Complex Interplay of CYP1A1 Genetic Polymorphism on Colorectal Cancer Susceptibility

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ABSTRACT

Colorectal cancer (CRC) is one of the most common malignancies in developed countries. It occurs via an interaction between an individual genetic background and environmental parameters such as dietary factors. A number of studies have suggested that dietary procarcinogens, such as heterocyclic amines, N-nitroso compounds and polycyclic aromatic hydrocarbons, might be related to the carcinogenesis of CRC. The carcinogens that cause the development of CRC enter the body as procarcinogens via transporters and are activated to carcinogens or eliminated by various enzymes. These toxicokinetic-related proteins are also controlled by our genetic background (e.g. by genetic polymorphisms).

Aim of work: To analyze the common genetic polymorphisms in the genes for the metabolic enzymes CYP1A1 in an attempt to elucidate the association between these polymorphisms and sporadic cases of colorectal carcinoma among Egyptian patients.

Subjects and Methods: This is a prospective controlled study where we investigated the association between polymorphism of cytochrome P4501A1 (m2) and colorectal cancer using PCR-RFLPS. Over a period of twenty one months, the present study included forty patients with colorectal carcinoma diagnosed by histopathological examination of tumor biopsy and not on chemotherapy or radiotherapy. Twenty healthy subjects, matched for age and sex were included as a control group. For all subjects, peripheral blood samples were assayed for genetic polymorphism of CYP1A1.

Results: Our results showed that a statistically significant association existed between CYP1A1 variant genotype (p=0.015 and the odds ratio OR=0.105 with the 95% confidence interval 0.028-0.390) and colorectal cancer. Conclusion: The studied polymorphism may be associated with the risk of development of colorectal carcinoma in Egyptian patients however, large scale studies are essential to confirm that association.

Keywords: Colorectal cancer, CYP1A1, genetic polymorphism

INTRODUCTION

Colorectal cancer (CRC) is the third leading cause of cancer-related deaths in both men and women in industrialized countries. In 2000, CRC accounted for 9.4% of the world’s new cancers, with 945,000 cases diagnosed and 7.9% of the world’s cancer deaths, with 492,000 deaths. In 2008, CRC represented 8.9% of all cancers with lung and breast cancers...
having higher incidence (14.5% and 10.8%, respectively)\(^{(1,2)}\). CRC is more prevalent in North America, Argentina, Australia, New Zealand and parts of Europe, and for this reason it is commonly regarded as a western lifestyle disease.\(^{(3)}\)

CRC is one of the most common malignant neoplasms in Egypt, representing 6.5% of cancers and tends to occur in younger Egyptians with no specific predilection to age. However, 29% of the tumors occurred in patients under 30 years and 26% of patients were aged 60 years. The median age of all patients with CRC was 48 years which is younger than that in developed countries.\(^{(4,5)}\)

It is widely accepted that colorectal carcinogenesis is a multistep process involving the inactivation of a variety of tumor-suppressor and DNA-repair genes and simultaneous activation of certain oncogenes.\(^{(6)}\)

Cancer risk resulting from human exposure to exogenous chemicals (xenobiotics), like polycyclic aromatic hydrocarbons (PAH), which are ubiquitous environmental, dietary, and tobacco carcinogens may vary according to the ability to clear the xenobiotics from the body.\(^{(1,2,6)}\)

Polymorphisms in the genes that encode enzymes involved in the metabolism of PAHs (xenobiotic-metabolizing enzymes), such as the cytochrome P450 group (CYP) and the glutathione-S-transferase group (GST), result in varying activity levels of these enzymes, which can then influence xenobiotic clearance. The metabolism of PAHs involves both activation (phase I) and clearance (phase II) reactions by these enzymes. During activation, reactive intermediates are formed that can bind to DNA and result in adducts that cause mutations if not repaired, thereby initiating carcinogenesis.\(^{(7)}\)

It is likely that the expression and activity levels of the xenobiotic-metabolizing enzymes determine the relative level of activation and detoxification of carcinogens. These levels are important because increased levels of activation, decreased detoxification or both may increase colorectal cancer incidence.\(^{(8)}\)

The cytochrome P450 family is a large and diverse group of enzymes. The function of most CYP enzymes is to catalyze the oxidation of organic substances. The substrates of CYP enzymes include metabolic intermediates such as lipids, steroidal hormones, as well as xenobiotic substances such as drugs.\(^{(9,10)}\)

Genes encoding CYP enzymes and the enzymes themselves are designated with the abbreviation CYP, followed by an Arabic numeral indicating the gene family (e.g. CYP1, CYP3), a capital letter A,B,C indicating the subfamily (e.g. CYP1A, CYP3A) and another numeral for the individual gene/isoenzyme/isoform (e.g. CYP1A1, CYP3A4).\(^{(11)}\)

These enzymes are variably distributed in tissues, but are mainly present in the liver, the main organ involved in drug and toxin removal, but a remarkable amount is also found in the small intestine. CYP is present in the microsomal part of the cytoplasm.\(^{(12)}\)

Cytochrome P450 CYP1A1, is one of the three members of the CYP1 family, which is found mainly in extrahepatic tissues and participates in
the metabolism of a vast number of xenobiotics, as well as a small number of endogenous substrates. Among the different reactions catalyzed by CYP1A1, hydroxylation at a vacant position of an aromatic ring is considered to be the hallmark for the reactive conversion products that can cause oncogenic mutations in experimental animals and humans. (13)

The CYP1A1 gene, located on chromosome 15, band 15q 22-24, comprises seven exons and six introns and spans 5810 base pairs. In humans, CYP1A1 is under regulatory control of the aryl hydrocarbon receptor, a transcription factor that regulates gene expression. (14)

CYP1A1 expression is mediated through a specific cytosolic receptor, the aryl hydrocarbon receptor or AhR. The transcription of CYP1A1 is inhibited by the AhR-related factor aryl hydrocarbon receptor repressor or AhRR, which localizes in the nucleus in the form of a dimeric protein. (15, 16)

Regarding CYP1A1 polymorphism, several mutations in the gene have been described. The four principal sequence variants are a thymine to cytosine substitution at the 3’ end of the gene (mutation 1) that gives rise to a Mspl restriction enzyme site, an adenine to guanine substitution (mutation 2), resulting in a Ile-462 Val exchange in the heme binding region of exon 7, an African American-specific thymine to cytosine substitution (mutation 3) in intron 7, and cytosine to adenine variant (mutation 4) resulting in a threonine 461 aspargine amino acid change in the heme-binding site of the enzyme. (17,18)

METHODOLOGY

Sixty subjects were chosen over a 21 month period (1st February 2009-31st October 2010) from the Department of General Surgery at Cairo University Hospital.

Subjects were divided into 2 groups:

- Group 1 included forty patients newly diagnosed with CRC (22 males and 18 females). The mean age was 48.5 ± 11.57 years (range from 17-70 years). Sixteen patients were smokers (40%) and only two patients gave a positive family history of colorectal cancer.

- Group 2 included twenty apparently healthy individuals to serve as controls (12 males and 8 females). The mean age was 48.85 ± 12.5 years (range from 18-65 years). Eight persons were smokers and none gave a positive family history of colorectal cancer.

All patients were subjected to full history taking and complete clinical examination. In Group 1 patients, radiological assessment including pelvi-abdominal scanning, chest radiography, abdominal sonography, sigmoidoscopy and colonoscopy as well as biopsy and histopathological examination were done.

From each participant in the study, 9 ml of venous blood were drawn and divided into 6ml of blood dispensed into 2 sterile EDTA vacutainer tubes and mixed properly (one for DNA extraction was done without delay, the other for CBC), and the other 3ml of blood were dispensed into sterile plain vacutainer tubes, centrifuged for 10 minutes at
3000rpm, and the separated serum was used for tumor marker evaluation and routine chemistry analysis.

All sixty subjects underwent laboratory investigations including CBC, kidney and liver functions, tumor markers (CEA and CA 19-9), genetic analysis of CYP1A1 gene polymorphism for detection of CYP1A1 A4887G (m2) using PCR-RFLP.

Genomic DNA extraction from peripheral blood leucocytes was done using Qiagen GmbH (Hoffman La Roche AG Max-Volmer-Strabe 4-40724-Hilden-Germany). The analysis of C.T transversion at position 4887 in exon 7 of CYP1A1, which results in the replacement of Thr by Asn at residue 461 in the heme binding region of the enzyme, was performed using PCR-RFLP as described by Cascorbi and co-workers (19).

Extracted DNA samples were amplified using M2F, 5’-CTGTCTCCCTCTGGTTACAGGAA GC and M2R, 5’-TTCCACCCGTTCAGCAGGATA GCC primers PCR was performed for 40 cycles with denaturing at 95°C for 1 min, annealing at 56°C for 1 min and extension at 72°C for 1 min. The PCR products were then digested with Bsa1 enzyme (New England-Bio Labs Catalog No. 18006327799) at 37°C overnight, then subjected to electrophoresis in ethidium-bromide-stained 3% agarose gel and visualized by ultraviolet light transillumination to detect m2 mutation.

**Statistical analysis:** All our data were statistically described in terms of range, mean standard deviation (+/- SD), frequencies and related frequencies when appropriate.

Comparison of quantitative variables between the study groups was done using Mann Whitney U test for independent samples when not normally distributed. For comparing categorical data, Chi square test was performed. Exact test was used when the expected frequency is less than 5. Multivariate logistic regression analysis was done to rest the effect of all important factors on the occurrence of CRC in the study sample. A probability value (p value) less than 0.05 was considered statistically significant.

**RESULTS**

In the current study both groups were age and sex matched (p›0.05) and no significant difference in smoking could be detected (p=0.22). Concerning the site of the carcinoma, we detected a higher prevalence of distal tumors in the sigmoid colon and rectum (57.5%). The main presenting symptom in our patients was bleeding per rectum (37.5%), diarrhea (25%), abdominal pain (22.5%), and constipation (15%).

On comparing the levels of the tumor markers CEA and CA 19-9 between cases and controls, a statistically significant difference was detected between the two groups (p=0.042 and 0.0264), respectively.

Biopsies from our patients revealed adenocarcinoma in 87.5% (35/40), mucinous adenocarcinoma in 7.5 % (3/40) and signet cell ring adenocarcinoma in 5% (2/40) of cases.

The BSA I RFLP analysis for CYP1A1 polymorphism revealed three genotypes; Ile/Ile; homozygous for the presence of the cut site
(homozygous wild-type allele) with two bands at 150 bp and 65 bp, Ile/Val heterozygous (heterozygous genotype) for the presence of the cut site with three bands at 204 bp, 150 bp and 65 bp and Val/Val homozygous (homozygous rare mutant allele) type for the absence of the cut site with an undigested band at 204 bp (Figure 1).

In CYP1A1 gene polymorphism, the cases included 5/40 (12.5%) with CYP1A1 (m2) mutation compared to 1/20 (5%) in the control group. A statistically significant difference was detected on comparing the incidence of CYP1A1 mutation between the two groups; \( p=0.015 \) and the odds ratio \( \text{OR}= 0.105 \) with the 95% confidence interval (C.I)(0.028-0.390). (Table 1)

No statistically significant differences were found between the incidence of CYP1A1 mutation and the age of patients (\( p=0.296 \)), smoking (\( p=0.051 \)), site of the tumor whether proximal or distal tumors (\( p=0.904 \)). (Tables 2, 3, and 4)

On investigating the association between CYP1A1 mutation and sex of CRC patients, it was found that 5/5 (100%) of patients with the mutation were males, while none of the patients having the mutation was a female. This difference was statistically significant; \( p=0.031 \). (Table 5)

All five patients with the CYP1A1 mutation had adenocarcinoma; two patients were 55 years old, while the other three patients were 42, 50 and 59 years old. Two tumors were found in the rectum, while the other three tumors were detected in the cecum, descending colon, and sigmoid colon. High levels of CEA (above 5ng/ml) were detected in three patients (60%), whereas elevated levels of CA 19-9 (above 40 U/ml) were found in four patients (80%).

The association between CYP1A1 polymorphism and the pathological type of malignancy showed no statistical significance; \( p=0.665 \), in which 100% of patients with variant genotype had adenocarcinoma and none had mucinous or signet ring cell adenocarcinoma. (Table 6)
Figure (1): gel electrophoresis showing the CYP1A1m2 mutation gene polymorphism after BstDI digestion.

- Lane 1: DNA marker
- Lanes 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 18, 19, 20, 21, 22 and 23: are homozygous wild types (Ile/Ile) (two bands at 150 bp and 65 bp)
- Lanes 11, 15, 17: are heterozygous for the mutation (Ile/Val), (three bands at 204 bp, 150 bp, and 65 bp)
- Lane 16: homozygous for the mutation (Val/Val) (one band at 204 bp)

Table (1): Frequency of CYP1A1 genotypes among the two groups

<table>
<thead>
<tr>
<th>CYP1A1 Genotypes</th>
<th>Cases (n=40)</th>
<th>Controls (n=20)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ile/Ile</td>
<td>35 (87.5%)</td>
<td>19 (95%)</td>
<td>0.015 (S)</td>
</tr>
<tr>
<td>Ile/Val</td>
<td>4 (10%)</td>
<td>1 (5%)</td>
<td></td>
</tr>
<tr>
<td>Val/Val</td>
<td>1 (2.5%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Gene polymorphism</td>
<td>5 (12.5%)</td>
<td>1 (5%)</td>
<td></td>
</tr>
</tbody>
</table>

Table (2): Frequency of CYP1A1 Genotypes according to the ages in cases

<table>
<thead>
<tr>
<th>CYP1A1 Genotypes</th>
<th>Wild type (n=35)</th>
<th>Variant (n=5)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age ≤ 50</td>
<td>19 (54.3%)</td>
<td>2 (40%)</td>
<td>0.296</td>
</tr>
<tr>
<td>Age &gt; 50</td>
<td>16 (45.7%)</td>
<td>3 (60%)</td>
<td></td>
</tr>
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</table>
Table (3): Frequency of CYP1A1 Genotypes according to smoking

<table>
<thead>
<tr>
<th>CYP1A1 Genotypes</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type (n=35)</td>
<td>Variant (n=5)</td>
</tr>
<tr>
<td>Number (%)</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>23 (65.7%)</td>
</tr>
<tr>
<td>Positive</td>
<td>12 (34.3%)</td>
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Table (4): Frequency of CYP1A1 Genotypes according to the site of malignancy

<table>
<thead>
<tr>
<th>CYP1A1 Genotypes</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type (n=35)</td>
<td>Variant (n=5)</td>
</tr>
<tr>
<td>Number (%)</td>
<td></td>
</tr>
<tr>
<td>Proximal</td>
<td>15 (42.9%)</td>
</tr>
<tr>
<td>Distal</td>
<td>20 (57.1%)</td>
</tr>
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</table>

Table (5): Frequency of CYP1A1 genotypes among both sexes

<table>
<thead>
<tr>
<th>CYP1A1 Genotypes</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Wild Type (n=35)</td>
<td>Variant (n=5)</td>
</tr>
<tr>
<td>Number (%)</td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>17 (48.6%)</td>
</tr>
<tr>
<td>Females</td>
<td>18 (51.4%)</td>
</tr>
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</table>

Table (6): Frequency of CYP1A1 genotypes according to the pathological type of the tumor

<table>
<thead>
<tr>
<th>CYP1A1 Genotypes</th>
<th>P</th>
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<tbody>
<tr>
<td>Wild type (n=35)</td>
<td>Variant (n=5)</td>
</tr>
<tr>
<td>Number (%)</td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>30 (85.7%)</td>
</tr>
<tr>
<td>Mucinous Adenocarcinoma</td>
<td>3 (8.6%)</td>
</tr>
<tr>
<td>Signet cell ring carcinoma</td>
<td>2 (5.7%)</td>
</tr>
</tbody>
</table>
DISCUSSION

Colorectal carcinoma diagnosis is often made at a too late stage inducing a poor prognosis, emphasizing the need for prevention and early diagnostic tools. Metabolic enzymes, including phase I and phase II enzymes, are involved in activation and detoxification of xenobiotics, which play an important role in the pathogenesis of colorectal cancer. Cytochrome P450, including family 1, subfamily A, polypeptide 1 (CYP1A1), is one of the phase I enzymes, metabolizing a large number of endogenous and exogenous substances, such as polycyclic aromatic hydrocarbons, heterocyclic amines, aromatic amines, and N-nitrosamines. Thus, CYP1A1 plays an important role in human susceptibility to colorectal cancer due to various exogenous factors.\(^{(20, 21, 22)}\)

CONCLUSION

In conclusion, CYP1A1 Ile462Val polymorphism may contribute to colorectal cancer risk, however, further study is needed with a large-scale case-control sample to validate the identified risk in our current study. In addition, potential gene-gene and gene-environment interactions should be taken into account when the relation between CYP1A1 Ile462Val polymorphism and colorectal cancer risk is further evaluated.

REFERENCES


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تأثير تعدد الأشكال الوراثية لـ GSTP1 على حدوث الإصابة بسرطان القولون والمستقيم

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سرطان القولون والمستقيم هو أحد الأورام الجينية الأكثر شيوعا في مصر ويمثل 5% من حالات السرطان. غالبًا ما يحدث في الشباب المصريين مع عدم الخلايا من سلالة مستقرة، حيث أن 29% من المرضى كانوا أعمارهم أقل من 30 سنة و26% من المرضى كانوا أكبر من 60 سنة، مع توزيع العمر في البداية المبكرة، ثم تقليلها في البداية المبكرة، ثم تقليلها في البداية المبكرة، ثم تقليلها في البداية المبكرة، ثم تقليلها في البداية المبكرة.

يعد GSTP1 جينًا مهمًا في بعض الأورام، بما في ذلك سرطان المريء، حيث يلعب دورًا في منع نمو الأورام والحد من النمو السريع. قد تؤثر تعدد الأشكال الوراثية لـ GSTP1 على حدوث الإصابة بسرطان القولون والمستقيم.

شملت هذه الدراسة 30 شخصًا أُجريت على الكروموزوم 15 وتمتدًا قاعدة إلى 58 كيلوببتيد ووجدت في العديد من الطفرات منها الطفرة 1 والتي تشير إلى الوراثة وجدت هذه الدراسة تقييمًا وجدت ارتباطًا مستقلًا بين تعدد الأشكال لـ GSTP1 وآكيتات القولون والمستقيم في المرضى المصريين.

أظهرت النتائج وجود ارتباط ذات دلالة إحصائية بين تعدد الأشكال الوراثية لـ GSTP1 وحدوث سرطان القولون والمستقيم في المرضى المصريين مما يجهد عامل خطر محتمل لتحدث المرض.