Association of Arg72Pro of P53 Polymorphism with Colorectal Cancer Susceptibility Risk in Malaysian Population

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Abstract

Background: Colorectal cancer (CRC) results from the interaction between environmental exposures and genetic predisposition factors. Aims: A case control study was designed and to investigate the genotype frequencies of P53 Arg72Pro polymorphism in Malaysian CRC patients and healthy controls and to determine the associated risk of this polymorphism with CRC predisposition. Methods: In this case-control study, peripheral blood samples of 202 sporadic CRC patients and 201 normal controls were collected, DNA extracted and genotyped using the polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) technique. Results: Genotype analysis showed the frequency of homozygous variant (Pro/Pro) genotype (21%) to be significantly higher in cases compared to controls (13%), (p=0.013). On examining the association between variant genotypes and CRC risk, the Pro/Pro homozygous variant genotype showed significantly higher risk association with CRC susceptibility (OR: 2.047, CI: 1.063-4.044, p=0.033). When stratified according to age, we observed that, individuals aged above 50 years and carriers of pro/pro genotype had significantly higher risk with OR: 3.642, CI: 1.166-11.378, p=0.026. Conclusions: Our results suggest that the codon 72 SNP which results in amino acid substitution of Arginine to Proline in cell cycle regulatory gene P53, is associated with sporadic CRC risk and carriers of Pro/Pro genotype and more than 50 years old may have high susceptibility.

Keywords: Colorectal cancer - TP53 codon 72 - Malaysia

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). The incidence of CRC is increasing in developing countries including Malaysia. In Malaysia, CRC has become the second commonest cause of cancer related mortality after breast cancer and has become the most common cancer in men and second in women (Malaysia Cancer Statistics, 2006). Being a complex and multifactorial disease, its etiopathogenesis involve interaction between environmental and genetic factors. Age, gender environmental factors such as diet, tobacco smoke and alcohol consumption (Giovannucci, 2001; Terry et al., 2001; Neagoe et al., 2004; Yeh et al., 2005; Stern et al., 2007) in interaction with genetic factors have been shown to increase the risk of colorectal cancer (de la Chapelle, 2004).

Tumour suppressor genes play important role in mediating cellular responses to genotoxic insults through its effects on gene transcription, DNA synthesis and repair, genomic stability and apoptosis (Vogelstein and Kinzler, 1992). The most common mutated gene in various cancers is P53 gene which is involved in 50% cancers. Being a guardian of the genome, p53 is involved in G1 arrest which facilitate DNA repair during replication or in induction of apoptosis and cell cycle regulation. In sporadic colorectal cancer, 75% of the tumours had been reported to have inactivation of p53 (Kressner et al., 1999). This inactivation could be single base substitution or loss of alleles with inactivation by viral or cellular proteins (Tommasino et al., 2003).

Genetic variation like Single Nucleotide Polymorphisms (SNPs) in candidate genes such as DNA damage and tumour suppressor genes are thought to play an important role in individual variation in colorectal cancer susceptibility. Genetic association studies have focused on SNPs as important tools for targeting the genes responsible for cancer susceptibility (Cao et al., 2009). A SNP at the codon 72, located at exon 4 of P53 gene (Lima-Ramos et al., 2008) has been studied significantly for its association with various types of cancer such as colorectal, breast and other types of cancer (Tenesa et al., 2008;
Table 2. Genotype and Allele Frequencies of Arg72Pro of P53 Polymorphism in Colorectal Cases and Controls

<table>
<thead>
<tr>
<th></th>
<th>Cases (n=202)</th>
<th>Controls (n=201)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genotype</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>70 (35%)</td>
<td>75 (37%)</td>
<td>0.578</td>
</tr>
<tr>
<td>Arg/Pro</td>
<td>88 (44%)</td>
<td>101 (50%)</td>
<td>0.179</td>
</tr>
<tr>
<td>Pro/Pro</td>
<td>44 (21%)</td>
<td>25 (13%)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td><strong>Allele</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg allele</td>
<td>0.564</td>
<td>0.624</td>
<td></td>
</tr>
<tr>
<td>Pro allele</td>
<td>0.436</td>
<td>0.376</td>
<td></td>
</tr>
</tbody>
</table>

*p-value < 0.05, statistically significant

Tomlinson et al., 2008; Zhu et al., 2007). Several studies showed positive association between this polymorphism with colorectal cancer susceptibility and the rest, failed to ascertain this relationship. Previous studies also have shown that, Arg72Pro polymorphism of P53 varies in different ethnic and population groups. Since no reports are available from Malaysian population, we conducted a case control study to investigate the allele and genotype frequencies and the contribution of variant genotype of Arg72Pro of P53 gene in modifying the susceptibility risk in Malaysian sporadic CRC patients.

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 Raja Perempuan Zainab II and Hospital Sultanah Bahiyah, Kedah, Malaysia. Genotyping was carried out at the Human Genome Center, Universiti Sains Malaysia. Two hundred and two (202) histopathologically confirmed sporadic CRC patients and 201 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 and selected by 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 P53 Arg72Pro 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 -200C until used for genotyping. Genotyping of SNP P53 Arg72Pro was carried out employing Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). Briefly, the region of interest was PCR amplified using appropriate primers (Foward: 5’-TCA AAC ATC CTG TCC CTA CT -3’, Reverse: 5’-CTG CGA TTA AAG GCT GTG GA -3’) which generated a 458 bp product containing the polymorphic site. The PCR reactions were carried out in a 25 µl volume of 1X PCR Buffer, 2.0mM of MgCl2, 0.5mM dNTPs, 0.4mM of each primers and 1U of AmpliTag gold polymerase with a denaturation of 94˚C for 5 min, followed by 35 cycles at 94˚C for 30 s, 57˚C for 30 s and 72˚C for 30 s and finally 5 min at 72˚C. Following amplification, PCR products were digested using BstUI restriction enzyme (New England BioLab) for 1 hour at 600C and electrophoresed on 2% agarose gel. The Arg allele was cleaved by BstUI and yielded two small fragments (321 and 137 bp). The Pro allele was not cleaved by BstUI and had a single 458 bp band. The heterozygous contained three bands (458, 321 and 137 bp). The genotype categorized as wildtype (Arg/Arg), heterozygous (Arg/Pro) and homozygous variant (Pro/Pro) based on band sizes are shown in Figure 1.

Statistical Analysis

The difference in distribution of genotypes, gender and age between cases and controls were assessed using Chi Square, χ2 test. The Odds Ratios (ORs) and 95% Confidence Interval (CI) were calculated by using binary logistic regression (SPSS version 18) adjusted by sex and age to assess the risk association. All statistical tests were two sided, and statistical significance was determined as p<0.05.

Results

In this case control study, cases involved 202 histopathologically confirmed sporadic CRC patients (113 males and 89 females) and controls comprised of 201 healthy normal individuals (95 males and 106 females).
The mean age was 60.36 ± 12.32 years for the cases and 55.01 ± 12.17 years for the controls. The distribution of gender and age group among study subjects are shown in Table 1. There was significant difference in the incidence of CRC between age groups (p-value < 0.001). CRC incidence was higher among individuals more than 50 years compared to those less than 50 years. When the incidence of CRC was compared between males and females, a slight preponderance of CRC was observed among males, however difference was not statistically significant (p=0.081).

Table 2 shows the genotypes and allele frequencies of Arg72Pro of P53 polymorphism in colorectal cases and controls. In colorectal cancer cases, the frequencies of genotypes of Arg/Arg, Arg/Pro and Pro/Pro were 70 (35%), 88 (44%) and 44 (21%) respectively whereas in the controls, the genotype frequencies were 75(37%), 101(50%) and 25(13%) respectively. On comparing the genotype frequencies between cases and controls, the frequency of Pro/Pro genotype was significantly higher in cases (p-value: 0.013). The frequencies of minor allele (Pro allele) in cases and controls were 0.436 and 0.376 respectively.

The risk association of Arg72Pro of P53 gene polymorphism with colorectal cancer susceptibility was examined using Binary Logistic Regression analysis and deriving Odds Ratios (ORs). All ORs were calculated relative to subjects with the major allele Arg/Arg genotype as a reference. Table 3 shows the association of P53 Arg72Pro genotypes with colorectal susceptibility risk. The homozygous variant (Pro/Pro) genotype showed significantly higher risk for colorectal cancer susceptibility with adjusted OR: 2.047, (95% CI: 1.063-4.044 and p-value = 0.033).

Additionally, we stratified our study subjects to investigate the relationship of the SNP studied with other confounding factors like gender and age with colorectal cancer susceptibility risk and the results are shown in Table 4. Age wise stratification showed that, carriers of Pro/Pro genotype with age 50 years and above had a significantly higher risk for colorectal cancer susceptibility ( OR: 3.642, 95% CI 1.166-11.378). When stratified according to gender and age, males with age more than 50 years and carriers of Pro/Pro genotype also showed high risk for colorectal cancer susceptibility (OR: 6.387, 95% CI: 0.766-53.289), but was statistically insignificant. This could be due to the wide range of CI observed due to the presence of Pro/Pro genotype in only 1 normal control male aged more than 50 years. We also found that, Arg/Pro genotype displayed a protective effect against colorectal development for individuals with age more than 50 years old and especially for males with age more than 50 years old with OR: 0.519 and 0.389 respectively.

**Discussion**

Progression of sporadic colorectal cancer involve accumulation of multiple somatic mutations in cells, inactivation of DNA damage repair genes and tumour suppressor genes as well as activation of oncogenes. Interaction of endogenous and exogenous factors which lead to DNA damages will cause the abnormalities in the human genome, especially in genes that involved in DNA repair pathways. DNA damage should be repaired before the cell proceeds for cell replication and division. Failure of DNA repair mechanism will lead to accumulation of DNA damage which turns to mutation. Defects in DNA repair pathways may lead to colorectal cancer. Therefore our study suggests that Arg72Pro polymorphism of P53 gene may contribute to the risk toward colorectal cancer development.
damage repair genes will also lead to involvement of 
P53 tumour suppressor gene to overcome the damages 
by inducing apoptosis or programmed cell death. P53 
gene is involved in cell cycle regulation, controlling DNA 
repair and apoptosis. Single nucleotide polymorphism in 
P53 may completely disrupt the function of the protein, 
resulting in high rate of uncontrolled cell growth or cancer 
and thus contribute to large number of tumours.

Amino acid substitution from arginine (CGC) to 
proline (CCC) at codon 72 has been identified and 
shown to alter the p53 protein structure (Matlashewski 
et al., 1987; Koushik et al., 2006). Studies have shown 
that these two types of proteins are different in biology 
and biochemical pathways (Thomas et al., 1999). Study 
conducted by Pim and Banks, demonstrated that, these 
two proteins, either Arginine or Proline have different 
results in alteration of primary structure of protein. Their 
results showed that Arg72 allele is more efficient than the 
Pro72 allele at inducing apoptosis (Pim and Banks, 2004). 
Marin et al. showed that, p53 mutant inactivated the p73 
protein and showed less effectiveness and low efficiency 
compared with Arg allele during apoptosis (Marin et al., 
2000). It has been reported that, Arg72 is better than Pro72 
in inducing apoptosis as Arg72 have a greater ability 
to localize to the mitochondria and enhance apoptosis whereas variant of p53 is more potent in binding with p73 and 
neutralizing p73 to induce apoptosis (Dumont et al., 
2003). These studies have demonstrated that functional differences exist between these two alleles of the P53 
gene. Genetic variations in P53 can result in the damage 
being left un repaired and inefficient apoptosis which can 
lead to un regulated cell growth and cancer.

In this case control study, we investigated the 
genotype frequencies and associated causal role of the 
common SNP Arg72Pro of P53 in sporadic colorectal 
predisposition in Malaysian population since no previous 
reports are available. We observed significant association 
between SNP Arg72Pro of P53 gene and colorectal cancer susceptibility. P53 codon 72 Pro/Pro genotype 
showed significantly higher risk for colorectal cancer susceptibility. This clearly indicated that, individuals who have Pro/Pro genotype have a twofold higher risk for colorectal cancer development compared to individuals 
who have Arg/Arg genotype.

Our results are concordant with few other studies that 
also showed significant risk association. Study conducted 
by Zhu et al. in Chinese population showed that, carriers of 
Arg/Pro and Pro/Pro genotype had a higher risk of colorectal cancer development with OR: 1.60 and 2.37 (Zhu et al., 2007). Significant association of variant Pro/ 
Pro genotype with increased risk of colorectal cancer was reported by Cao et al.(2009) in a Korean population 
and Sameer et al. (2010) in a Kashmiri population. When 
analyzed based on the age-gender factors, no significant 
associations were found between these two confounding 
factors on CRC susceptibility (Zhu et al., 2007; Cao et 
al., 2009, Sameer et al., 2010). However, in the present 
study, we found a significant association on age and CRC 
susceptibility. Out of 202 CRC patients, 82% were above 
50 years old and 18% were less than 50 years.

Study conducted by Joshi et al. (2010) found that 
men with Pro/Pro genotype and Pro allele showed 
significantly higher risk for colorectal cancer development 
compared to women. They combined the genotype of P53 
polyorphism with MDM2 polymorphism and found an 
association between these two polymorphisms with 
colorectal cancer susceptibility. Individuals who carried 
the Pro allele of P53 and guanine allele of SNP309 
showed significantly higher risk with OR: 1.67, CI: 1.11- 
2.51 (Joshi et al., 2010). Van Heemst et al., did a formal 
large meta-analysis of published results from the literature 
and studied the impact of P53 Pro/Pro and Arg/Arg 
polymorphisms upon the frequency of developing cancers 
and upon the longevity of the population under study. 
From the analysis, they concluded that individuals with 
a Pro/Pro genotype had an increased risk of developing 
a cancer over their lifetimes compared to individuals 
with an Arg/Arg genotype. These researchers interpreted 
that the Arg/Arg genotype has a higher apoptotic rate in 
response to stress and so protects against cancer better 
(Van Heemst et al., 2005).

Kaushik et al. (2006) did not find significant overall 
association between P53 Arg72Pro genotype and 
colorectal cancer but found significant association with 
colorectal adenoma risk. When stratified by gender, an 
increased risk of proximal colorectal cancer in women 
distal colon cancer in men were observed (Koushik et al., 
2006). Higher risk association between CRC and 
P53 Arg72Pro polymorphisms were reported by Perez 
et al.(2006) in an Argentinean population and also by 
Mammano et al. (2009) in Italian population.

Few other studies have reported contradictory findings 
also. Tang et al. (2010) conducted a meta-analysis 
involved 17 case control studies and with a total of 
3537 colorectal cancer cases and 5168 controls as study 
subjects. They did not find any significant association of 
Pro/Pro genotype of P53 with colorectal cancer when 
compared with Arg/Arg genotype. In this study, risk for 
Pro/Pro genotype was OR: 1.02, (CI: 0.80-1.29) and for 
Arg/Pro, the OR was 1.00, CI: (CI: 0.86-1.16) . Similarly 
Economopoulos et al. also did not find any significant 
risk association when they conducted a Meta-analysis 
study involving 19 Caucasian ,6 Chinese and 2 mixed 
populations (Economopoulos et al., 2010).

The difference in results on risk association between 
the present study and other previous studies might be 
explained by difference in groups studied or populations, 
and also differences in environmental exposure and 
lifestyle factors. Small sample size and or inadequate 
controlling for certain confounder factors such as gender 
and age also might have contributed to differing results 
and lack of association. Further studies exploring the 
interaction of P53 Arg72Pro with other genes involved 
in DNA repair pathways, either singly or in combination, 
and also correlating with environmental interactions such 
as smoking, alcohol consumption, and dietary habits 
as well as clinico pathological characteristics, would be 
beneficial in deriving more accurate risk predictive 
markers. In conclusion, our study provides evidence 
that P53 Arg72Pro polymorphism may contribute to the 
etiology of sporadic colorectal cancer in the Malaysian 
population and individuals who are above 50 years old
and carriers of Pro/Pro genotype especially have a higher risk for colorectal cancer susceptibility.

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**References**


