Polymorphism of Selected Genes Coding for Xenobiotic-Metabolizing Enzymes and Susceptibility to Colorectal Cancer in a Saudi Population

Mansour A. Alghamdi

Department of Environmental Sciences, Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdulaziz University, KSA

Abstract. Colorectal cancer (CRC) is a threatening health problem in Saudi Arabia. Polymorphism of genes coding for xenobiotic-metabolizing enzymes (XME) represent significant risk for a variety of cancers related to the interaction of environmental and genetic factors. The present study aimed to investigate the contribution of CYP1A1 and NAT2 genes polymorphism in CRC. We conducted a hospital-based case-control study in Saudi population. Samples from buccal swabs were obtained from 116 de novo diagnosed cases of CRC and 72 controls. Seven polymorphisms in the two genes analysed: CYP1A1 T3801C, CYP1A1 C2453A, CYP1A1 A2455G, NAT2 G590A, NAT2 C481T, NAT2 G857A, and NAT2 A803G were identified. Among selected genes, CYP1A1 A2455G and NAT2 A803G genotypes demonstrated significant differences ($p < 0.05$, 0.001) and greater risk for developing CRC comparing to the wild genotype (OR: 7.65, 12.13 and 8.18 for GA, AA and GA+AA respectively for CYP1A1 A2455G; and 3.65, 4.24 and 4.17 for GA, GG and GA+GG respectively for NAT2 A803G). Remaining loci demonstrated no significance with risk. Of the seven polymorphisms screened, CYP1A1 A2455G and NAT2 A803G may be of importance to the colon carcinogenesis. Further large-scale studies are needed.

Keywords: genes, polymorphism, colorectal cancer.

Introduction

Colorectal cancer (CRC) is a major cause of mortality and morbidity worldwide. In Saudi Arabia, the incidence of CRC is increasing. According to the latest report of the National Cancer Registry (Al-Eid *et al.*, 2011), CRC is the first most common cancer among Saudi males (12.9%) and the third among the Saudi females (9.4%), being the second most common malignancy among Saudis for all ages (10.3%). Sporadic CRC is a typical multifactorial disease, arising from environmental exposures (air pollution, dietary carcinogens, cigarette smoke), susceptibility genes and the interaction between genotypes and environments (Khabaz, 2012 and Hezova *et al.*, 2012)). Exposure to polycyclic aromatic hydrocarbons (PAHs) and heterocyclic amines (HCAs) have long been suspected to play a

prominent role in CRC aetiology (Al-Saleh *et al.*, 2008).

Genes coding for xenobiotic-metabolizing enzymes (XME) act as low-penetrance genes, and their functional polymorphisms confer significant risks for a variety of cancers related to the interaction of environmental and genetic factors (Agundez, 2004 and Ladero *et al.*, 2012). Two important groups participate in the conversion of xenobiotics to more water soluble metabolites: the cytochrome P450 family and the N-acetyltransferases.

Among the members of Cytochromes P450 isozymes, CYP1A1, participates in the metabolism of a vast number of xenobiotics, and is important for the conversion of PAHs such as benzo[a]pyrene to mutagenic benzo[a]pyrene diol epoxide (BPDE)

(Gelboin, 1980). In addition, CYP1A1 is one of the more significant P450 enzymes involved in the process of metabolic activation of many other environmental carcinogens (Androutsopoulos *et al.*, 2009). The CYP1A1 T3801C variant involves a thymidine (T) to cytosine (C) substitution at position 3801 of the 3' non coding region downstream of the polyadenylation site (Androutsopoulos *et al.*, 2009), and it has been associated with increased carcinogen activation (Landi *et al.*, 1994). The CYP1A1 A2455G leads to an Ile462Val substitution in the heme-binding domain of exon 7, with a concurrent increase in catalytic activity of the protein, while C2453A leads to a Thr461Asn substitution in the same domain as the previously mentioned polymorphism, with currently unknown consequences for the function of the encoded protein (Georgiadis *et al.*, 2005).

The N-acetyltransferase 2 (NAT2), is expressed in the liver and the intestinal mucosa and catalyzes N- and O-acetylation of certain pro-carcinogens, heterocyclic aromatic amines and aromatic amines. N-acetylation takes place in the liver and results in N-hydroxi-metabolites which are excreted into the gastrointestinal lumen and are O-acetylated at the level of the colorectal mucosa, forming covalent compounds that will bind to the DNA chain and will induce gene alterations and then formation of cancerous cells (Osian *et al.*, 2010). Genetically determined differences in acetylation capacity may alter exposure to carcinogens and thus influence individual susceptibility to environmental exposures, suggesting that polymorphisms in NAT2 enzyme plays important role in colorectal carcinogenesis (Lilla *et al.*, 2006). Individuals are divided in three principal phenotypes according to their genotype in fast acetylators, intermediated acetylators or slow acetylators. The NAT2 C481T, G857A, A803G and G590A genotypes are associated with slow

acetylation (Woolhouse *et al.*, 1997; Osian *et al.*, 2010).

In this paper, we used the PCR-RFLP method to study the polymorphisms of the 2 genes coding for xenobiotic-metabolizing enzymes (CYP1A1 and NAT2), and whether they represent a risk factor for colorectal cancer in Saudis.

Materials and Methods

Study population

De novo diagnosed cases of CRC treated in the King Abdulaziz University Hospital, Jeddah, Saudi Arabia were enrolled in this study. Samples from buccal swabs were obtained from 116 unrelated consecutive patients (60 males and 56 females) with colorectal cancer and 72 healthy individuals. They were included in the study after giving informed written consent. The colorectal cancer diagnosis was based on the histopathology of the patient. Control samples were obtained from healthy blood donors visiting the Blood Bank of the same hospital. The study proposal was scrutinized and approved by King Abdulaziz University Research Ethics Committee (protocol number 758-12, approval date 14/3/2012).

Genotyping

Genomic DNA was extracted from buccal swabs using DNA Blood Mini kit (Qiagen), following the manufacturer's protocol. The presence or absence of the polymorphisms was determined by PCR-RFLP analyses, using different enzymes according to the SNP to detect. PCR reactions were performed in 25 µl containing 6 µg of genomic DNA, 0,8 mM each primer, 200 µM of each dNTPs, 1,5 mM MgCl₂ and 1 U Promega GoTaq DNA Polymerase. After denaturation for 3 min at 93°C, each PCR was performed for 35 cycles of 1 min at 93°C, 1 min at 59°C and 1 min at 72°C. Final elongation step was 10 min at 72°C. We used the same PCR conditions to

amplified all genes studied. Negative nontemplate controls were included in each PCR and as an internal control we used β -globin gene.

PCR products were directly analyzed on a 2% agarose gel, except PCR products NAT2 C481T and NAT2 G857A, which were analyzed on a 1% agarose gel due to PCR failure.

Once we checked the PCR products, they were incubated with specific restriction enzymes in order to determine the polymorphism of interest. CYP1A1 A2455G were incubated for one hour at 65°C with 0.25 ul of BsrDI, NAT2 G590A for 4 hours at 37°C with 0.25 ul of Taq I, and all the others products for 1 hour at 37°C with 0.25 of the corresponding enzymes. The same aliquot of DNA was used to analyse all SNPs, and the digestions were terminated by taking out of the bath the samples and putting them at -20°C. The digested products were revealed in 3% agarose gel except for NAT2 C481T that were revealed in 1,5% agarose gel.

Based on the RFLP technique, we were able to identify seven polymorphisms in the two genes analysed: CYP1A1 T3801C, CYP1A1 C2453A, CYP1A1 A2455G, NAT2 G590A, NAT2 C481T, NAT2 G857A, and NAT2 A803G. Primers and restriction enzymes used to detected polymorphisms in the genes are presented in table 1.

Table 1. Primers and restriction enzymes used to detected polymorphisms in the genes.

Polymorphism	Primer Sequence	Enzyme
CYP1A1 T3801C	GGCTGAGCAATCTGA CCCTA TAGGAGTCTTGTCTCA TGCCT	Msp I
CYP1A1 A2455G	CTGTCTCCCTCTGGTT ACAGGAAGC	BsrD I

CYP1A1 C2453A	TTCCACCCGTTGCAGC AGGATAGCC	Bsa I
NAT2 G590A	CCTGGACCAAATCAG GAGAG	Taq I
NAT2 A803G	ACACAAGGGTTTATTT TGTTCC	Dde I
NAT2 C481T	GTCACACGAGGAAAT CAAATGC	Kpn I
NAT2 G857A	GTTTTCTAGCATGAAT CACTCTGC	BamH I

CYP1A1 T3801C

Amplification was done by incubation with Msp I enzyme. This endonuclease has CCGG as recognition sequence, and since in this case the wild type genotype is TT, it would only cut with at least one C allele. Hence, in the TT samples, we observed one fragment of 880 bp, while in the TC and CC genotype, there were three (880 bp, 692 bp and 190 bp) and two bands (692 bp and 190 bp) respectively.

CYP1A1 C2453A

We incubated the patients and control samples with Bsa I, an enzyme that has GGTCTC sequence as recognition site. In this case, the wild type genotype is CC and its presence is known by the appearance of two bands of 156 bp and 50 bp. In the CA genotype, we observed 3 fragments of 204 bp, 156 bp and 50 bp; and for the homozygous AA, the Bsa I could not cut so there was only the 204 bp band.

CYP1A1 A2455G

To determine the CYP1A1 A2455G polymorphisms, the PCR products were incubated with the BsrD I endonuclease, that recognizes the GCAATG sequence. In the wild type genotype AA, this enzyme would cut the amplicon giving place to two fragments of 149 bp and 55 bp. If the sample was heterozygous AG, three bands of 204

bp, 149 bp and 55 bp will be observed. Lastly, the enzyme couldn't cut the homozygous genotype GG, and therefore in these cases there was only the 204 fragment.

NAT2 G590A

Samples were incubated with Taq I, which TCGA is the recognition sequence. Since the amplicon analyzed in this case, had more than one site of recognition for this endonuclease, the pattern of bands observed was more sophisticated than in the others analyses. We therefore concentrated on specific fragments in order to identify the polymorphism of interest. If the genotype was wild type (GG), the pattern consisted of three bands: 170 bp, 141 bp and 109 bp. In the GA genotype, four bands of 283 bp, 174 bp, 142 bp and 109 bp were found. Finally, for the homozygous AA, two fragments of 283 bp and 142 bp were observed.

NAT2 C481T

The Kpn I endonuclease was used to analyse this polymorphism. This enzyme recognises the GGTACC sequence. The wild type genotype CC was identified with the observation of two fragments of 661 bp and 550 bp. In the heterozygous CT genotype there were 3 bands of 1211 bp 661 bp and 550 bp and for the TT samples only the 1211 bp fragment was observed.

NAT2 G857A

The BamH I endonuclease, which has the GGATCC sequence as recognition site, was used to identify this polymorphism. The pattern of bands in the wild type genotype GG was two bands of 925 bp and 286 bp, in the heterozygous GA three bands (1211 bp, 925 bp and 286 bp) and in the AA genotype only one band of 1211 bp, because the BamH I could not cut neither allele.

NAT2 A803G

To determine the NAT2 A803G polymorphism, the samples were incubated with Dde I. This enzyme has the CTNAG recognition sequence and therefore, when the wild type genotype GG was present, three bands of 300 bp, 99 bp and 25 bp were obtained. If the genotype was GA, fragments of 300 bp, 297 bp, 128 bp, 99 bp, and 25 bp were expected. However, 25 bp bands were not observed in the 3% agarose gel; and the 300 bp and the 297 bp fragments, the difference being only 3bp, were not differentiated. Finally, for the AA genotype there were only two bands: 297 bp and 128 bp.

Statistical analysis

Statistical analyses were performed using the SNPStats statistical analysis software package (Sole, *et al.* 2006) and GraphPad Prism statistical software (GraphPad Software, San Diego, CA). SNPStats was used to estimate the haplotype analysis and linkage disequilibrium in both investigated groups. Hardy-Weinberg equilibrium was tested for each polymorphism, calculating the chi-square test. All investigated polymorphisms were in Hardy-Weinberg equilibrium. The chi-square test, with one or two degrees of freedom (df), or Fisher's exact test was used to examine differences of genotype and allele frequencies between patients and controls. Odds ratios (OR) and 95% confidence interval (CI) were calculated. P values less than 0.05 were considered to be statistically significant.

Results

The personal characteristics of the individuals included in the study are presented in Table 2. No significant difference in the age of participants between the different groups was detected. Concerning the tumor site, the number of cases with non sigmoid cancer was

higher among male. Adenocarcinoma was the prevalent type among both sexes. Therefore, the results of the polymorphism among males and females were pooled both in cases and controls.

Table 2. Characteristics of the individuals included in the study.

	Patients (n=116)		Controls (n=72)	
	Male (n=60)	Female (n=56)	Male (n=37)	Female (n=35)
Age years	55.1 ± 9.6	52.5 ± 12.9	54.9 ± 10.2	56.9 ± 11.7
Tumor site				
Non sigmoid	24	17	-	-
Sigmoid	19	18	-	-
Rectum	17	21	-	-
Histopathology				
Adenocarcinoma	56	54	-	-
Mucin adenocarcinoma	3	2	-	-
Undifferentiated small cell carcinoma	1	0	-	-

Table 3 provides detailed information on the frequency of the multiple genotypes analysed. One hundred sixteen CRC samples were included in this study and were analysed for 7 SNPs in 2 genes. Association between polymorphism and the risk to develop colon cancer was assessed by comparing cases with the control group consisting of 72 individuals.

Table 3. Distribution of polymorphism in colorectal cancer patients and healthy population (n=188).

Polymorphism	Genotype	Patients (n=116)	Control (n=72)	OR (95% CI)	P
CYP1A1 T3801C	TT	42 (70%)	34 (65.4%)	1	
	TC	14 (23.3%)	16 (30.8%)	0.74 (0.60-3.3)	0.58
	CC	4 (6.7%)	2 (3.8%)	1.74 (0.11-3.5)	0.46
	TC+CC	18 (30%)	18 (34.6%)	0.85 (0.56-2.7)	0.60
CYP1A1 C2453A	CC	32 (28.1%)	22 (30.6%)	1	
	CA	76 (66.7%)	50 (69.4%)	0.96 (0.50-1.83)	0.59
	AA	6 (5.3%)	0 (0%)	0.00 (0.00-NA)	0.56

	CA+AA	82 (71.9%)	50 (69.4%)	0.89 (0.46-1.69)	0.72
CYP1A1 A2455G	GG	19 (16.4%)	46 (63.9%)	1	
	GA	82 (70.7%)	24 (33.3%)	7.65 (2.66-19.25)	0.000
	AA	15 (12.9%)	2 (2.8%)	12.13 (2.68-44.46)	0.000
	GA+AA	97 (83.6%)	26 (36.1%)	8.18 (3.00-20.11)	0.000
NAT2 G590A	AA	8 (12.5%)	12 (17.1%)	1	
	GA	52 (81.2%)	52 (74.3%)	0.67 (0.25-1.7)	0.63
	GG	4 (6.2%)	6 (8.6%)	1.00 (0.21-4.7)	0.59
	GA+GG	56 (87.5%)	58 (82.9%)	0.69 (0.26-1.8)	0.45
NAT2 C481T	CC	38 (35.9%)	34 (54.8%)	1	
	CT	46 (43.4%)	18 (29%)	1.44 (0.21-0.89)	0.064
	TT	22 (20.8%)	10 (16.1%)	1.21 (0.21-1.22)	0.059
	CT+TT	68 (64.2%)	28 (45.2%)	1.46 (0.24-0.87)	0.055
NAT2 G857A	GG	42 (36.2%)	28 (38.8%)	1	
	GA	46 (39.7%)	31 (43.1%)	0.63 (0.28-1.6)	0.094
	AA	28 (24.1%)	13 (18.1%)	1.54 (0.26-1.32)	0.076
	GA+AA	74 (63.8%)	44 (61.2%)	0.57 (0.25-1.12)	0.099
NAT2 A803G	AA	86 (74.1%)	66 (91.7%)	1	
	GA	26 (22.4%)	5 (6.9%)	3.65 (1.39-9.57)	0.006
	GG	4 (3.4%)	1 (1.4%)	4.24 (2.45-16.91)	0.000
	GA+GG	30 (25.8%)	6 (8.3%)	4.17 (1.6-10.81)	0.002

Discussion

The incidence of CRC in the Kingdom of Saudi Arabia has been on a constant rise over the past few years with a prediction of increase in the coming years (Ibrahim *et al*, 2008; Mosly and Al Ahwal, 2012). This increase was reported to be over a three-fold incidence rise in males from 3.2% (Al-Eid and Arteh, 2004) to 11.2% (Haya and Al-Eid, 2007) within around 7 years. A parallel trend in females was observed from 2.7% to 8.8% for the same period. The increasing incidence was referred to demographic changes and the implementation of Western lifestyle (WCRF, 2014).

Little of the genetic variation in CRC has been explained and it is likely that many more variants remain to be identified (Figueiredo *et al*, 2011). In Saudi Arabia, few are the studies dealing with gene polymorphism and the susceptibility to CRC. No association could be detected between the susceptibility to CRC and resistin promoter C-180G genotype (Al-Harithy and Al-Ghafari, 2010), PARP-1 Val762Ala (Alshammari *et al*, 2014), GSTP1 Ile105Val (Khabaz, 2012) and autozygosity (Siraj *et al*, 2012). On the other hand, MMP-

2C-qa306T ANP (Saeed *et al*, 2013 a), PARP1 Lys933Asn and Parp-1 Lys945Asn (Alshammari *et al*, 2014), rs 961253 Lys25Arg (Khan *et al*, 2014), CYP1A1, CYP2E1 and GSTM1 (Saeed *et al*, 2013b) were significantly associated with CRC.

A mass evidence shows susceptibility to cancer is mediated by alterations in detoxifying capacity of genetically determined factors that play a role in cellular defence mechanism against endogenous and exogenous substances, many of which have carcinogenic potential (Khabaz, 2012).

Activation of procarcinogens is mediated mainly by cytochrome P-450 enzymes, phase I biometabolism of xenobiotics, (Koutros *et al*, 2014). Among phase I enzymes, the CYP1A1 gene was studied in the present work. Only one of the three studied SNPs, the CYP1A1 A2455G variant (minor allele), emerged as a risk allele in colorectal cancer patients. Polymorphisms of CYP1A1 A2455G involves the A>G transition in codon 462 (exon 7) leading to the substitution of isoleucine to valine (Ile462Val). As a result, the enzyme is activated and higher amount of carcinogenic molecules are obtained, especially from polycyclic aromatic hydrocarbons. These may be involved in the carcinogenesis of intestinal mucosa (Akiyama *et al*, 2003). The contribution of CYP1A1 A2455G polymorphism to colorectal cancer risk was documented in many previous studies (Gil *et al*, 2012; Jin *et al*, 2011; Zheng *et al* 2012; Gil *et al* 2014).

On the other hand, the present study failed to detect any significant change when CYP1A1 T3801C was analysed. This is in agreement with studies in other populations, for example, no statistically significant differences in CYP1A1 T3801C 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). But other studies showed that CYP1A1 T3801C is significantly associated with CRC in Saudi population (Saeed *et al*, 2014b) and in Japanese living in Hawaii (Nisa *et al*, 2010). It is noteworthy to mention here that allelic frequency of CYP1A1 T3801C in Arabs was reported to be only comparable to that reported in Africans; and that the allelic frequency of CYP1A1 A2455G, while closer to Caucasians, is significantly different from all other populations (Bu *et al*, 2004).

Elimination of environmental chemicals proceeds through the complex detoxification mechanisms and is associated with phase II biometabolic enzymes (Koutros *et al*, 2014). The impact of genetic variation in phase II xenobiotic clearance enzymes has been attributed among others to genes as NAT2 (N-acetyl transferase 2) which was analysed in the present study, and GSTT1 (glutathione S-transferase T1) (Sachse *et al*, 2002). The four studied SNPs of the NAT2 gene included in the present study are associated with slow acetylation (Osian *et al*, 2010). Slow acetylator status in Arabs was found to be similar to Caucasians and significantly higher than in Asians (Bu *et al*, 2004). Only the NAT2 A803G showed significant variations in CRC patients. An impaired detoxification of aromatic amines such as 4-aminobiphenyl, for which N-acetylation is an important detoxification pathway (Hein *et al*, 1993), may explain the findings of greater risk among NAT slow acetylators to develop CRC. This finding is in line with several previous studies (Tiemersma *et al*, 2004; Welfare *et al*, 1997; Moslehi *et al*, 2005; Da Silva *et al*, 2011; Lilla *et al*, 2012; Nothlings *et al*, 2009; Andersen and Vogel 2015).

Although it is difficult to arrive at a definite conclusion from a small data set, our preliminary results support the hypothesis of the potential role of polymorphism of genes

coding for XME in the colon carcinogenesis. Obviously, further studies with large-scale studies including other XME genes are needed to a better understanding of the role of genetic factors in CRC aetiology in Saudi population.

Acknowledgement

The author acknowledges Prof. Suhad Bahajri, Department of Clinical Biochemistry, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia, for her valuable assistance in sample collection.

References

- Agundez, J. (2004) Cytochrome P450 gene polymorphism and cancer. *Curr. Drug Metab.* **5**: 211-224.
- Al-Eid, H. and Arteh, S. (2004) *Cancer Incidence Report 1999-2000*. National Cancer Registry.
- Al-Eid, H.S., Bazarbashi, S. and Al Zahrani, A. (2011) *Cancer Incidence Report, Saudi Arabia 2005*. Saudi Cancer Registry website. Available at: <http://www.scr.org.sa/reports/>. Assessed on December 29, 2014.
- Al-Harithy, R. and Al-Ghafari, A. (2010) Resistin in human colon cancer. Increased expression independently of resistin promoter C-180G genotype. *Saudi Med J* **31**(5): 495-500.
- Al-Saleh, I., Arif, J., El-Doush, I., Al-Sanea, N., Jabbar, A., Billedo, G., Shinwari, N., Mashhour, A. and Mohamed, G. (2008) Carcinogen DNA adducts and the risk of colon cancer: case-control study. *Biomarkers* **13**(2): 201-216.
- Alshammari, A., Shalaby, M., Alanazi, M. and Saeed, H. (2014) Novel mutations of the PARP-1 gene associated with colorectal cancer in the Saudi population. *Asian Pac J Cancer Prev* **15**(8): 3667-3673.
- Andersen, V. and Vogel, U. (2015) Interactions between meat intake and genetic variation in relation to colorectal cancer. *Genes Nutr.* **10**(1): 448.
- Androtsopoulos, V., Tsatsakis, A. and Spandidos, D. (2009) Cytochrome P450 CYP1A1: wider roles in cancer progression and prevention. *BMC Cancer* **9**: 187.
- Boccia, S., Sayed-Tabatabaei, F.A., Persiani, R. and *et al.* (2007) Polymorphisms in metabolic genes, their combination and interaction with tobacco smoke and alcohol consumption and risk of gastric cancer: a case-control study in an Italian population. *BMC Cancer* **7**: 206.
- Bu, R., Gutierrez, M., Al-Rasheed, M., Belgaumi, A. and Bhatia, K. (2004) Variable drug metabolism genes in Arab population. *Pharmacogenomics J.* **4**: 260-266.
- Da Silva, T., Felipe, A., De Lima, J., Oshima, C. and Forones, N. (2011) N-acetyltransferase 2 genetic polymorphisms and risk of colorectal cancer. *World J Gastroenterol.* **17**(6): 760-765.
- Figueiredo, J., Lewinger, J.P., Song, C., Campbell, P., Conti, D., Edlund, C., Duggan, D., Rangrej, J., Lemire, M., Hudson, T., Zanke, B., Cotterchio, M., Gallinger, S., Jenkins, M., Hopper, J., Haile, R., Newcomb, P., Potter, J., Baron, J., Le Marchand, L. and Casey, G. (2011) Genotype-environment interactions in microsatellite stable/ microsatellite instability-low colorectal cancer: Results from a genome-wide association study. *Cancer Epidemiol Biomarkers* **20**(5): 758-766.
- Gelboin, H.V. (1980) Benzo[alpha]pyrene metabolism, activation and carcinogenesis: role and regulation of mixed-function oxidases and related enzymes. *Physiol Rev.* **60**, (4): 1107-1166.
- Georgiadis, P., Topinka, J., Vlachodimitropoulos, D., Stoikidou, M., Gioka, M., Stephanou, G., Autrup, H., Demopoulos, N., Katsouyanni, K., Sram, R. and Kyrtopoulos, S. (2005) Interactions between CYP1A1 polymorphisms and exposure to environmental tobacco smoke in the modulation of lymphocyte bulky DNA adducts and chromosomal aberrations. *Carcinogenesis* **26**(1): 93-101.
- Gil, J., Gaj, P., Misiak, P., Ostrowski, J., Karpinski, P., Jarczyńska, A., Kielan, W. and Sasiadek, M. (2014) CYP1A1 Ile462Val polymorphism and colorectal cancer risk in Polish patients. *Med Oncol.* **31**: 72.
- Gil, J., Ramsey, D., Stembalska, A., Karpinski, P., Pesz, K.A., Laczmanska, I., Leszczynski, P., Grzebieniak, Z. and Sasiadek, M.M. (2012) The C/A polymorphism in intron 11 of the XPC gene plays a crucial role in the modulation of an individual's susceptibility to sporadic colorectal cancer. *Mol Biol Rep.* **39**(1):527-34.
- Haya, S. and Al-Eid, H. (2007) *Cancer Incidence and Survival Report*. Saudi Cancer Registry.
- Hein, D.W., Doll, M.A., Rustan, T.D. and *et al.* (1993) Metabolic activation and deactivation of arylamine carcinogens by recombinant human NAT1 and polymorphic NAT2 acetyltransferases. *Carcinogenesis* **14**:1633-8
- Hezova, R., Bienertova-Vasku, J., Sachlova, M., Brezkova, V., Vasku, A., Svoboda, M., Radova, L., Kiss, I., Vyzula, R. and Slaby, O. (2012) Common polymorphisms in GSTM1, GSTT1, GSTP1, GSTA1 and susceptibility to colorectal cancer in the Central European population. *European J of Med. Res.* **17**:17.
- Ibrahim, E., Zeeneldin, A., El-Khodary, T. and *et al.* (2008) Past, present and future of colorectal cancer in the Kingdom of Saudi Arabia. *Saudi J Gastroenterol* **14**: 178-182.
- Jin, J.Q., Hu, Y.Y., Niu, Y.M., Yang, G.L., Wu, Y.Y., Leng, W.D. and Xia, L.Y. (2011) CYP1A1 Ile462Val

- polymorphism contributes to colorectal cancer risk: a meta-analysis. *World J Gastroenterol.* **17**(2):260–6.
- Khabaz, M.** (2012) The GSTP1 Ile105Val polymorphism is not associated with susceptibility to colorectal cancer. *Asian Pacific J Cancer Prev.* **13**: 2949-2953.
- Khan, W., Abduljaleel, Z., Alanazi, M. and Elrobh, M.** (2014) Evidence of colorectal cancer risk associated variant Lys25Ser in the proximity of human bone morphogenetic protein 2. *Gene* **522**(1): 75-83.
- Koutros, S., Andreotti, G., Berndt, S.I., Hughes, Barry. K., Lubin, J.H., Hoppin, J.A., Kamel, F., Sandler, D.P., Burdette, L.A., Yuenger, J., Yeager, M., Alavanja, M.C. and Freeman, L.E.** (2011) Xenobiotic-metabolizing gene variants, pesticide use, and the risk of prostate cancer. *Pharmacogenet Genomics.* **21**(10):615–23.
- Ladero, J., Agundez, J., Martinez, C., Amo, G., Ayuso, P. and Garcia-Martin, E.** (2012) Analysis of the functional polymorphism in the cytochrome P450 CYP2C8 gene rs11572080 with regard to colorectal cancer risk. *Frontiers in Genetics* **3**:278.
- Landi, M.T., Bertazzi, P.A., Shields, P.G., Clark, G., Lucier, G., Garte, S.J., Cosma, G. and Caporaso, N.E.** (1994) Association between CYP1A1 genotype, mRNA expression and enzymatic activity in humans. *Pharmacogenetics* **4**(5): 242-246.
- Lilla, C., Verla-Tebit, E., Risch, A., Jager, B., Hoffmeister, M., Brenner, H. and Chang-Claude, J.** (2006) Effect of NAT1 and NAT2 genetic polymorphisms on colorectal cancer risk associated with exposure to tobacco smoke and meat consumption. *Cancer Epidemiol Biomarkers Prev.* **15**(1): 99-107.
- Moslehi, R., Chen, J., Hein, D.W. and et al.** (2005) Cigarette smoking, NAT1 and NAT2 susceptibility haplotypes, and the risk of advanced colorectal adenoma. *Proc Am Assoc Cancer Res* **46**:769.
- Mosli, M. and Al-Ahwal, M.** (2012) Colorectal cancer in the Kingdom of Saudi Arabia: Need for screening. *Asian Pac J Cancer Prev.* **13**: 3809-3813.
- Nisa, H., Kono, S., Yin, G. and et al** (2010) Cigarette smoking, genetic polymorphisms and colorectal cancer risk: the Fukuoka colorectal cancer study. *BMC Cancer* **10**: 274.
- Nothlings, U., Yamamoto, J., Wilkens, L., Murphy, S., Park, S., Henderson, B. and et al.** (2009) Meat and heterocyclic amine intake, smoking, NAT1 and NAT2 polymorphisms, and colorectal cancer risk in the lultirthnic cohort study. *Cancer Epidemiol Biomark Prev.* **18**(7): 2098-2106
- Osian, G., Procopciuc, L., Vlad, L., Lancu, C., Criesta, P., Mocan, T. and Mocan, L.** (2010) NAT2 polymorphisms and sporadic colorectal cancer survival. *J Gastrointestin Liver Dis.* **19**(4): 361-368.
- Sachse, C., Smith, G., Wilkie, M.J., Barrett, J.H., Waxman, R., Sullivan, F., Forman, D., Bishop, D.T. and Wolf, C.R.** Colorectal Cancer Study Group. (2002). A pharmacogenetic study to investigate the role of dietary carcinogens in the etiology of colorectal cancer. *Carcinogenesis* **23**(11):1839–49.
- Saeed, H., Alanazi, M., Nounou, H., Shalaby, M., Semlali, A., Azzam, N., Aljebreen, A., Alharby, O., Parine, N., Shaik, J. and Arafaha, M.** (2013b) Cytochrome P450 1A1, 2E1 and GSTM1 gene polymorphisms and susceptibility to colorectal cancer in the Saudi population. *Asian Pacific J Cancer Prev* **14**: 3761-3768.
- Saeed, H., Alanazi, M., Parine, N., Shaik, J., Semlali, A., Alharbi, O., Azzam, N., Aljebreen, A., Almadi, M. and Shalaby, M.** (2013a) Matrix metalloproteinase-2 (-1306 c)t promoter polymorphism and risk of colorectal cancer in the Saudi population. *Asian Pac J Cancer Prev* **14**(10): 6025-6030.
- Siraj, A., Khalak, H., Sultana, M., Al-Rasheed, M., Bavi, P., Al-Sanea, N., Al-Dayel, F., Uddin, Sh., Alkuraya, F. and Al-Kuraya, K.** (2012) Colorectal cancer risk is not associated with increased levels of homozygosity in Saudi Arabia. *Genet Med* **14**(8): 720-728.
- Slattery, M.L., Samowitz, W., Ma, K. and et al.** (2004) CYP1A1, cigarette smoking, and colon and rectal cancer. *Am J Epidemiol.* **160**: 842-52.
- Tiemersma, E.W., Bunschoten, A., Kok, F.J., Glatt, H., de Boer, S.Y. and Kampman, E.** (2004). Effect of SULT1A1 and NAT2 genetic polymorphism on the association between cigarette smoking and colorectal adenomas. *Int J Cancer* **108**:97–103.
- WCRF, W.C.R.F.** (2014, 1/22/2014). *World Cancer Research Fund International*. <http://www.wcrf.org/> World Cancer Research Fund International. Accessed on January 5th, 2015.
- Welfare, M.R., Cooper, J., Bassendine, M.F. and Daly, A.K.** (1997) Relationship between acetylator status, smoking, and diet and colorectal cancer risk in the north-east of England. *Carcinogenesis* **18**:1351–4.
- Woolhouse, N.M., Qureshi, M.M., Bastaki, S.M., Patel, M., Abdulrazzaq, Y. and Bayoumi, R.A.** (1997) Polymorphic N-acetyltransferase (NAT2) genotyping of Emiratis, *Pharmacogenetics*, **7**, (1): 73-82.
- Ye, Z. and Parry, J.M.** (2002) Genetic polymorphisms in the cytochrome P450 1A1, glutathione S- transferase M1 and T1, and susceptibility to colon cancer. *Teratog Carcinog Mutagen.* **22**: 385-92.
- Zheng, Y., Wang, J.J., Sun, L. and Li, H.L.** (2012) Association between CYP1A1 polymorphism and colorectal cancer risk: a meta-analysis. *Mol Biol Rep.* **39**(4):3533–40.

تعدد الأشكال لبعض الجينات المختارة التي تشفر إنزيمات أيض الدخيل الحيوي والقابلية لسرطان القولون والمستقيم في مجتمع سعودي

منصور أحمد الغامدي

قسم العلوم البيئية، كلية الأرصاد والبيئة وزراعة المناطق الجافة،

جامعة الملك عبدالعزيز، جدة، المملكة العربية السعودية

المستخلص. يعتبر سرطان القولون مشكلة صحية منذرة في المملكة العربية السعودية. ويمثل تعدد الأشكال في الجينات المشفرة لإنزيمات أيض الدخيل الحيوي خطراً معنوياً للعديد من أنواع السرطان المرتبطة بالتفاعل بين العوامل البيئية والجينية. وتهدف الدراسة الحالية إلى بحث مشاركة تعدد الأشكال للجينات CYP1A1، و NAT2 في الإصابة بسرطان القولون والمستقيم. طرق البحث: تم القيام بدراسة "عينة الحالة المراقبة في مستشفى" لدى مجتمع سعودي. تم جمع عينات مسحية من الفم لمائة وستة عشر حالة سرطان القولون والمستقيم حديثة التشخيص، واثنين وسبعين فرد كعينة ضابطة. تم تحليل سبعة أشكال تعددية للجينات CYP1A1، و NAT2، وهي: CYP1A1 T3801C، CYP1A1 C2453A، CYP1A1 A2455G، NAT2 G590A، NAT2 C481T، NAT2 G857A، and NAT2 A803G. أظهرت النتائج أن الأشكال التعددية CYP1A1 A2455G، و NAT2 A803G كانت مختلفة معنوياً ($p < 0.05$, 0.001) وتمثل خطراً أكبر في تكوين سرطان القولون والمستقيم عند المقارنة بالشكل التعددي البري (معامل الخطر: ٧,٦٥، ١٢,١٣، ٨,١٨، للـ GA، و AA، و GA+AA بالترتيب للشكل التعددي CYP1A1 A2455G، و ٣,٦٥، ٤,٢٤، ٤,١٧، للـ GA، و GG، و GA+GG بالنسبة للشكل التعددي NAT2 A803G. أما المواقع الأخرى فلم تظهر أي خطر معنوي. والخلاصة، أنه من الأشكال التعددية السبعة التي تم كشفها، قد تكون الأشكال التعددية CYP1A1 A2455G، و NAT2 A803G أهمية في عملية سرطنة القولون. وهناك حاجة لمزيد من الدراسات على نطاق أوسع.

//الكلمات الدالة: الجينات، والتعدد الشكلي، وسرطان القولون والمستقيم.