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CYP19A1 gene polymorphism and colorectal cancer etiology in Saudi population: case–control study

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Background: Considerable interest is directed toward the enzyme aromatase (CYP19A1) and the development of cancer, due to CYP19A1's role in estrogen biosynthesis. Several cancers display excessive intra-tumor accumulation of estrogens, and aromatase inhibitors are used for treatment. The CYP19A1 gene exhibits polymorphism and mutations that can alter its expression or aromatase activity and influence estrogen production. We designed this study to investigate the link between CYP19A1 polymorphism and susceptibility to colorectal cancer (CRC) development in Saudis.

Patients and methods: Blood samples from 100 CRC patients and 100 healthy controls were drawn for DNA extractions. Three polymorphic sites, rs4774585, rs936308, and rs4775936, were genotyped using Taqman genotyping by real-time polymerase chain reaction. Allelic and genotype frequencies were calculated and compared in the two groups.

Results: All single nucleotide polymorphisms (SNPs) were polymorphic in Saudis, and comparison of allele frequencies showed several differences when compared to other populations. None of the SNPs were associated with the risk of CRC development in Saudis ($P>0.05$). Some gender and location (colon or rectal) differences were observed.

Discussion: The results of this study highlighted the genetic heterogeneity existing between populations in the prevalence of different SNPs and their relation to disease state. It showed that, although rs4774585, rs936308, and rs4775936 are involved in CRC development in several populations, their role is not significant in the etiology of CRC in Saudis; however, some SNPs do increase susceptibility or resistance to CRC development as judged from the odds ratio. Further large-scale studies are warranted to clarify the role of the CYP19A1 development in CRC.

Keywords: aromatase, *CYP19A* gene, polymorphism, SNPs, colorectal cancer, rs4774585, rs936308, rs4775936

Introduction

In Saudi Arabia, the second most common cancer, in both men and women, is colorectal cancer (CRC) and it also comprises the second leading cause of cancer-related deaths in this population.[1] The clinical characteristics of CRC are variable and depend largely on the location, that is, colon or the rectum, where cancer develops.[2] The malignant transformation initiates on the inner lining of the colon or the rectum, as a noncancerous growth called a polyp, which develops slowly, over a period of 10–20 years to the malignant state.[2,3]

During the last few years, interest has been directed toward the role of estrogens in the development of CRC. Some of the earlier reports pointed to a protective role as it was shown that men have lower estrogen levels and a higher susceptibility to colon cancer compared to women,[4] and women on hormone replacement therapy (HRT) had

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even lower susceptibility compared to their counterparts not receiving HRT.[5] Studies showed the presence of different isoforms of the estrogen receptor (ER) and the wild type, ERβ, was shown to be overexpressed in the colonic tissue.[6] The ERβ was protective against cancer development, and an inverse relationship was reported to exist between tumor progression and ERβ expression in the CRC cells compared to the normal cells in the same patient.[7] More recent studies demonstrated that total estrogens (estrone and estradiol) were 2- to 2.4-fold higher in the CRC tissue compared to the adjacent normal tissue.[8,9] The intra-tumoral estrogens were locally synthesized and were shown to be significantly associated with poorer survival.[6] Two major pathways were reported to be involved in the local synthesis of estrogens, that is, the aromatase pathway and the sulfatase pathway.[6,10] Though the major intra-tumoral synthetic partway was the sulfatase pathway, the levels of aromatase (EC 1.14.14.1; CYP19A1) were shown to be significantly elevated in the CRC tissue.[8] English et al[11] demonstrated that intra-tumoral aromatase was very active in the colon epithelial and carcinoma tissue and was responsible for the conversion of androstenedione to estrone. A significant association was demonstrated between the levels of total estrogens and the clinical outcome in CRC.[6] Similar finding in other malignancies, including breast cancer,[12–14] gastric cancer,[15] ovarian cancer,[16] and others,[17] points to the significance of estrogens in cancer development. Since the intra-tumoral aromatase is elevated, a treatment strategy using aromatase inhibitors to decrease the level of estrogens was adopted for several of the cancers.[18,19]

Aromatase is considered in several studies as a possible candidate involved in the development of CRC.[20–24] The enzyme is located in the estrogen-producing cells on the endoplasmic reticulum and brings about the conversion of the C19 androgens: androstenedione to estrone and testosterone to estradiol. Several studies discuss its clinical significance.[25,26] It is encoded by the CYP19A1 gene (cytochrome P450 family 19 subfamily A member 1) located at chromosome 15q21.2.[27–29] Several polymorphisms have been reported in the promoter and intronic regions of CYP19A1 gene that may affect the gene product resulting in either increased or decreased aromatase activity. The role of CYP19A1 polymorphisms has been evaluated in breast cancer, endometrial cancer, prostate cancer, Alzheimer's disease, cardiovascular disease, and obesity.[30–35] Certain single nucleotide polymorphisms (SNPs) show a positive correlation, whereas others show a protective effect against the development of these pathological states.

We hypothesized that polymorphisms in CYP19A1 might be associated with the development and outcome in CRC. We designed the present case–control study to investigate three SNPs in the CYP19A1, that is, rs4774585, rs936308, and rs4775936, in Saudi CRC patients. The SNPs were selected based on database NCBI/dbSNP (https://www.ncbi.nlm.nih.gov/snp)[38] and previous population studies that have focused on other types of cancers and diseases in which these SNPs were reported to show a functional effect on the development of these diseases.[36] Selection of each SNP was also based on their location in the gene, where it may play a role in the regulation of CYP19A gene expression. In this paper, we report frequencies of three polymorphisms in the CYP19A1 in normal healthy Saudis and patients suffering from CRC. We also show the differences that exist in the frequency of different alleles of these SNPs in Saudis compared to other populations. We believe this is the first report exploring genetic variations in CYP19A1 in CRC in Saudis.

Patients and methods

Study population

The study was reviewed and approved by the institutional review board of the ethics committee at King Khalid University Hospital (KKUH), Riyadh, Saudi Arabia. The study population comprised 100 cases diagnosed with CRC at the endoscopy service at KKUH, Riyadh. A group of 100 age-matched normal healthy individuals, also attending the endoscopy unit for other reasons and with normal colonoscopy, were recruited as controls. The objectives of the study were explained to both groups, and written informed consent was obtained. Demographic data and clinical data of the patients were collected from the participants and entered on specially designed spreadsheets.

SNP selection and genotyping

Blood was collected from patients and controls, by venepuncture, in EDTA tubes and stored at 4°C until required for analysis. To extract genomic DNA from whole blood, QIAmp DNA blood mini kits (Qiagen, Valencia, CA, USA) were used following the protocol provided by the manufacturer. TaqMan genotyping assay kits (Thermo Fisher Scientific, Waltham, MA, USA) were used for genotyping of the three SNPs (rs4774585, rs936308, and rs4775936). All probes used were those designed by Thermo Fisher Scientific (C__27892984_10, C___1664163_10 and C__11301451_10) following the protocol provided by the manufacturer. The reaction mixture (11 µL) consisted of 5.5 µL of TaqMan PCR master mix, 0.26 µL of SNP assay, and 2 µL of DNA (20 ng)

and the volume was made up by adding 3.25 µL of water. For the PCR amplification, LightCycler 480 Instrument II Real-Time PCR System was used, following the protocol: initial denaturation at 94°C for 15 minutes, followed by 45 cycles at 94°C for 15 seconds and at 60°C for 1 minute.

Statistical methods used for data analysis

The genotypes and allele frequencies of the three SNPs were calculated manually. Fisher's exact test (two-tailed) was applied to obtain the χ^2, odds ratios (OR), and 95% CI, to determine the significance of the difference between the results of the CRC patients and controls. The results were subjected to statistical analysis using the Statistical Package for the Social Sciences (version 21) (SPSS Inc., Chicago, IL, USA). Student's *t*-test was used to determine the significance of the difference between demographic data in the patients and controls.

Results

The demographic and clinical characteristics of CRC patients and the control group were obtained from interviewing the patients and from their hospital files and are presented in Table 1.

The Hardy–Weinberg model was applied to the data, and the results showed that the genotype distributions were consistent with the expected values ($P>0.05$). The OR of CRC association were calculated using the homozygous wild-type allele as a reference for the other two genotypes. The genotype and allele frequencies of the analyzed SNPs, the OR and the significance levels (*P*) for each SNP investigated are presented in Table 2. The genotype frequencies of GG, AG, and AA for rs4774585 were 46%, 46%, and 8%, respectively, in the CRC patients, and 48%, 41%, and 11%, respectively, in the control group. The frequencies of GG, GC, and CC for rs936308 were 5%, 35%, and 60%, respectively, in CRC patients and 9%, 34%, and 57%, respectively, in the control group. Although the frequencies of the CC, CT, and TT genotypes of rs4775936 were 37%, 44%, and 19%, respectively, in CRC cases, and 35%, 47%, and 18% respectively, in the control group. These genotype and allele frequencies for rs4774585, rs936308, and rs4775936 did not show a statistically significant difference between the CRC patients and the healthy control group.

Table 1 Demographic characteristics of colorectal cancer patients and normal control group

Parameters	Normal control (N=100)	Cancer patients (N=100)	*P*-value
Age (years) (mean ± SD)	53.06±1.5	55.50±1.3	>0.05
Sex			>0.05
Male	58	60	
Female	42	40	
Smoking	5	5	>0.05
Non-smoking	95	95	

The patients were stratified according to whether the cancer was in the colon or the rectum, and for each location, the genotype and allele frequencies were calculated, separately for each SNP. Table 3 presents the results for the three SNPs in colon cancer compared to the results in rectal cancer patients. It also shows the results in the colon cancer patients and rectal cancer patients, compared to the results in the control group. The AG genotype of rs4774585 occurred at a significantly higher frequency in the rectal cancer patients compared to the colon cancer patients. None of the other genotypes or alleles of the studied SNPs showed a significant difference between the two groups. When compared to the results in the control group, the CG genotype for rs936308 occurred at significantly higher frequency in the rectal cancer patients. Frequencies of all other genotypes and alleles, for the three SNPs, did not differ in the colon cancer and rectal cancer patients when compared to the results in the control group.

In an attempt to determine gender influence on the frequency of CYP19A genotypes in CRC, the male and female patients were grouped separately, and the frequency of occurrence of the genotype and allele was calculated and compared to the results in the control groups of the same gender. Table 4 presents the findings and shows the significantly lower frequency of the genotype "AA" of rs4774585 in the male CRC patients compared to the control ($P=0.04$), and the genotype "CT" of rs4775936 in female CRC patients compared to the controls. Frequencies of the other genotypes and alleles of the three studied SNPs were similar in the two genders, compared to their sex-matched controls.

The allele frequencies of the three SNPs in healthy Saudis were compared to those reported for other populations in the NCBI SNP database. The results are presented in Table S1.

Discussion

Extensive investigations have reported genetic variations in different genes that may be related to cancer susceptibility and many positive associations have been identified. The aromatase (CYP19A1) gene has been investigated in several studies, where some of the common polymorphisms have been investigated but the results obtained are inconsistent

Table 2 Genotype and allele frequencies of three SNPs in CYP19A1 gene in the CRC patients and controls

SNP	Variation	Case	Control	OR	CI	χ^2	*P*-value
rs4774585	GG	46 (46)	48 (48)	Ref			
	AG	46 (46)	41 (41)	1.17	0.65–2.09	0.28	0.59
	AA	8 (8)	11 (11)	0.76	0.28–2.05	0.30	0.58
	AG + AA	54 (54)	52 (52)	1.08	0.62–1.88	0.08	0.77
	G	138 (69)	137 (68.5)	Ref			
	A	62 (31)	63 (31.5)	0.977	0.64–1.49	0.01	0.91
rs936308	GG	5 (5)	9 (9)	Ref			
	CG	35 (35)	34 (34)	0.97	0.53–1.77	0.01	0.94
	CC	60 (60)	57 (57)	1.89	0.59–5.99	1.21	0.27
	CG + GG	40 (40)	43 (43)	0.88	0.50–1.55	0.19	0.66
	G	45 (22.5)	52 (26)	Ref			
	C	155 (77.5)	148 (74)	1.21	0.76–1.91	0.67	0.41
rs4775936	CC	37 (37)	35 (35)	Ref			
	CT	44 (44)	47 (47)	0.88	0.47–1.60	0.15	0.70
	TT	19 (19)	18 (18)	0.99	0.45–2.20	0.00	0.99
	CT + TT	63 (63)	65 (65)	0.91	0.51–1.63	0.09	0.76
	C	118 (59)	117 (58.5)	Ref			
	T	82 (41)	83 (41.5)	0.98	0.65–1.45	0.01	0.92

Abbreviations: CRC, colorectal cancer; OR, odds ratio; Ref, reference; SNP, single nucleotide polymorphism; CI, confidence interval.

in regard to their possible association with CRC risk, the location of the tumor, levels of sex hormones, and survival rate of the patients. Population-related variations are also frequent. Hence, aromatase has become the target for considerable research in the exploration of cancer association and a potential target for therapeutic interventions due to the significant role it plays in the synthesis of estrogens.[25,26] The intra-tumoral concentration of estrogens is elevated in several cancers, including colon cancer. Estrogen levels are higher in aromatase positive cancer cells, and a study by Niikawa et al[37] confirmed that aromatase levels were higher in the men compared to women, even those in their postmenopausal state. This elevated level of aromatase may be a consequence of elevated gene expression in the cancer tissue and the increase in amount, or enzyme activity may result in increased production of estrogens. Elevated aromatase immunoreactivity in the colon cancer tissue was demonstrated, confirming an increased amount of aromatase. Furthermore, a close association was shown between aromatase mRNA and the levels of estradiol in the tissue. Niikawa et al[37] and Sato et al[8] showed that there was a five fold increase in aromatase mRNA in cancerous compared to the adjacent noncancerous tissue. All of these point to an elevated aromatase gene expression, and this may be due to mutations or SNPs in the regions of the gene responsible for control of gene expression. With this background, we have explored three polymorphisms in aromatase gene and have selected two SNPs in the promoter region and one in the intronic region of the CYP19A1 gene. The selection was based on the fact that these regions in the gene may influence the rate of gene expression.

Of the three SNPs investigated, two of the SNPs, rs4774585, an A > G transition and rs936308, C > G transversion, are located in the promoter region; while the third SNP rs4775936 is located in the intronic region and is a C > T transition.

The SNPs, rs4774585, and rs936308 were studied to detect if the promoter region mutation has an influence on the gene expression of CYP19A1 gene and whether it plays a role in the development of CRC. Our results showed that these SNPs exhibit polymorphism in Saudis; however, the frequency of the genotypes did not differ when the CRC patients were compared to controls. When stratified by sex, AA genotype of rs4774585 showed a significant difference between CRC patients and controls in the male group (*P*-value =0.04). The presence of "A" allele in the homozygous state (AA) decreased the OR to 0.28, compared to 3.55 in the homozygous GG and 1.52 in the heterozygous AG state, suggesting a negative association with CRC development in males and exhibits a recessive inheritance. Comparison of the frequencies of G and A in Saudis with those reported by NCBI[36] in different population groups showed several differences compared to the Hispanic and Asian populations, but the frequencies in Saudis were similar to those reported for Europeans and Sub-Saharan Africans.[36] For the SNP rs936308, the ancestral allele G was found to occur at a low frequency in the Saudi population both the CRC and the control group, compared to the results in other populations.

Table 3 Comparison of the genotype and allele frequencies of three SNPs in CYP19A1 gene in the colon and rectum cancer patients and the control group

SNP	Variation	Case[a]		Control	Colon vs rectal				CRC vs control			
		Colon	Rectal		OR	CI	χ^2	*P*-value	OR	CI	χ^2	*P*-value
rs4774585	GG	46 (70.7)	–	48 (48)	Ref				Ref			
		–	15 (45.4)									
	AG	11 (16.9)	–	41 (41)	4.46	1.70–11.6	9.86	0.001	1.05	0.54–2.04	0.03	0.86
		–	16 (48.4)						1.24	0.55–2.83	0.28	0.59
	AA	8 (14)	–	11 (11)	0.76	0.14–4.01	0.10	0.75	0.84	0.28–2.51	0.09	0.76
		–	2 (6.06)						0.58	0.11–2.92	0.44	0.50
	AG + AA	19 (29.2)	–	52 (52)	2.90	1.21–6.92	5.97	0.014	1.21	0.42–3.46	0.13	0.71
		–	18 (54.5)						1.10	0.50–2.44	0.06	0.78
	G	103 (56.9)		137 (68.5)	Ref				Ref			
			46 (69.6)									
	A	27 (18.46)		63 (31.5)	1.65	0.84–3.25	2.18	0.13	0.966	0.60–1.55	0.02	0.88
			20 (30.3)						0.94	0.51–1.72	0.03	0.855
rs936308	GG	4 (0.06)		9 (9)	Ref				Ref			
			1 (3.03)									
	CG	22 (33.8)		34 (34)	3.81	0.39–37.0	1.50	0.22	0.94	0.48–1.85	0.03	0.87
			21 (63.6)						3.201	1.37–7.44	7.65	0.005
	CC	38 (58.4)		57 (57)	1.15	0.11–11.4	0.02	0.90	1.53	0.44–5.35	0.47	0.49
			11 (33.3)						1.73	0.19–15.12	0.26	0.61
	CG + GG	27 (41.5)		43 (43)	1.15	0.11–11.4	0.02	0.90	1.50	0.44–5.11	0.44	0.50
			22 (66.6)						2.65	1.16–6.04	5.56	0.01
	G	30 (23.0)		52 (26)	Ref				Ref			
			23 (34.8)									
	C	98 (75.3)		148 (74)	0.57	0.29–1.09	2.86	0.09	1.17	0.69–1.96	0.36	0.54
			43 (65.1)						0.65	0.36–1.19	1.92	0.16
rs4775936	CC	31 (47.6)		35 (35)	Ref				Ref			
			16 (48.4)									
	CT	22 (33.8)		47 (47)	0.96	0.37–2.48	0.00	0.94	0.88	0.47–1.60	0.15	0.70
			11 (33.3)						0.51	0.21–1.23	2.24	0.13
	TT	12 (18.4)		18 (18)	0.96	0.30–3.06	0.00	0.95	0.99	0.45–2.20	0.00	0.99
			6 (18.1)						0.72	0.24–2.18	0.32	0.57
	CT + TT	34 (52.3)		65 (65)	0.96	0.41–2.24	0.01	0.94	0.91	0.51–1.63	0.09	0.76
			17 (51)						0.57	0.25–1.26	1.91	0.16
	C	84 (64.6)		117 (58.5)	Ref				Ref			
			43 (65.1)									
	T	46 (35.3)		83 (41.5)	0.97	0.52–1.81	0.01	0.94	0.77	0.48–1.21	1.24	0.26
			23 (34.8)						0.75	0.42–1.34	0.92	0.33

Note: [a]The total number of patients is not 100, since the location was not available for a few patients.
Abbreviations: CRC, colorectal cancer; OR, odds ratio; Ref, reference; SNP, single nuclear polymorphism; CI, confidence interval.

When the frequencies of G and C in Saudis were compared with different populations, several differences were identified with the Asians, and Sub-Saharan African populations, although a similarity was seen to the reports for Europeans and Hispanic populations.[36] When the patients were grouped based on the location of cancer, that is, colon or rectum, the GC genotype showed a significant difference between the patients with rectal cancer compared to the control group (*P*-value =0.0056). The presence of homozygous GG state decreased the OR to 0.57 compared to 1.73 in the homozygous CC. Hence, the allele "G" seems to have a negative association with rectal cancer development.

For rs4775936, when the samples were grouped according to the gender, the frequency of CT heterozygous showed a statistically significant difference between female CRC patients and the controls (*P*=0.029). The presence of homozygous TT state decreased the OR to 0.68, whereas the OR was 1.4 in the homozygous CC state, thus showing a negative association of the T allele and CRC development in females.

Conclusion

This study has shown that the studied SNPs exhibit polymorphism in Saudis. It has confirmed our earlier observations during our studies that the frequencies of different SNPs

Table 4 Genotype and allele frequencies of three SNPs in CYP19A1 gene in females and males colorectal cancer patients and the control group

SNP	Variation	Case[a]	Control	OR	CI	χ^2	*P*-value
rs4774585		Females					
	GG	18 (42.85)	29 (49.15)	Ref			
	AG	20 (47.61)	12 (28.33)	1.11	0.49–2.52	0.06	0.80
	AA	4 (9.52)	1 (1.69)	6.44	0.66–62.3	3.22	0.07
	AG + AA	24 (57.14)	13 (22.03)	1.28	0.58–2.85	0.39	0.53
	G	56 (66.66)	70 (59.32)	Ref.			
	A	28 (33.33)	14 (11.86)	1.40	0.76–2.58	1.18	0.27
		Males					
	GG	27 (46.55)	19 (46.34)	Ref			
	AG	26 (44.82)	12 (29.26)	1.52	0.61–3.75	0.85	0.35
	AA	4 (6.89)	10 (24.39)	0.28	0.07–1.03	3.90	0.04
	AG + AA	29 (50)	22 (53.65)	0.96	0.42–2.14	0.01	0.91
	G	80 (68.96)	50 (60.97)	Ref			
	A	56 (48.27)	32 (39.02)	0.66	0.36–1.20	1.81	0.17
rs936308		Females					
	GG	3 (7.14)	4 (6.77)	Ref			
	CG	11 (26.19)	23 (38.98)	0.54	0.22–1.31	1.83	0.17
	CC	28 (66.66)	32 (54.23)	1.16	0.24–5.66	0.04	0.84
	CG + GG	13 (30.95)	27 (45.76)	0.59	0.26–1.34	1.57	0.20
	G	17 (20.23)	31 (26.27)	Ref			
	C	67 (79.76)	69 (58.47)	1.40	0.71–2.74	0.99	0.32
		Males					
	GG	3 (5.17)	5 (12.19)	Ref			
	CG	23 (39.65)	11 (26.82)	1.63	0.67–3.97	1.18	0.27
	CC	32 (55.17)	25 (60.97)	2.13	0.46–9.79	0.98	0.32
	CG + GG	26 (44.82)	22 (53.65)	1.27	0.56–2.86	0.33	0.56
	G	29 (25.38)	21 (25.60)	Ref			
	C	87 (75.61)	61 (74.39)	1.03	0.53–1.97	0.01	0.92
rs4775936		Females					
	CC	20 (47.61)	17 (28.81)	Ref			
	CT	14 (33.33)	32 (54.23)	0.37	0.15–0.91	4.73	0.02
	TT	8 (19.04)	10 (16.94)	0.68	0.21–2.21	0.45	0.50
	CT + TT	22 (52.38)	42 (71.18)	0.44	0.19–1.01	3.74	0.05
	C	54 (64.28)	66 (55.93)	Ref.			
	T	30 (35.71)	52 (44.06)	0.70	0.39–1.25	1.42	0.23
		Males					
	CC	18 (31.03)	18 (43.9)	Ref			
	CT	29 (50)	16 (39.02)	1.81	0.74–4.43	1.71	0.19
	TT	11 (18.96)	7 (17.07)	1.57	0.49–4.96	0.60	0.44
	CT + TT	40 (68.96)	23 (56.09)	1.73	0.75–3.99	1.72	0.18
	C	65 (56.03)	52 (63.41)	Ref			
	T	51 (43.96)	30 (36.58)	1.36	0.76–2.42	1.08	0.29

Note: [a]The total number of patients is not 100, since the gender was not recorded for a few patients.
Abbreviations: OR, odds ratio; Ref, reference; SNP, single nucleotide polymorphism; CI, confidence interval.

in Saudi population differ considerably when compared to other populations thus any potential therapeutic development targeting this pathway in other populations might not be applicable to this population and emphasizes the notion of personalized treatment targets. Studies on a larger population-based levels are required to confirm our findings and investigations on ethnic groups within Saudi Arabia may provide insight into the role of polymorphisms in *CYP19A1* gene in CRC development. Furthermore, Saudis may be carrying SNPs that are specific for this population and to identify these SNPs in *CYP19A1* gene, future studies have to be directed to the sequencing of the gene.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Annual Cancer Incidence Report for Saudi Cancer Registry (SCR) Saudi Health Council. Riyadh: Kingdom of Saudi Arabia; 2011.
2. Winawer SJ, Zauber AG. The advanced adenoma as the primary target of screening. *Gastrointest Endosc Clin N Am*. 2002;12:1–9.
3. Stryker SJ, Wolff BG, Culp CE, Libbe SD, Ilstrup DM, MacCarty RL. Natural history of untreated colonic polyps. *Gastroenterology*. 1987; 93(5):1009–1013.
4. Ries LA, Wingo PA, Miller DS, et al. The annual report to the nation on the status of cancer, 1973–1997, with a special section on colorectal cancer. *Cancer*. 2000;88(10):2398–2424.
5. Rossouw JE, Anderson GL, Prentice RL, et al. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *JAMA*. 2002;288(3):321–333.
6. Sato R, Suzuki T, Katayose Y. Steroid sulfatase and estrogen sulfotransferase in colon carcinoma: regulators of intratumoral estrogen concentrations and potent prognostic factors. *Cancer Res*. 2009;69(3): 914–922.
7. Bardin A, Boulle N, Lazennec G, Vignon F, Pujol P. Loss of ER beta expression as a common step in estrogen-dependent tumor progression. *Endocr Relat Cancer*. 2004;11(3):537–551.
8. Sato R, Suzuki T, Miura K, et al. Aromatase in colon carcinoma. *Anticancer Res*. 2012;32(8):3069–3075.
9. Mauri D, Pavlidis N, Polyzos NP, Ioannidis JP. Survival with aromatase inhibitors and inactivators versus standard hormonal therapy in advanced breast cancer: meta-analysis. *J Natl Cancer Inst*. 2006; 98(18):1285–1291.
10. Sasano H, Suzuki T, Nakata T, Moriya T. New development in intracrinology of breast carcinoma. *Breast Cancer*. 2006;13(2):129–136.
11. English MA, Stewart PM, Hewison M. Estrogen metabolism and malignancy: analysis of the expression and function of 17beta-hydroxysteroid dehydrogenases in colonic cancer. *Mol Cell Endocrinol*. 2001;171(1–2):53–60.
12. Chetrite GS, Cortes-Prieto J, Philippe JC, Wright F, Pasqualini JR. Comparison of estrogen concentrations, estrone sulfatase and aromatase activities in normal, and in cancerous, human breast tissues. *J Steroid Biochem Mol Biol*. 2000;72(1–2):23–27.
13. Pasqualini JR, Chetrite G, Blacker C, et al. Concentrations of estrone, estradiol, and estrone sulfate and evaluation of sulfatase and aromatase activities in pre- and postmenopausal breast cancer patients. *J Clin Endocrinol Metab*. 1996;81(4):1460–1464.
14. Suzuki T, Miki Y, Moriya T, et al. 5Alpha-reductase type 1 and aromatase in breast carcinoma as regulators of *in situ* androgen production. *Int J Cancer*. 2007;120(2):285–291.
15. Izawa M, Inoue M, Osaki M, et al. Cytochrome P450 aromatase gene (*CYP19*) expression in gastric cancer. *Gastric Cancer*. 2008;11(2): 103–110.
16. Cunat S, Rabenoelina F, Daures JP, et al. Aromatase expression in ovarian epithelial cancers. *J Steroid Biochem Mol Biol*. 2005;93(1): 15–24.
17. Honma N, Hosoi T, Arai T, Takubo K. Estrogen and cancers of the colorectum, breast, and lung in postmenopausal women. *Pathol Int*. 2015;65(9):451–459.
18. Goss PE, Ingle JN, Pritchard KI, et al. Extending Aromatase-Inhibitor Adjuvant Therapy to 10 Years. *N Engl J Med*. 2016;375(3):209–219.
19. Hong Y, Chen S. Aromatase inhibitors: structural features and biochemical characterization. *Ann N Y Acad Sci*. 2006;1089:237–251.
20. Honma N, Hosoi T, Arai T, Takubo K. Estrogen and cancers of the colorectum, breast, and lung in postmenopausal women. *Pathol Int*. 2015; 65(9):451–459.
21. Foster PA. Oestrogen and colorectal cancer: mechanisms and controversies. *Int J Colorectal Dis*. 2013;28(6):737–749.
22. Lin JH, Manson JE, Kraft P, et al. Estrogen and progesterone-related gene variants and colorectal cancer risk in women. *BMC Med Genet*. 2011;12:78.
23. Lundgreen A, Herrick JS, Kadlubar S, Caan BJ, Potter JD, Wolff RK. Variation in the CYP19A1 gene and risk of colon and rectal cancer. *Cancer Causes Control*. 2011;22(7):955–963.
24. Slattery ML, Lundgreen A, Herrick JS, et al. Variation in the CYP19A1 gene and risk of colon and rectal cancer. *Cancer Causes Control*. 2011; 22(7):955–963.
25. Simpson ER, Clyne C, Rubin G, et al. Aromatase a brief overview. *Ann Rev Physiol*. 2002;64(1):93–127.
26. Czajka-Oraniec I, Simpson ER. Aromatase research and its clinical significance. *Endokrynol Pol*. 2010;61(1):126–134.
27. Simpson ER, Mahendroo MS, Means GD, et al. Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. *Endocr Rev*. 1994;15(3):342–355.
28. Chen SA, Besman MJ, Sparkes RS, et al. Human aromatase: cDNA cloning, Southern blot analysis, and assignment of the gene to chromosome 15. *DNA*. 1988;7(1):27–38.
29. Sebastian S, and Bulun SE. A highly complex organization of the regulatory region of the human CYP19 (aromatase) gene revealed by the Human Genome Project. *J Clin Endocrinol Metab*. 2001;86(10): 4600–4602.
30. Yang L, Wang XY, Li YT, et al. CYP19 gene polymorphisms and the susceptibility to breast cancer in Xinjiang Uigur women. *Genet Mol Res*. 2015;14(3):8473–8482.
31. Yang HP, Gonzalez BJ, Li Q, et al. Common genetic variation in the sex hormone metabolic pathway and endometrial cancer risk: pathway-based evaluation of candidate genes. *Carcinogenesis*. 2010;31(5): 827–833.
32. Ersekerci E, Sofikerim M, Taheri S, Demirtas A, Halis F. Genetic polymorphism in sex hormone metabolism and prostate cancer risk. *Genet Mol Res*. 2015;14(3):7326–7334.
33. Chace C, Pang D, Weng C, et al. Variants in CYP17 and CYP19 cytochrome P450 genes are associated with onset of Alzheimer's disease in women with Down syndrome. *Alzheimers Dis*. 2012;28(3):601–612.
34. Sowers MR, Randolph JF, Zheng H, et al. Genetic polymorphisms and obesity influence estradiol decline during the menopause. *Clin Endocrinol (Oxf)*. 2011;74(5):618–623.
35. Alanazi M, Al-Jafari A, Mobeirek, et al. Significant contribution of promoter region polymorphism in CYP19 (aromatase) gene to cardiovascular disease in Saudis. *Exp Clin Cardiol*. 2014;20:6748–6766.
36. Database of Single Nucleotide Polymorphisms (dbSNP) [webpage on the Internet]. Bethesda, MD: National Center for Biotechnology Information, National Library of Medicine. (dbSNP Build ID: 142). Available from: http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=4774585. Accessed October 23, 2016.
37. Niikawa H, Suzuki T, Miki Y, et al. Intratumoral estrogens and estrogen receptors in human non–small cell lung carcinoma. *Clin Cancer Res*. 2008;14(14):4417–4426.
38. NCBI SNP database. Available from: https://www.ncbi.nlm.nih.gov/snp. Accessed September 5, 2016.

Supplementary material

Table S1 Allele frequencies in the three studied SNPs in Saudis compared to frequencies in populations reported in NCBI SNP database[1]

Populations	rs4774585			Significance compared to Saudi population*	rs936308			Significance compared to Saudi population*	rs4775936			Significance compared to Saudi population*
	Chromosome sample number	A	G		Chromosome sample number	C	G		Chromosome sample number	C	T	
Saudi Arabian	200	0.315	0.685		200	0.740	0.260		200	0.585	0.415	
European	1,006	0.219	0.780	0.0055	1,006	0.8399	0.1600	0.0007	1,006	0.567	0.432	0.6987
African	1,322	0.150	0.849	<0.0001	1,322	0.3026	0.697	0.2557	1,322	0.892	0.1074	<0.0001
American	694	0.273	0.726	0.2931	694	0.7839	0.216	0.2298	694	0.716	0.2839	0.0006
South American	978	0.181	0.819	<0.0001	978	0.672	0.3272	0.0738	978	0.6933	0.3067	0.0035
Asia	172	0.005	0.994	<0.0001	170	0.5882	0.4117	0.0001	172	0.6220	0.3779	0.4607

Note: *This study.

Abbreviation: SNP, single nucleotide polymorphism.

Reference

1. NCBI SNP database. Available from: https://www.ncbi.nlm.nih.gov/snp. Accessed September 5, 2016.