The resulting genotypes were categorized into wildtype homozygous (Thr/Thr), heterozygous (Thr/Met) and homozygous variant (Met/Met).**

Results and conclusion: **The distribution of genotypes (Thr/Thr, Thr/Met and Met/Met) among CRC cases (83%, 16%, 1% respectively) was not significantly different from those among controls (79%, 21%, 0% respectively). On examining the association between the variant genotypes and CRC risk, the variant genotype either single or in combination did not show significant association with CRC susceptibility risk suggesting that the XRCC3 codon 241 polymorphism does not convey moderate increase in susceptibility to CRC in Malaysian population. Lack of association could be attributed to the small sample size, interaction of other polymorphic DNA repair genes and also low frequency of variant allele for the polymorphism studied in this population.**

KEY WORDS

colorectal cancer, DNA repair gene, XRCC3, polymorphism, susceptibility risk

INTRODUCTION

Colorectal cancer (CRC) is the second to fourth most common cancer in developed countries. Worldwide, 875,000 or more people are diagnosed with CRC annually (De la Chapelle, 2004). CRC represents a complex, multifactorial disease and its etipathogenesis include environmental factors such as dietary factors, life style habits and carcinogen exposure on one hand and genetic predisposition on the other hand (de Jong *et al.*, 2002; De la Chapelle, 2004). Growing evidence suggest that genetic predisposition acts via a combination of high risk variants in a set of low and medium penetrance genes. Humans, who are routinely exposed to mutagens and environment carcinogens such as aromatic amines via diet (through overcooked, charred and preserved meat) and polycyclicaromatichydrocarbon (PAH) from tobacco smoke, are at an increased risk of CRC (Norat *et al.*, 2002). These chemicals when consumed can produce DNA adducts and lead to DNA damage (Vinies *et al.*, 1996). In order to protect the genome against the deleterious effects of carcinogens present in the diet as well as environment, the human body has evolved a host of metabolic enzymes, DNA repair enzymes and other protective enzymes (Yeh *et al.*, 2005).

DNA repair plays a significant role in protecting the genome from damage by endogenous and environmental agents. Distinct pathways, each involving numerous factors, have evolved to perform DNA repair (Friedberg *et al.*, 2002). Genes involved in nucleotide excision repair (NER), recombination repair (RR), base excision repair (BER) and mismatch repair (MMR) pathways have critical roles in protection against cancers. Genetic variations in some DNA repair gene in each of these pathways in the normal population appears to influence cancer susceptibility in exposed individuals (Berwick and Vinies, 2000; Goode *et al.*, 2002). A large number of single nucleotide polymorphisms (SNPs) in DNA repair genes have been determined among individuals. Polymorphisms in DNA repair genes and differences in repair capacity between individuals have been widely documented.

X-Ray Cross Complementing group 3 (XRCC3) gene which is located at chromosome 14q32.3, and related to Rad51, is required for the information of the protein complex necessary for homologous recombination repair (HRR) of Double Strand Break (DSB) and cross-links. XRCC3 plays a key role in maintaining chromosomal integrity and preventing mutations, chromosomal instability and carcinogenesis

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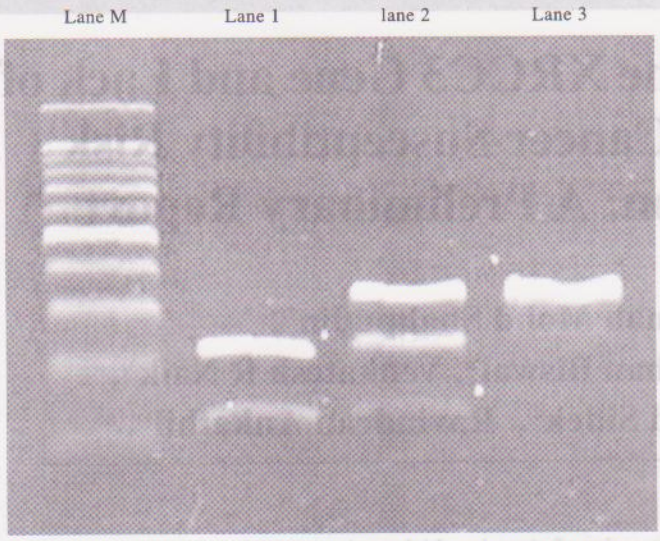

Figure 1. Representative gel of PCR-RFLP analysis of the Thr241Met polymorphism of the XRCC3 gene

Lane M: 100 bp marker
Lane 1: Variant allele genotype (230 and 106 bp)
Lane 2: Heterozygous variant allele genotype (336, 230 and 106bp)
Lane 3: Wildtype allele genotype (336bp)

(Liu *et al.*, 1998, Khanna and Jackson, 2001, and Brenneman *et al.*, 2000). The main polymorphism in this gene involves the change of Threonine (Thr) to Metheonine (Met) of amino acid at codon 241 in exon 7 (Shen *et al.*, 1998), which may affect the coding enzyme's function and/or its interaction with other proteins involved in the DNA repair (Matullo *et al.*, 2001). The variant allele Met is associated with relatively high DNA adduct level in lymphocyte DNA, indicating relatively low DNA repair capacity (Matullo *et al.*, 2001 and Shen *et al.*, 1998). What is the frequency of this polymorphism in Malaysian population and does this polymorphism contribute to CRC susceptibility risk in Malaysia population? No data are available as no previous study has been undertaken. In order to address these issues, a case control study was undertaken to examine the frequency of polymorphism at codon 241 of the DNA repair gene, XRCC3 and to investigate the associated susceptibility risk for CRC in Malaysian population.

MATERIALS AND METHODS

Study subjects

The study was approved by Research Review Board and Ethics Committee of Universiti Sains Malaysia, Kelantan and Ministry of Health, Malaysia. For this Hospital based, case control study, subjects were recruited from Hospital Universiti Sains Malaysia (HUSM), Hospital Kota Bharu and Hospital Alor Setar, Kedah. The genotyping study was carried out at the Human Genome Center, School of Medical Sciences, Universiti Sains Malaysia, Kelantan. One hundred eighteen (118) colorectal cancer patients with hispathologically confirmed diagnosis and an equal number of age and sex matched healthy normal controls were recruited as study subjects. Cases with known (as indicated in the pathology reports) familial adenomatous polyposis, ulcerative colitis or Crohn's disease or any other previous malignancy were excluded. Controls were normal healthy individuals, volunteers who visit HUSM for other problems unrelated to colorectal cancer, matched to cases by sex and five (5) year age group, using the same eligibility criteria as those used for cases. Controls were biologically unrelated to the study patients and were cancer free participants. Epidemiological data was collected from patients using a pre-structured questionnaire which included socio-demographic status, physical status, dietary factors, occupation, tobacco/alcohol habits, previous illness, radiation exposure etc.

DNA extraction and genotyping

Three (3) ml of whole blood was collected from all study participants in sterile EDTA-coated vacutainer. DNA was extracted using the QIAamp DNA Mini Kit (Qiagen) and stored at -20℃ until used for genotyping. Genotyping of XRCC3 gene was carried out by using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). Briefly, PCR primers for the XRCC3 Thr241Met (Foward: 5-GCTCGCCTGGTGGTCATCGACTCG-3, Reverse: 5-AAGAGCACAGTCCAGGTCAGCTG-3) were used to generate 336 bp product containing the polymorphic site. The PCR reactions were carried out in a 25 μl of volume of 1X of PCR buffer, 0.4 mM of each primers, 0.5 mM dNTPs, 2.0 mM of $MgCl_2$ and 1 U of *Taq* polymerase, with a denaturation of 94℃ for 5 min, followed by 35 cycles at 94℃ for 30 s, 66℃ for 30 s and 72℃ for 30 s and finally 5 min at 72℃. Following amplification, PCR products were digested using *NlaIII* restriction enzyme (*New England BioLab*) for 1 hour at 37℃ and electrophoresed on 3% agarose gel. The genotypes were categorized into wildtype homozygous (Thr/Thr), heterozygous (Thr/Met) and homozygous variant (Met/Met). The wild-type homozygous was identified by 336 bp banding patterns, the heterozygous by 336, 230 and 106 bp and homozygous variant by 230 and 106 bp. (Figure 1)

Table1. Genotype frequencies of XRCC3 Thr241Met in cases and controls

Genotype XRCC3 Thr241Met	Cases n = 118	Controls n = 118	*P*-value
(Thr/Thr)	98(83%)	93(79%)	0.406*
(Thr/Met)	19(16%)	25(21%)	0.315*
(Met/Met)	1(1%)	0(0%)	-

*($P > 0.05$, not significant)

Statistical Analysis

The Odds Ratios (ORs) and 95% Confidence Interval (CI) were calculated by using unconditional logistic regression analysis to assess the relationship between XRCC3 Thr241Met polymorphism and CRC. The X^2 test was used to compare the distribution of the genotype of CRC cases and controls. All P-values were two-sided and the test was conducted by SPSS software.

RESULT

In this case control study, out of the 118 CRC patients, 69 were males and 49 were females, (mean age 59.2 years) and among the 118 normal controls, 65 were males and 53 were females (mean age 41.3 years). Among the CRC cases, 98 (83%) showed XRCC3 Thr/Thr genotype, 19 (16%) showed XRCC3 Thr/Met genotype and 1 (1%) showed XRCC3 Met/Met genotype. In the case of controls, 93 (79%) showed XRCC3 Thr/Thr genotype, 25 (21%) showed XRCC3 Thr/Met genotype and none (0%) showed XRCC3 Met/Met genotype. The genotype frequencies of XRCC3 genotypes among cases and controls are shown in Table 1. On comparing the frequencies of the various genotypes between cases and controls, there was no significant difference between the two groups ($p > 0.05$). The association between the polymorphic genotypes and CRC risk was determined by performing logistic regression analysis. As the homozygous variant genotype frequency was very low in cases and nil in controls. Odds Ratios (ORs) were calculated for combined variant genotypes (Thr/Met + Met/Met) relative to the major allele Thr/Thr genotype. The variant genotype combinations did not show any significant association with CRC susceptibility risk. The associated risk of polymorphic genotypes with CRC is shown in Table 2.

DISCUSSION

In recent years, a great deal of attention has been devoted to the role of DNA repair genes as CRC risk modulators. Molecular genetic epidemiological studies investigating the possible associations between common DNA repair SNPs and risk of CRC can provide a useful insight into the relationship between cancer and individual susceptibility in response to DNA damage. XRCC3 encodes for protein participating in homologous recombination repair (HRR) of DNA double-strand breaks. It is member of an emerging family of

Table 2. Association between XRCC3 polymorphic genotypes and CRC susceptibility risk

Genotype XRCC3 Thr241Met	Cases n = 118	Controls n= 118	OR (95% CI)	*P*-value
(Thr/Thr)	98	93	Reference	-
(Thr/Met)	19	25	0.721 (0.373-1.396)	0.332*
(Met/Met)	1	0	-	-
Thr/Met + Met/Met	20	25	0.759 (0.395-1.458)	0.406*

*($P > 0.05$, not significant)

Rad-51 related proteins that may take part in homologous recombination to repair DSB and maintain genome integrity (Brenneman et al., 2000). XRCC3 deficient cells exhibited defect in Rad51 focus formation after radiation damage and demonstrated genetic instability and increased sensitivity to DNA damaging agents (Griffin *et al.*, 2002). Carriers of the variant allele of XRCC3 Thr241Met had relatively high DNA adduct levels in lymphocyte DNA, indicating that this polymorphism was associated with relative low DNA repair capacity (Matullo *et al.*, 2001). Therefore, XRCC3 has been considerable interest as a candidate susceptibility gene for cancer.

A large number of molecular epidemiologic studies have been performed to evaluate the role of Arg399Gln of XRCC3 gene polymorphism with colorectal cancer. Mort *et al.*, (2003) observed a significant over-representation of XRCC3 241T allele in CRC patients compared to controls (OR, 1.52; 95% CI, 1.04-2.22). Completely opposite results for the same SNP were found by Jin *et al.*, (2005) in a study on 140 CRC cases and 280 cancer-free controls from China. Carriers of the variant M allele showed a higher CRC risk (OR, 3.13; 95% CI, 1.41-6.95). Similar outcome was reported by Krupa and Blasiak, (2004), with a surprisingly strong association (OR, 9.45; 95% CI, 8.77-11.65).

In our study on Malaysian CRC patients, no risk for CRC predisposition was evident for the XRCC3 Thr241Met polymorphism. Few other studies did not find any association of this polymorphism with CRC susceptibility risk (Bigler *et al.*, 2005; Stern *et al.*, 2005; Moreno *et al.*, 2006; Yeh *et al.*, 2006). XRCC3 T241M polymorphism was associated with CRC risk, but with opposite directions, as reported by Mort *et al.*, (2003); Krupa and Blasiak, (2004); Jin *et al.*, (2005). In the study of Jin *et al.*, (2005), the carriers of XRCC3 241M allele nonsmokers and non-alcohol drinkers, showed an increased CRC risk. In this case, the stratification reduced drastically the number of observations within each group.

Three studies carried out by Yeh *et al.*, (2005, 2005, 2006), did not find any association for XRCC3 T241M, but a stratification of the patients for meat consumption revealed that individuals with 241TT genotype and low consumption of meat had an increased risk of CRC. The combination of XRCC3 T241M wild type genotype and CYP1A1*2C variant GG genotype was associated with increased CRC risk in women. In this case, the stratification for dietary/lifestyle risk factors and gender was justified by the large size of the cohort (> 700 individuals). The effect of age did not emerge, except in study by Jin *et al.*, (2005), where XRCC3 241M allele was associated with CRC risk among older individuals (> 60 years).

Inconsistent data with positive and negative association could be due to several reasons; first, DNA damage repair is a complex process involving interaction between several genes in various DNA repair pathways (Miller *et al.*, 2001). DSB involves at least two pathways; homologous recombination and non-homologous end joining repair that involves more than 16 proteins, including product of the breast cancer genes BRCA1 and BRCA2, XPD, XRCC3 and also several molecules (O'Driscoll and Jeggo, 2006). Secondly it could be due to small sample size. Increasing the number of sample size will increase the power of this study. Thirdly, very low frequency of variant allele in a population could also be a reason for lack of association. All the above factors might have contributed for this lack of association of XRCC3 Thr241Met polymorphism with susceptibility risk in Malaysian CRC patients.

To summarize, in this preliminary study, we determined the frequencies of XRCC3 Thr241Met genotype in Malaysian population and its associated risk with CRC susceptibility. Our results suggest that, XRCC3 Thr241Met polymorphism does not convey moderate increase in susceptibility to CRC in Malaysian population. The study needs to be extended to large number of samples size which can increase the power of the study and also needs to explore the polymorphic genotype combinations of XRCC3 and other DNA repair genes.

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