Cytochrome P450 1A1, 2E1 and GSTM1 Gene Polymorphisms and Susceptibility to Colorectal Cancer in the Saudi Population

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Abstract

Background: The Saudi population has experienced a sharp increase in colorectal and gastric cancer incidences within the last few years. The relationship between gene polymorphisms of xenobiotic metabolizing enzymes and colorectal cancer (CRC) incidence has not previously investigated among the Saudi population. The aim of the present study was to investigate contributions of CYP1A1, CYP2E1, and GSTM1 gene polymorphisms.

Materials and Methods: Blood samples were collected from CRC patients and healthy controls and genotypes were determined by polymerase chain reaction restriction fragment length polymorphism and sequencing.

Results and Conclusions: CYP2E1*6 was not significantly associated with CRC development (odd ratio=1.29; confidence interval 0.68-2.45). A remarkable and statistically significant association was observed among patients with CYP1Awt/*2A (odd ratio=3.65; 95% confidence interval 1.39-9.57). The GSTM1*0/*0 genotype was found in 2% of CRC patients under investigation. The levels of CYP1A1, CYP2E1 and GSTM1 mRNA gene expression were found to be 4, 4.2 and 4.8 fold, respectively, by quantitative real time PCR. The results of the present case-control study show that the studied Saudi population resembles Caucasians with respect to the considered polymorphisms. Investigation of genetic risk factors and susceptibility gene polymorphisms in our Saudi population should be helpful for better understanding of CRC etiology.

Keywords: Cytochrome P450 - xenobiotic - colorectal cancer - single nucleotide polymorphism

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types of cancers. The CYP1A1, an inducible CYP, is important for the conversion of carcinogenic polycyclic aromatic hydrocarbons (PAHs) such as benzo[a]pyrene to mutagenic benzo[a]pyrene diol epoxide (BPDE) (Gelboin, 1980). The CYP1A1 gene is located on the long arm of chromosome 15q22-qter (Corchero et al., 2001). A phenotypic polymorphism in CYP1A1 inducibility was first reported in 10% of Caucasians who showed much higher CYP1A1 activity in lymphocytes after exposure to inducer than the non exposed group (Kellerman et al., 1973). In genotyping studies, two closely linked polymorphisms of the CYP1A1 gene have been well studied in Caucasian and Oriental populations, the 3'-flanking region Mspl site that associated with increased sensitivity to inducer in some studies and the exon 7 Ile-Val substitution that appears to result in higher enzyme activity in vitro (Hayashi et al., 1991). Individuals possessing the CYP1A1*2A allele might thus exhibit higher rates of carcinogen activation than individuals with the wild-type allele. The CYP2E1 enzyme, a member of the cytochrome P450 superfamily, is a natural ethanol-inducible enzyme that is involved in the metabolic oxidation of low molecular weight carcinogens such as N-nitrosoamines, benzene and vinyl chloride (Liu et al., 2009). CYP2E1 gene is located on the 10q24.3-qter. It is 18,754 bp long consisting of nine exons and eight introns, which encodes a 493 amino acid protein. CYP2E1 gene contains six restriction fragment length polymorphisms, of which the Rsal/PstI polymorphism in its 50-flanking region has been shown to affect its transcriptional level. The variant type of this polymorphic site can enhance the transcription and increase the level of CYP2E1 activity in vitro (Hayashi et al., 1991). A nucleotide substitution (7632T>A) in intron 6 of the CYP2E1 causes the absence of a DraI restriction enzyme site (CYP2E1*6 allele, rs. 6413432). The effect of this mutation on enzyme activity is still not yet fully elucidated. However, one study showed a trend to lower 6-OH-chlorzoxazone/chlorzoxazone plasma ratios in healthy subjects possessing at least one CYP2E1*6 allele compared with wild type subjects (Haufroid et al., 2002). Moreover, induction of CYP2E1 by ethanol seems to be less efficient in patients with the mutated genotype in comparison with the wild-type genotype (Lucas et al., 1995). Glutathione S-transferases (GSTs) are family of phase II inducible enzymes that are essential in carcinogen detoxification. They catalyze the conjugation of a variety of different compounds with the endogenous tripeptide glutathione (GSH).

In humans GSTM1 gene is polymorphic and mapped on chromosome 1p13.3 (Gao et al., 2010). Both the expression and the protein level of GST isozymes vary between individuals, making them liable to the toxic effects of environmental carcinogens. Elevated levels of GSTs (especially GSTP) have been found in human cancer tumours compared with normal tissues (Ketterer et al., 1992). One of these GSTs is the GSTM, a protective enzyme that detoxifies a number of chemical carcinogens such as benzo[a]pyrene diol epoxide (BPDE) (Chang and Yang, 2000). An inherited homozygous deletion of the gene (GSTM*0/*0 genotype) causes deficiency in enzyme activity. Individuals possessing this genotype have reduced carcinogen-detoxification ability and are theoretically at a high risk of cancer development. Case control studies have shown that some of the mentioned gene polymorphisms are associated with a significant increase in the risk of cancer including CRC in some populations (Chang and Yang, 2000).

In the Kingdom of Saudi Arabia, the relationship between gene polymorphisms of CYPs and GSTs and cancer incidence has not been investigated previously. The aim of the present study was to test for potential association between the CYP1A1*2A, CYP2E1*6 and GSTM1*0/*0 polymorphisms and the risk of CRC in Saudi population.

**Materials and Methods**

**Samples collection**

This study was conducted after review and approval of the Institutional Review Board of the Ethics Committee at King Khalid University Hospital in Riyadh, KSA. Blood samples were collected from 100 confirmed colon cancer patients (65 males and 27 females, age range, 26-80 years; mean age, 58.4 years) and 79 healthy controls matched for age and sex. The control samples were collected from subjects referred to the hospital for general medical checkups. Colon cancer tissue samples were also collected from 12 unrelated colorectal cancer patients 8 males and 4 females and histologically normal tissues in the distant margin to the tumour were collected at the time of surgery from the patient who undergoing resection of colorectal tumours. The diagnosis of cancer was based on standard clinical, endoscopic, radiological, and histological criteria. Clinical and demographic characteristics were recorded, including age at diagnosis, gender, family history, smoking habits, disease behaviour, disease location, and need for surgery. Tissue samples to be used for RNA analysis were immediately submerged in RNAlater solution (Ambion, Courtabeuf, France) to avoid RNA degradation, stored at 4°C for 24 h, and then stored at -20°C until needed. Genomic DNA was isolated from blood samples using QIAampR DNA Blood Min Kit Cat. No. 51106 (Qiagen Co., Germany). Samples of 30-60 mg of the preserved colorectal tissues were homogenized in RLT lyses buffer (Qiagen Co., Germany) supplemented with 1% 2-mercaptoethanol, using a rotor-stator homogenizer. Total RNA was extracted using the RNeasy Mini kit (Qiagen Co., Germany), with a DNA digestion step, according to the manufacturer’s instructions. Elution was performed with 50 µl nuclease-free water. Concentration, purity, and quality of the isolated RNA were determined using the Agilent 2100 Bioanalyzer System and Agilent Small RNA analysis kit according to instruction provided by the manufacturer (Agilent Technologies, Waldbronn, Germany). The RNA Integrity Numbers, RIN were ranged from 6.4-8.6 in CRC and normal control samples. Total RNA in aliquots of 1 µg was retro-transcribed into single-stranded c-DNA using the ImProm-II Reverse Transcription System (A3800, Promega USA). Complementary DNA was synthesized by reverse transcription and used as a template for the quantification of CYP1A1, 2E1 and GSTM1 gene.
expression levels.

Cell lines and culture conditions

LoVo (human colon supraclavicular lymph node metastasis), HCT-116 (human epithelial colorectal carcinoma) and SW480 (human colorectal adenocarcinoma) cell lines were obtained from Dr Abdellilah Aboussékdra Research Laboratories, King Faisal hospital Riyadh, Arabia Saudi. Human lung adenocarcinoma epithelial cell line (A549) was obtained from ATCC (ATCC No.CCL-185TM). These cell lines were cultured in Dulbecco’s medium (DMEM) supplemented with 100 IU/mL of penicillin G and 10% fetal bovine serum. The medium was changed three times a week and when the culture reached 90% confluence, the cells were detached from the flasks using a 0.05% trypsin–0.1% ethylenediaminetetraacetic acid (EDTA) solution, washed twice and finally resuspended in DMEM-supplemented medium at a final concentration of 106 cells/mL to use for RNA isolation and cDNA synthesis.

Genotyping

Genotyping for the CYP1A1*2A allele (6235T>C; rs4646903) was achieved by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Briefly, a 340bp DNA fragment containing the polymorphic MspI restriction site, corresponding to the 3’ end of CYP1A1, was amplified using the following primers: 5’-CAGTGAGAGGTGATGCCGCT-3’ and 5’-TAGGAGTCTTGTCTCATGAC-3’. The PCR mixture was digested overnight at 37°C with 20 units of MspI and the amplified products were run on an agarose gel. The digestion products were subjected to electrophoresis on 2.5% agarose gel. The size of the products were determined by including 100 bp DNA ladder on the gel and visualization using AlphaImager (Alpha Innotech Version 2.0.0). Finally, 20 µl of each PCR product was digested overnight at 37°C with 20 units of DraI restriction enzyme and analyzed on 2.5% agarose gel.

Genotyping for the GSTM1 gene deletion (GSTM1*0/*0 genotype) was performed by PCR as previously described (Darazy et al., 2011). A 219 bp ampiclon of the GSTM1 gene was amplified using forward and reverse primers: 5’-GAACTCCTGAAAAGCTAAAGC-3’ and 5’-GTTGGGGTCAAATATACGGTG-3’. In addition, a 268 bp ampiclon of the β-globin gene (5’-CACCCTCATCACTGACC-3’ and 5’-GAGAGCCAGGAGGCGATC-3’) was used as an internal positive control. The PCR mixture contained 30 pmole of each primer and 100 ng of genomic DNA. The same PCR program was applied as for the other two genes except that theannealing temperature was 55°C.

Sequencing of PCR products

Sequencing of the PCR products was carried out according to Sanger et al. (1977), using the MegaBACE 1000 DNA Sequencing System (Pharmacia/Amersham Co.). The chain termination sequencing reaction was conducted utilizing the DYEnamic ET terminator kit as an integral part of the MegaBACE 1000 DNA sequencing system. The sequencing reaction products were purified using DyeEx 2.0 Spin Kit (63206 Qiagen Co., Germany) and applied to MegaBace 1000 Sequencing machine.

Quantitative real-time RT-PCR

Quantitative PCR (qPCR) was carried out as previously described (Lionel et al., 2010). mRNA transcripts for CYP1A1, CYP2E1 and GSTM1 were measured using the Applied Biosystem 7500 Fast real-time PCR detection system. Reactions were performed using a PCR SYBR Green supermix from Applied Biosystem. Primers used were as follows; CYP1A1 forward and reverse primers: 5’- GAATAGGGATGAGTGACGTG-3’, 5’- ACACCTTCACCCCTCATGCTA-3’; CYP2E1: 5’- TAAATGGAGCTCTAGCTCGC-3’, 5’- CAGGATGTAGATTGACCT-3’; GSTM1: 5’- GTGGGGAAGACAGAAGAGG-3’, 5’- AATCTCGATGTTGAGCATC-3’; GAPDH: 5’- GTATGCGTCAAGAACACTATG-3’, 5’- ATGCCAGTGAGCT-3’. Primers were added to the reaction mix at a final concentration of 250 nM. Five microlitres of each cDNA sample was added to a 20 µl PCR mixture containing 12.5 µl of SYBR Green supermix (Applied Biosystem), 0.5 µl of specific primers (1A1, 2E1, GSTM1 or GAPDH) (eurofins MWG/Operon) and 7 µl of RNase/DNase-free water. Each reaction was performed in a 7500 fast real time PCR Thermal Cycler. The thermocycling conditions for CYP1A1 and CYP2E1 were established as 5 min at 95°C, followed by 40 cycles of 15 s at 95°C, 30 s at 58°C, and 30 s at 72°C, with each reaction done in triplicate. The specificity of each primer pair was verified by the presence of a single melting temperature peak. GAPDH produced uniform expression levels varying by less than 0.5 CTS between sample conditions and was therefore used as a reference gene for this study. The amplified products were run on an agarose gel to confirm that there were no spurious products amplified during the cycles. Results were analysed using
Detection of cytochrome P450 1A1 by immunohistochemistry

Slides were deparaffinized in three changes of xylene for 5 min each. They were hydrated in decreasing concentrations of ethanol and rinsed in 1X PBS. A hydrophobic barrier created around the section using an Immurev pen (Dako, Cambridgeshire, UK). Antigen retrieval was performed by immersing the slides in 0.01 M citrate buffer pH 6.0 and heating for 2-3 minutes microwaving at 100% power followed by 10-30 minutes at 20-30% power using an 800-900 Watt maximum capacity microwave oven. Endogenous peroxidase was quenched with 3% H2O2 for 6 min at room temperature. Slides were incubated overnight at 4°C with a 1:50 dilution of CYP1A1 (H-7) rabbit polyclonal antibody raised against amino acids 246-315 mapping to an internal region of CYP1A1 of human origin (Santa Cruz Biotechnology, INC). A biotin-streptavidin detection system was employed with diaminobenzidine (DAB) as the chromogen. Slides were washed twice with PBS and incubated with the linking reagent (biotinylated anti-rabbit IgM antibody) for 1 hour at room temperature. After rinsing in 1X PBS, the slides were incubated with the peroxidase-conjugated streptavidin label for 20 min. The sections were again rinsed with 1X PBS and incubated with DAB for 10 min in the dark. After chromogen development, slides were washed in two changes of water for 8 min each and counterstained with 0.2% methyl green (MD Supplies, UK) in sodium acetate buffer, pH 4.0. The sections were rinsed with 1X PBS and incubated with DAB for 10 min in the dark. After chromogen development, slides were washed in two changes of water for 8 min each and counterstained with 0.2% methyl green (MD Supplies, UK) in sodium acetate buffer, pH 4.0. The sections were then dehydrated, cleared in xylene, and mounted with DPX mounting medium (Raymond A. Lamb Laboratory supplies, UK).

Microscopical analysis

Two investigators independently evaluated CYP1A1 staining under a light microscope at a magnification of 10X and 40X. Five images of representative areas were acquired for each specimen.

Statistical analysis

Genotype and allelic frequencies were computed and were checked for deviation from Hardy-Weinberg equilibrium (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl). Case-control and other genetic comparisons were performed using the chi-square test and allelic odds ratios (OR), and 95% confidence intervals (CI) were calculated by Fisher’s exact test (two-tailed). Statistical analysis was done using SPSS 16.0 for Windows. We considered p-value of <0.05 as significant.

Results

It should be noted that the molecular tools used for the genotyping do not determine whether other polymorphisms in the same gene are also present in the tested subject. The wild type (wt) denotation thus refers to the wild type allele at the investigated polymorphic site only, regardless of other unstudied polymorphisms for that particular gene. Furthermore, in this study, there were no significant differences in the distribution of genotypes for the three investigated genes when male groups were compared with females groups (data not shown).

CYP1A1 genotyping

The CYP1A1 genotypes are illustrated in Figure 1. The distribution of CYP1A1 genotypes among the tested subjects and statistical analysis of the obtained data are detailed in Table 1. The frequency of the CYP1A1 wt/*2A allele was 22.3% and 6.05% in controls. The distribution of CYP1A1 wt/*2A genotype reflected a statistically significant increase of cancer risk associated with this genotype (OR=3.65; CI=1.39-9.57; \( \chi^2 = 7.59 \) and \( p=0.0058 \)). The distribution of CYP1A1 wt/*2A and *2A/*2A genotype in patients was also statistically different from the control healthy individuals (OR=4.17; CI=1.6-10.81; \( \chi^2 = 9.63 \) and \( p=0.0019 \)).

CYP2E1 genotyping

The CYP2E1 genotypes are illustrated in Figure 2A and B. The distribution of CYP2E1 genotypes among the tested subjects and the statistical analysis of the obtained data are detailed in Table 1. The frequency of the CYP2E1*6 allele was 24.46% in CRC patients and 35.44% in the healthy controls. The homozygous variant allele, CYP2E1*6/*6 was detected in 5.31% of the CRC patients but not in the control group. The distribution of CYP2E1 wt/*6 genotype in CRC patients was not statistically different from that of healthy controls (Fisher’s exact p=0.17), the OR being 1.57 (95% CI: 0.81-3.05). Moreover, the results remained statistically non-significant when both CYP2E1*6/*6 and wt/*6 were considered together as one group (OR=1.29 at CI 0.68-2.5).

Table 1. Statistical Analysis and Distribution of CYP1A1*2A (MspI, 6235T>C, rs4646903 and CYP2E1*6 (DraI, 7632 T>A rs6413432 Genotypes for Colorectal Cancer Patients and Controls

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases No. (Freq)</th>
<th>Control No. (Freq)</th>
<th>OR</th>
<th>95%CI</th>
<th>( \chi^2 )</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2E1 (A&gt;T) rs6413432:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA (wt)</td>
<td>66 (0.70)</td>
<td>51 (0.65)</td>
<td>Ref</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AT (wt/2E*6)</td>
<td>23 (0.25)</td>
<td>28 (0.35)</td>
<td>1.570</td>
<td>0.81-3.05</td>
<td>1.82</td>
<td>0.17</td>
</tr>
<tr>
<td>TT (variant) (2E<em>6/2E</em>6)</td>
<td>5 (0.05)</td>
<td>0</td>
<td>0.117</td>
<td>0.006-2.172</td>
<td>3.2</td>
<td>0.95</td>
</tr>
<tr>
<td>AT+TT (wt/2E<em>6 and 2E</em>6/2E*6)</td>
<td>28 (0.3)</td>
<td>28 (0.35)</td>
<td>1.290</td>
<td>0.68-2.45</td>
<td>0.63</td>
<td>0.42</td>
</tr>
<tr>
<td>CYP1A1 (C&gt;T) rs6466903:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC (wt)</td>
<td>70 (0.745)</td>
<td>73 (0.924)</td>
<td>Ref</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT (wt/*2A)</td>
<td>21 (0.223)</td>
<td>6 (0.076)</td>
<td>3.650</td>
<td>1.39-9.57</td>
<td>7.59</td>
<td>0.0058</td>
</tr>
<tr>
<td>TT (variant) (+2A/*2A)</td>
<td>3 (0.032)</td>
<td>0</td>
<td>4.245</td>
<td>2.452-16.91</td>
<td>7.93</td>
<td>0.00002</td>
</tr>
<tr>
<td>CT+TT (wt/*2A and *2A/*2A)</td>
<td>24 (0.255)</td>
<td>6 (0.076)</td>
<td>4.170</td>
<td>1.6-10.81</td>
<td>9.63</td>
<td>0.0019</td>
</tr>
</tbody>
</table>

*N, number of cases; OR, odd ratio; p, Fisher’s exact p; CI, confidence interval; wt, wild-type allele for investigated polymorphism; CRC, colorectal cancer; Ref, referent.
Analysis of genotype combinations for the three polymorphic sites in the three investigated genes was conducted (data not shown). None of the tested subjects carried the variant allele for the three gene polymorphisms simultaneously. All other combinations between the genotypes of the three genes showed no significant variation from the results of the analysis of each gene separately.

**Gene expression analysis of CYP1A1, 2E1 and GSTM1 by quantitative real time PCR**

The levels of gene expression of CYP1A1, 2E1 and GSTM1 were determined in twelve colon cancer samples and twelve normal tissues in the distant margin to the tumour using qPCR. Results are shown in Figure 3A. It was observed that CYP1A1, CYP2E1 and GSTM1 were highly expressed in colon cancer tissues as compared with normal control adjacent tissues and their expression levels were found to be 4, 4.2 and 4.8 times as compared to the control respectively. Gene expression levels of CYP1A1, CYP2E1 and GSTM1 were also examined in colon cancer cell lines LoVo, HCT-116 and SW480 and in human lung adenocarcinoma epithelial cell line (A549). It was noticed that CYP1A1 was highly expressed in LoVo and HCT-116 cell lines as compared with SW480 and A549 cell lines.

Figure 1. (A) Agarose gel (2.5%) Electrophoresis for PCR Products of CYP1A1 in Colon Cancer Patients Samples (Lanes 2-5) and Control Samples (Lanes 6-8). Lane 1 Represents 100 bp DNA Molecular Weight Markers. (B) Agarose Gel (2.5%) Electrophoresis for PCR Products of CYP1A1 in Colon Cancer Patients Samples (2-14) Digested with MspI Prior to Electrophoresis. (C) Polyacrylamide Gel (12.5%) Electrophoresis for Genotyping Results of CYP1A1 of Colon Cancer Patients (Lanes 1-21). The amplicon is subjected to digestion with MspI prior to electrophoresis. The 340 bp uncut amplicon (upper band) reveals CYP1A1 wt/wt homozygous wild type, the mutant homozygous CYP1A1*2A/*2A shows two fragments (200 and 140 bp), and the heterozygous genotype CYP1A1wt/*2A presents three fragments (the uncut 340-bp fragment and two restriction fragments of 200 and 140 bp). Lanes 1-3 represent control uncut PCR products. Lanes 4 and 21 represent 50 bp DNA ladder molecular weight markers.

Figure 2. (A) Agarose gel (2.5%) Electrophoresis for PCR Products of CYP2E1 in Colon Cancer Patients Samples (Lanes 2-9) and Control Samples (Lanes 10-16). Lane 1 Represents 100 bp DNA Molecular Weight Markers. (B) Agarose gel (2.5%) Electrophoresis for PCR Products of CYP2E1 in Colon Cancer Patients Samples (2-12) Digested with DraI Prior to Electrophoresis. (C) Agarose gel (2.5%) Electrophoresis for PCR Products of Genotyping Results of 10 Samples for GSTM1 (Lanes 2-11).

The presence of a 219 bp amplicon reveals the GSTM1 wt/wt and wt/*0; absence of amplicon reveals the variant genotype GSTM1*0/*0. The 268 bp amplicon of β-globin is the internal positive control. Lanes 1 and 12 represent 100 bp DNA molecular weight markers.

Figure 3. (A) Real Time PCR Analysis of CYP1A1, CYP2E1 and GSTM1 mRNA in Normal (control) and Colon Cancer Tumor Tissues. (B) Expression of CYP1A1, 2E1 and GSTM1 in Different Cancer Cell Lines as Compared to GAPDH.

**GSTM1 genotyping**

The GSTM1 genotype is illustrated in Figure 2C. Among the CRC patients, 2% were homozygous for the deletion polymorphism (GSTM1*0/*0). On the other hand, the control group included 100% GSTM1 positives and possessed at least one copy of the GSTM1 gene (either GSTM1 wt/wt or GSTM1 wt/*0).

GSTM1 genotyping

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GSTM1 genotyping

2.45) as shown in Table 1.
a single T to C substitution that results in a polymorphic
at nucleotide 3801 in the 3' non-coding region containing
and Shimada, 1998; Wenlei et al., 2012). The SNP located
has been implied to associate with cancer risk (Guengerich
carcinogen activation/detoxification and modulate DNA
expression levels and functions, may increase or decrease
genes encoding the enzymes, possibly by altering their
activation or deactivation. Genetic variations in these
by the activation of enzymes involved in carcinogens
2001). Moreover, susceptibility to cancer is determined
among variations in human genomes, and it has been
demonstrated that some SNPs are strongly associated
diseases (Brookes, 1999; Roses, 2000; Sachidanandam et
(299x682)agreed with studies in other populations, for example
statistically significant eight fold increased in risk for CRC
was reported in Japanese living in Hawaii and homoyzous
for this allele (Nisa et al., 2010). No statistically significant
differences in CYP1A1 Mspl genetic variations have been
shown in Italy (Boccia et al., 2007), the United Kingdom
(Ye and Parry, 2002) and in Lebanon (Slattery et al., 2004).
It has been reported that the detection of an association
between CYP1A1*2A and cancer could not be confirmed
in Caucasian populations because of the low frequency
of this allele among these population (Garte et al., 2001).
The frequency of the CYP1A1*2A allele is about 9.4%
in Caucasians, 35.8% in Asians, and 23.8% in Africans
(Wu et al., 2002). The CYP1A1*2A polymorphism has been
associated experimentally with increased catalytic activity
and individuals possessing CYP1A1*2A are expected
to exhibit high rates of carcinogen activation (Lindi et al.,
1994). The present study showed that the variant
CYP1A1*2A allele is significantly associated with CRC
in Saudi population (OR=3.65; CI=1.39-9.57). This is in
agreement with studies in other populations, for example
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(Lindi et al., 1994). The present study showed that the
CYP1A1 gene expression level was four times higher in
CRC compared to control normal tissues as measured by
qPCR (Figure 3A) indicated that the high mRNA level
for CYP1A1 was detected in tumour tissues as compared
with normal tissues. This finding was confirmed by
immunohistochemistry study using anti-cytochrome P450
1A1 antibody (Figure 4). It was clearly observed that colon
cancer tumour tissues expressed more CYP1A1 protein
than normal adjacent control tissues.

Many studies have investigated the relationship
between CYP2E1 gene variation and the risk of CRC. The
most extensively studied SNPs of CYP2E1 are Rsal/PstI
site in the 5’ flanking region and the DraI site in intron 6
(Hayashi et al., 1991; Liu et al., 2009). The present study
indicated that the polymorphism caused by the presence
or absence of a DraI site in CYP2E1 intron 6 has no
significant association with CRC in Saudi populations.
This is in agreement with a previous study about CRC
in a Taiwanese population (Dil Llio et al., 1987) and
Lebanese population (Darazy et al., 2011). The effect
of this mutation on CYP2E1 enzyme activity is still not
well established; however, an increased risk for CRC
was detected among Italian population ever drinkers carrying
CYP2E1*6 (Boccia et al., 2007). It seems that the low
frequency of this allele and the low number of samples
would make it difficult to detect an association with CRC
in Saudi population. Study of CYP2E1 expression by
qPCR showed a 4.2 fold induction in mRNA levels in CRC
samples as compared with control tissues (Figure 3A).

The genotyping for GSTM1 in the present study
was based on PCR assay that identified the GSTM1 and the GSTM1 (null/-) genotypes but did not distinguish homozygous wild type +/- and heterozygous +/- individuals. The present study demonstrated that the GSTM1*0/*0 genotype is found in 2% of CRC patients under investigation indicating low frequency of GSTM1 (null -/-) genotype in Saudi population. Meanwhile, the expression level of GSTM1 mRNA was found to be 4.8 times higher in CRC patients (Figure 3C) compared with normal control healthy adjacent tissues. GSTM1 is generally considered as a protective enzyme because it detoxifies a number of toxic and carcinogenic substances such as nitrosamines and PAHs including BPDE (Ketterer et al., 1992). Moreover, GSTM1 protect cells against endogenous agents, preventing DNA damage and carcinogenesis. The increase of GSTM1 gene expression in larger tumour could comply with an adaptive cellular response to disease progression and this response was less evident in the deletion variants. GSTM1 gene expression varies widely inter-individually and this may be due to tumour-specific expression or to metabolic specialization. Some of these variations are genetically linked and may affect an individual’s susceptibility for cancer (Katoh et al., 1996; Rawal et al., 1999; Martinez et al., 2006). GSTM1*0/*0 genotype was linked to an elevated risk of CRC in several populations such as the Spanish (Ates et al., 2005), Turkish (Kiss et al., 2004), and Italian (Sgambaro et al., 2002). It was linked also to an elevated risk of gastric cancer in Turkish and Iranian populations (Saadat and Saadat, 2001; Tamer et al., 2005). Other studies investigating the association between the GSTM1*0/*0 gene polymorphism and gastric cancer risk have reported conflicting results. Case-control studies in Korean and England populations reported no significant increase in the risk of gastric cancer or CRC (Welfare et al., 1999; Piao et al., 2009). Meta analysis of 49 published case-control and cohort studies showed that there was a significant association between the GSTM1*0/*0 genotype and gastric cancer among Asians, but not among Caucasians (Wang et al., 2010). Another meta analysis of 36 case-control studies observed significant associations with CRC in Caucasians but not in other ethnic groups (Gao et al., 2010). The population-dependent difference in term of association might be due to a difference in the frequency of this polymorphism among these populations as well as to other factors related to the diet and the environment. In addition, the presence of other genetic polymorphisms affecting XMEs similar to GSTM1 might also affect the strength of the association. For instance, GSTT1, which is involved in the detoxification of chemical carcinogens, also exhibits a gene deletion mutation. A case–control study in a Spanish population observed that the GSTT1*0/*0 genotype alone was associated with a 1.91-fold increase in the risk of CRC, but the combination of both GSTM1*0/*0 and GSTT1*0/*0 genotypes was associated with an even higher OR of 4.98 (Ates et al., 2005).

In conclusion, the work undertaken in this study raises a number of interesting observations. First, the CYP1A2*2A genotype is a significant risk factor for CRC in Saudi population. Second, the CYP2E1*6 and GSTM1 *0/*0 independently do not seem to be significantly risk factors in the analyzed population. Third, all genes under investigation were found to be highly expressed in CRC patients which is likely to be an important determinant in predicting the outcome of cancer chemotherapy. Finally, large-scale studies with more CRC patients are required to draw a clearer picture about the genetic factors of CRC etiology in Saudi population.

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