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ORIGINAL RESEARCH

Risk of Colorectal Carcinoma May Predispose to the Genetic Variants of the *GST, CYP450*, and *TP53* Genes Among Nonsmokers in the Saudi Community

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Purpose: Colorectal carcinoma (CRC) represents a considerable public health burden in Saudi Arabia. Several candidate genes and genetic variants have been associated with morbidity and mortality among patients with CRC. We explored whether allelic variants of the *GSTM1, GSTT1, CYP450* (rs4646903 and rs1048943), and *TP53* (rs1042522) genes predisposed nonsmoking Saudi individuals to increased risk for CRC.

Patients and Methods: DNA from buccal cells of 158 participants (80 with CRC and 78 healthy controls) were analyzed for five SNPs using conventional PCR and TaqMan genotyping assays. The SNPStats software was utilized to choose the best interactive inheritance mode for selected SNPs (https://www.snpstats.net).

Results: The mean age of diagnosis was 62.4±13.5 years (range, 40–83 years), with those aged 71–80 years and those aged 40–50 years accounting for the most diagnoses (35.7% and 28.6% of diagnosis, respectively). The *GSTM1* and *TP53* rs1042522 SNPs were associated with CRC (OR= 3.7; $P< 0.0001$, and OR= 1.6; $P= 0.033$, respectively). A plausible contribution to CRC was observed for the *GSTM1* and *TP53* rs1042522 SNPs ($x^2_{Yates}= 14.7$; $P= 0.00013$, and $x^2_{Yates}= 11.2$; $P= 0.0008$, respectively), while the *GSTT1* null variant did not affect risk. Heterozygosity in the *CYP450* (rs4646903 and rs1048943 SNPs) was associated with a significant risk for CRC. The *GSTM1*/*GSTT1* and *CYP450* rs4646903/rs1048943 SNP pairs were in linkage disequilibrium, and the associations were statistically significant ($P= 0.01$ and $P= 4.6\text{x}10^{-7}$, respectively).

Conclusion: The *GSTM1* and *TP53* rs1042522 variants can increase the development of CRC in Saudi nonsmokers. Even the presence of one copy of a variant allele in the *CYP1A1* gene can predispose CRC risk. Additional studies should also examine other SNP combinations with lifestyle factors that may help prevent, rather than facilitate, colorectal tumorigenesis.

Keywords: colorectal carcinoma, single nucleotide polymorphism, TaqMan genotyping, linkage disequilibrium, age at diagnosis, nonsmokers

Plain Language Summary

Colorectal cancer (CRC) is the second most common cancer in men and the third most common in women in Saudi Arabia. The current study focuses on specific genes predisposed to the CRC risk among nonsmokers. Multiple genes involved in CRC development with strong genetic impact have been identified in different cultures and ethnic peoples. The

heterogeneity and the interaction effects of genetic variants could play a considerable role in complex multifactorial disorders. Advanced methods such as whole-exome analysis may unveil further predictive factors and help explain a susceptibility to the disease risk. The increasing trend of CRC among young adults suggests that significant lifestyle modifications are necessary. Unfortunately, malnutrition or insufficient food will also inhibit cell apoptosis and thus enhance tumor cells' growth. Several popular foods and other dietary and lifestyle products and smoking habits are considered risk factors for CRC in young adults. Under the environmental pressure of malnutrition or insufficient nutrition, cancer cells grow better than normal cells.

Introduction

Genetic factors are known to cause the development of many common cancers. In some countries, genetic susceptibility accounts for 35% of colorectal carcinoma (CRC) cases,[1] most of which remain unexplained. In Saudi Arabia, CRC is the second most common cancer among men and the third most common among women.[2] In the past two decades, the CRC prevalence in Saudi Arabia has nearly doubled from 4.8% to 10.1%.[3,4] The differences in CRC prevalence among distinct countries, with marked regional variation, indicate that environmental factors, such as diet and exposure to carcinogens, could have an important role in cancer risk.[5]

The selection of candidate genes is always laborious, especially in multifactorial disorders and cancer in which exposure to endogenous and exogenous toxins is problematic. Thus, various members of the Phase I cytochrome P450 (CYP) and Phase II glutathione S-transferase (GST) gene families are of interest in various cancers. GSTs play significant roles in detoxifying environmental pollutants, carcinogenic compounds, reactive oxidative species to protect DNA from oxidative damage.[5–7] Loss or reduction of the enzyme activity inhibits toxin neutralization and may indirectly affect the risk of cancer development.[7] Several tumor studies focus on the most common GST classes, namely mu (μ) and theta (θ). *GSTM1* (MIM #138350) and *GSTT1* (MIM #600436) genes are commonly focused due to their high frequency of polymorphisms and broad expression in gastrointestinal tissue.[8–10]

CYPs are heme proteins with an important function in detoxifying, activating, and metabolizing several endogenous and exogenous toxins by adding an oxygen atom to their substrate (Stavrinou et al, 2015).[11–13] CYP1A1, an extra enzyme responsible for the aryl-hydrocarbon hydroxylase activity, was already seen in the context of CRC 28 years ago.[14,15] This enzyme is involved in the metabolic activation of several carcinogenic substances.[16] Moreover, CYP1A1 play a crucial role in the metabolic activation of polyaromatic hydrocarbons and heterocyclic amines, both known to cause CRC.[7,17] Although, there is potential support for several studies on smoking-related cancers and CYP1A1 polymorphisms,[18–20] few studies found no significant associations between CYP1A1 and enzyme inducibility.[16,21] Two common gene polymorphisms (*CYP1A1*2A* rs4646903 and *CYP1A1*2C* rs1048943) are correlated with a predisposition to different cancer types,[22] and a significant association has been observed between these *CYP1A1* variants and in situ CRC.[23]

Reports from various types of carcinomas also reported the importance of genes involved in cell cycle control.[24,25] p53 protein, a tumor suppressor, is considered one of the most significant cancer development drivers in various organs, including the colon.[26] *TP53* contributes to cell monitoring, including cell cycle control, DNA repair, genomic plasticity, differentiation, and cell apoptosis.[27] Once the cell is damaged, p53 protein changes the cell cycle or induces apoptosis by repairing DNA.[28] Otherwise, the genomic instability caused by the deletion of p53 may make tumor cells accumulate more cancer drivers, thus accelerating carcinogenesis, tumor metastasis, and drug resistance.[29] The *TP53* gene (*TP53*, MIM #191170) mutation rate in non-hypermutated CRC is about 60%, making *TP53* mutations the second most frequent mutations seen in CRC. The mutation rate of *TP53* is lower (almost 20%) in hypermutated CRC[30,31] but is significantly increased in advanced CRC patients (higher than 60%).[32]

Previous studies addressing the impact of polymorphisms in *GSTs, CYP450*, and *TP53* have found different effects on cancer types among different ethnic populations.[19,33–35] This study investigated associations between the common *GSTM1, GSTT1, CYP450* (rs4646903 and rs1048943), and *TP53* (rs1042522) polymorphisms and the risk of CRC in the non-smoking Saudi community.

Patients and Methods

Ethics Statement

This study was conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained for the study from the Institutional Biomedical Ethics Committee at Medicine college-Umm Al-Qura University (reference #HAPO-02K-012), licensed from the National Committee of Medical and

Bioethics-Riyadh (http://bioethics.kacst.edu.sa/About.aspx?lang=en-US). All individuals provided informed consent before enrollment in the study.

Study Population

Our study included eighty nonsmoking patients from the Oncology Department at King Abdullah City Hospital (Western region of Saudi Arabia, Mecca) who had CRC confirmed by a histopathological diagnosis of specimens collected during colonoscopy surgery (unpublished data). Individuals who currently or previously smoked were excluded from this study due to correlations between tobacco smoke and the examined polymorphic variants. Epidemiological data for eligible individuals, including sex, age, and history of histopathology other than CRC, were obtained. Patients who had undergone radiotherapy or chemotherapy before surgery were excluded. Nonsmoking individuals (n = 78; age 51–88 years) were selected as healthy controls if they had no clinical evidence of malignancies or ulcerative colitis.

DNA Isolation

Within the Molecular Diagnostic Laboratory in the Medicine College of Umm Al-Qura University, DNA was extracted from buccal cells (Oragene OGR-575 kit, DNA Genotek Inc., Ottawa, ON, Canada). Briefly, buccal cells were collected in the Oragene tube within 30 s and capped immediately. OGR-lysis buffer was added, and the cells were incubated in a 53°C water bath for an hour. DNA samples were precipitated by ethanol and dissolved in an aqueous elution buffer.[36]

Diplex Amplifications of *GSTM1*/*GSTT1* Loci

We investigated the *GSTM1* and *GSTT1* gene deletions using a diplex PCR strategy, as described by Hezova et al[37]. The amplicon fragments (215 bp and 480 bp) were separated on a 2% agarose/ethidium bromide gel and visualized. These fragments were aligned with internal control and a blank test to help confirm the successful PCR amplification.

TaqMan Genotyping Analysis

We implemented TaqMan Real-Time PCR assays (Fast Dx Real-Time PCR System, Model 7500, Thermo Fisher Inc., USA) to genotype the individuals for the *CYP450* (rs4646903, and rs1048943) and *TP53* rs1042522 SNPs. One hundred fifty-eight cases and controls and eight negative controls were loaded in a 96-well plate to validate the genotype results. Assays were repeated for 10% of the genotypes for confirmation.

Bioinformatics Analysis

We utilized Sorting Intolerant from Tolerant (SIFT) (https://sift.bii.a-star.edu.sg), Polymorphism Phenotyping v2 (PolyPhen-2) (http://genetics.bwh.harvard.edu/pph2/), MutationTaster (https://www.mutationtaster.org/ChrPos.html), Functional Analysis through Hidden Markov Models (FATHMM), Mutation Assessor, and the in-silico LoFtool to predict the effects of the SNPs on the functional proteins (Ensemble Variant Effect Predictor; https://www.ensembl.org/vep).

Statistical Analysis

Hardy-Weinberg equilibrium (HWE) was evaluated to detect any significant differences between the observed and expected genotypic distributions for CRC cases using the chi-square (χ^2) test (https://www.genecalculators.net/pq-chwe-genotypes.html). The SNPStats software (https://www.snpstats.net) was used to choose the best interactive model of inheritance for all the examined markers among cases and controls (adjusted by gender) and to examine linkage disequilibrium (LD) between the polymorphic markers. Moreover, the statistical significance of the LD was calculated based on the coefficient of LD (D') and the correlation coefficient between pairs of loci (r). All statistical parameters utilized in this study, including the odds ratio (OR), 95% confidence interval (CI), z-test, and χ^2-test in terms of *P*-value, were calculated for genotype distributions and allele frequencies with Social Science Statistics (https://www.socscistatistics.com/tests/) and MedCalc Statistical Software (https://www.medcalc.org).

Results

Characteristics of the Study Population

Eighty eligible Saudi nonsmokers with CRC (55 men: 25 women; a ratio of 2.2: 1) and 78 controls (54 men: 24 women; a ratio of 2.25: 1) were enrolled in the study. Thirty-nine individuals who had undergone radiotherapy or chemotherapy (n = 12), had ulcerative colitis (n= 21) or had other cancer types (n= 6) were excluded from the study (Figure 1).

The mean age of diagnosis was 62.4 ± 13.5 years (range, in the 40–83 years), with no significant

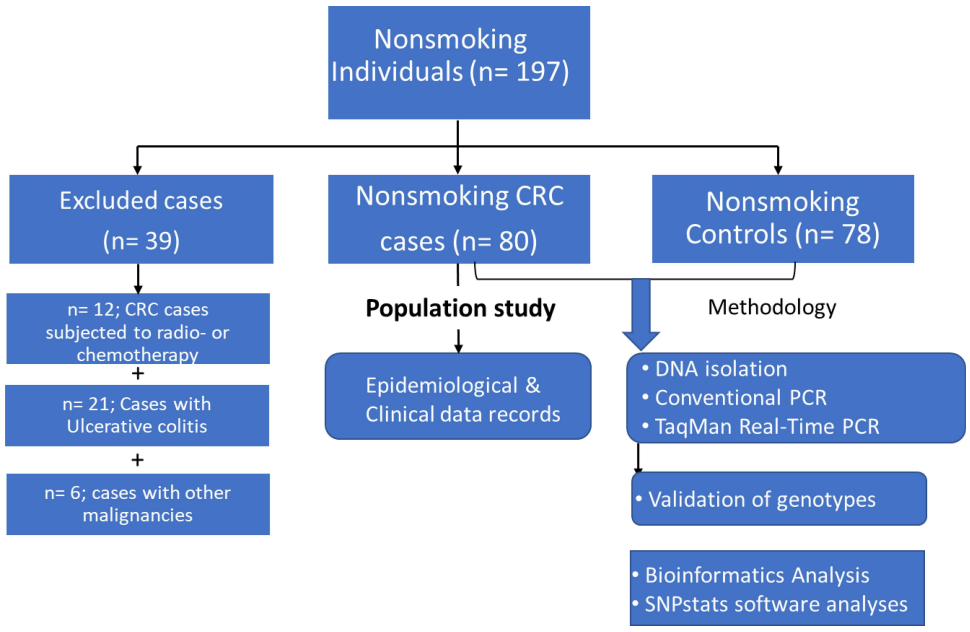

Figure 1 Flow chart of the eligible nonsmoking individuals and applied methodology in the study population.

difference (t = 0.84; P = 0.4) when compared with controls (64.0 ± 10.1 years). The mean age of diagnosis was lower among men than women (51.3 ± 18.23 years versus 54.8 ± 21.91 years), but not significantly (t = 0.97; P = 0.335). The frequency of CRC was highest in those aged 71–80 years (35.7%). The second highest frequency was in adults aged 40–50 years (28.6%), followed by those aged 51–60 (Figure 2). Several popular foods and other dietary and lifestyle products and habits are considered risk factors for CRC in young

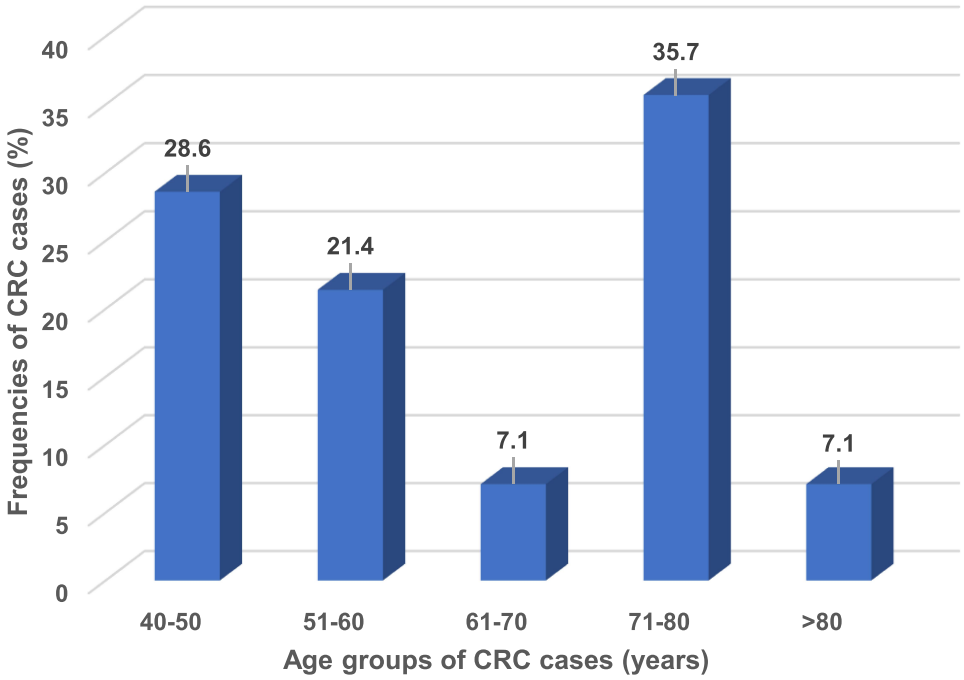

Figure 2 A schematic histogram showing the distribution of CRC cases (n = 80) according to their age groups. A maximum frequency of 35.7% is found at the 71–80 years age group. The young age group (40–50 years) showed a frequency of 28.6%.

Saudi adults. Thus, the high frequency in younger adults may highlight the importance of lifestyle modifications and physical activity for prevention.

Hardy-Weinberg Equilibrium

The genotypic distribution of the *CYP450* rs1048943 A>G SNP was consistent with HWE in both cases and controls ($\chi^2 = 0.86$; $P = 0.35$, and $\chi^2 = 0.29$; $P = 0.56$, respectively), but the *CYP450* rs4646903 T>C and *TP53* rs1042522 G>C SNPs deviated from HWE in cases and controls ($P > 0.05$) (Table 2). The disagreement of *CYP450* rs4646903 T>C with HWE might be due to the absence of the homozygous C/C genotype. For the *TP53* rs1042522 G>C SNP, the small number of cases with the normal G/G genotype compared with the G/C genotype might explain the deviation from the HWE. We could not examine HWE for the *GSTM1* or *GSTT1* genotypes, as the heterozygotes could not be tested using the conventional PCR protocol.

GSTM1/GSTT1 Variants in CRC

The frequencies of the *GSTM1* null allele were significantly higher in CRC cases than in controls (62.5% versus 28.6%; OR = 3.7 95% CI, 2.4–6.0, z = 5.5 and p = 0.0001. Results were similar for the *GSTT1* null allele (12.5% for cases versus 7.1% for controls; OR = 1.03, 95% CI, 0.5–2.0, z = 0.08, and z = 0.93 (Table 1). The genotypic distribution of the *GSTM1* null genotype showed a significantly higher frequency in CRC cases than in controls (62.5% versus 30.8%; $x^2_{\text{Yates}} = 14.70$, p = 0.00013) (Table 2). The *GSTM1* null allele was strongly associated with CRC (OR = 3.7; 95% CI, 2.4–6.0, z = 5.5; p = 0.0001). Neither the homozygous null *GSTT1* genotype "–/–" nor its allelic frequency "–" were significantly different in cases than in controls ($x^2_{\text{Yates}} = 0.54$, p = 0.46, and OR = 1.0; 95% CI, 0.5–2.0, $z = 0.08$; p = 0.93). However, the frequency of *GSTT1* null homozygotes was increased in cases when compared with controls (12.5% versus 7.7%) (Table 2).

CYP450 rs4646903 and rs1048943 Variants in CRC

Frequency differences between variant alleles in cases and controls were not statistically significant for the *CYP450* rs4646903 T/C (OR =1.20, 95% CI, 0.7–2.0, z = 0.90; p = 0.56) and rs1048943 A/G (OR = 1.7, 95% CI, 0.7–4.0, z = 1.2; z = 0.23) SNPs. Although these two SNPs were not associated with CRC, heterozygosity at each of the two loci was more common in cases than in controls (73.8% versus 38.5% and 18.8% versus 11.5%, respectively) (Table 2). The normal genotypes were less frequent in cases than in controls (56.3% versus 61.5% for rs4646903 T/T and 81.3% versus 88.5% for rs1048943 A/A).

Table 1 Allele Frequencies of the Examined SNPs in CRC Cases and Controls

Allele	CRC Cases n (Freq.)	Healthy Controls n (Freq.)	OR	z (*P* value)	95% CI
GSTM1:					
"+"	60 (37.5)	108 (69.2)	1		
"–"	100 (62.5)	48 (28.6)	3.7	5.5 (**< 0.0001**)	2.4–6.0
GSTT1:					
"+"	140 (87.5)	144 (92.3)	1 (reference)		
"–"	20 (12.5)	12 (7.1)	1.03	0.08 (0.93)	0.5–2.0
*CYP1A1*2A* rs4646903 T/C:					
T	125 (78.1)	126 (80.8)	1 (reference)		
C	35 (21.9)	30 (19.2)	1.20	0.9 (0.56)	0.7–2.0
*CYP1A1*2C* rs1048943 A/G:					
A	145 (90.6)	147 (94.2)	1 (reference)		
G	15 (9.4)	9 (5.8)	1.7	1.2 (0.23)	0.7–4.0
TP53 Cd72 rs1042522 G/C (*p*.P72R):					
G	70 (40.6)	87 (55.8)	1 (reference)		
C	90 (59.4)	69 (44.2)	1.6	2.1 (**0.033**)	1.0–2.5

Note: Bold numbers, statistically significant associations ($P < 0.05$).
Abbreviations: CRC, colorectal cancer; "+,+", homozygous present genotype; "–,–", homozygous null genotype; OR, odds ratio; CI, confidence interval.

TP53 rs1042522 G>C Variant in CRC

A statistically significant difference was found between allele frequencies of the *TP53* rs1042522 G/C polymorphism in cases versus controls (OR = 1.6, 95% CI, 1.0–2.5, p = 2.1, z = 0.033) (Table 1). Interacting the G/C versus G/G+C/C genotypes (overdominant model of inheritance) in cases and controls revealed a strongly significant difference within the rs1042522 SNP ($x^2_{\text{Yates}} = 11.20$, z = 0.0008). C/C homozygosity was associated with a three-

Table 2 Genotype Distributions of the Examined SNPs in CRC Cases and Healthy Controls (Adjusted by Gender)

Variable[a] (SNP ID)	CRC Cases (n= 80)	Healthy Controls (n= 78)	Statistics χ^2 (P-value)[a]	Statistics χ^2_{Yates} (P-value)[b]	HWE χ^2 (P-value)
	n (%)	n (%)			
GSTM1:					NA
"+/+"	30 (37.5)	54 (69.2)	15.84	1 (reference)	
"–/–"	50 (62.5)	24 (30.8)	(0.0001)	14.70 (**0.00013**)[b]	
GSTT1:					NA
"+/+"	70 (87.5)	72 (92.3)	0.993	1 (reference)	
"–/–"	10 (12.5)	6 (7.7)	(0.319)	0.54 (0.46)b	
*CYP1A1**2A rs4646903 T/C:					
T/T	45 (56.3)	48 (61.5)	0.44 (0.51)		6.27 (0.012)[d]
C/C	0 (0.0)	0 (0.0)	–		4.42 (0.035)[d]
T/C	35 (43.8)	30 (38.5)	0.46 (0.50)	1 (reference)	
T/T+C/C	45 (56.3)	48 (61.5)		0.26 (0.610)[b]	
*CYP1A1**2C rs1048943 A/G:					
A/A	65 (81.3)	69 (88.5)	1.6 (0.21)		0.86 (0.35)[c]
G/G	0 (0.0)	0 (0.0)	–		0.29 (0.56)[c]
A/G	15 (18.8)	9 (11.5)	1.6 (0.20)	1 (reference)	
A/A+G/G	65 (81.3)	69 (88.5)		1.10 (0.298)[b]	
TP53 rs1042522 G/C (*p*.P72R):					
G/G	20 (25.0)	18 (23.1)	0.078 (0.78)		4.54 (0.03)[d]
C/C	30 (37.5)	9 (11.5)	14.5 (**0.0001**)		8.26 (0.004)[d]
G/C	30 (37.5)	51 (65.4)		1 (reference)	
G/G+C/C	50 (62.5)	27 (34.6)	12.1 (0.0005)	11.20 (**0.0008**)[b]	

Notes: Bold numbers, statistically significant associations ($P < 0.05$); HWE, Hardy-Weinberg equilibrium. [a]Statistically significant difference between two genotypes in cases and controls. [b]Statistical difference between genotypes; T/C & (A/A+G/G) for rs4646903 SNP, A/G & (A/A+G/G) for rs1048943, and G/C & (G/G+C/C) for rs1042522, in cases compared to controls. The values of chi^2_{Yates}' corrections are used for continuity in a 2x2 contingency table [c]HWE is consistent at a marker with cases and controls ($P > 0.05$). [d] HWE is deviated at a marker with cases and controls ($P < 0.05$).

Abbreviations: NA, not available; CRC, colorectal cancer; "+,+", homozygous present genotype; "–,–", homozygous null genotype; OR, odds ratio; CI, confidence interval.

fold increase in risk of CRC in cases when compared with controls (37.5% versus 11.5%; x^2= 14.5; z = 0.0001), and G/G homozygosity was not associated with any difference in risk between cases and controls (25.0% versus 23.1%; x^2= 0.08; z = 0.78) (Table 2). At the *TP53* rs1042522 loci, G/C heterozygosity was more common in controls than in cases, suggesting a significant protective effect (65.4% versus 37.5%; x^2= 12.2; P = 0.0005).

Gene-Gene Interactions

Figure 3 presented the gene-network interaction of the examined genes was created with STRING software. The *CYP1A1* gene was exhibited to interact with the *GSTM1* gene strongly and, to a lesser extent, with the *TP53* gene. A weak gene-gene interaction was found between the *GSTM1-TP53* network. The scores of the gene-gene interactions among *CYP1A1-GSTM1, CYP1A1-TP53*, and

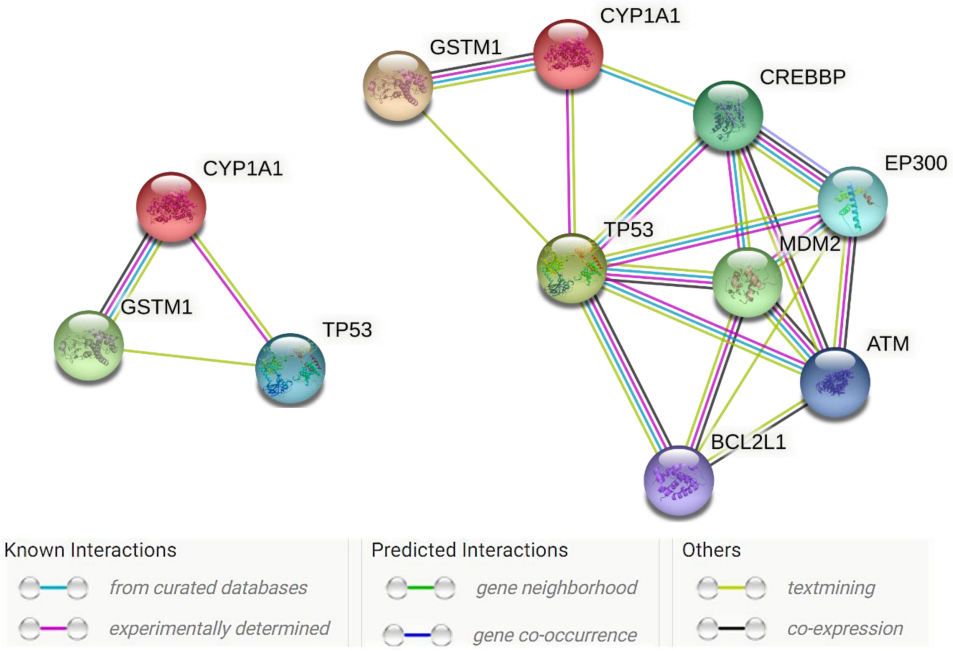

Figure 3 Gene-network interactions contained the CYP1A1, GSTM1, and TP53 genes examined in this study (left side) created with STRING (https://string-db.org/). On the Right side: More extended genes, namely, CREBBP, EP300, MDM2, ATM, and BCL2L1 genes, strongly interacted with the TP53 genes. Each node represents all the proteins produced by a single, protein-coding gene locus. Colored nodes describe proteins and the first shell of interactors. Edges represent protein-protein associations that are meant to be specific and meaningful, ie, proteins jointly contribute to a shared function; this does not necessarily mean they are physically binding each other.

GSTM1-TP53 were 0.962, 0.754 and 0.543, respectively (https://string-db.org/).

Linkage Disequilibrium

Table 3 shows the correlation coefficients of linkage disequilibrium among the examined SNPs. The *CYP450* rs4646903 and rs1048943 polymorphisms were in LD, and their association was statistically significant (D' = 0.999, r = 0.550; $P = 4.6x10^{-7}$) (Table 3). The *GSTM1* and *GSTT1* genetic loci were also found to be in LD with a statistically significant association (D' = 0.998, r = 0.280; $P = 0.010$).

Bioinformatics and Functional Data

Table 4 shows the predicted functional consequences of the examined SNPs. The missense *CYP450* rs1048943 SNP (c.1506A/G; p.I462V) was found to have "possibly damaging" effects on the functional protein according to the MutationaTaster and Mutation Assessor tools and a loss-of-function effect (score, 0.627; possibly damaging) according to the LoFtool. The missense *TP53* rs1042522 SNP (c.348G/C; p.P72R) was also predicted to have negative effects according to PolyPhen-2 (possibly damaging), FATHMM (score, –5.23; damaging), and the LoFtool (score, 0.00096; probably damaging). The *CYP450* rs4646903 SNP, supposed to form a complex with a lncRNA in the 3'untranslated region, was predicted to have a possibly damaging effect (score, 0.627, LoFtool) on the functional protein (https://www.ensembl.org/vep).

Discussion

Our hospital-based case-control study is the first investigation of *GSTM1/GSTT1, CYP450* (rs4646903 and rs1048943), and *TP53* (rs1042522) polymorphisms and CRC in Saudi non-smokers. Overall, our results provide strong evidence of an association between the *GSTM1* SNP and CRC risk and the *TP53* rs1042522 SNP and CRC risk. Moreover, we found strong LD between the polymorphic *GSTM1/GSTT1* and *CYP450* rs4646903/rs1048943 pairs. Although the *CYP450* rs4646903 and rs1048943 SNPs were not shown to be associated with CRC risk, heterozygosity at these two loci was more common in CRC cases than in controls.

The absence of an association between the null *GSTT1* allele and CRC in this study is aligned with the several other studies' results.[38,39] The null genotype of the *GSTM1* allele indicates a loss of the entire gene and is considered the most common polymorphism in CRC. Enzyme activities of GSTM1 are absent in individuals with a homozygous null allele.[40] The ability to detoxify carcinogens decreases in individuals with the null *GSTM1*

Table 3 Coefficients of Linkage Disequilibrium (LD) Among the *GSTM1*, *GSTT1*, *CYP450* (rs4646903 and rs1048943), and *TP53* rs1042522 SNPs

	GSTT1	***CYP450* (rs4646903)**	***CYP450* (rs1048943)**	***TP53 cd72* (rs1042522)**
GSTM1	−0.0407 0.998 0.280 0.0101	0.0204 0.176 0.103 0.3471	0.0170 0.417 0.134 0.2210	$1.67x10^{-16}$ $7.77x10^{-16}$ $6.73x10^{-16}$ 1.0000
GSTT1		−0.0192 0.996 −0.163 0.1256	−0.00676 0.994 −0.0894 0.4126	$1.11x10^{-16}$ $2.33x10^{-15}$ $7.56x10^{-29}$ 1.0000
CYP450 (rs4646903)			0.0569 0.999 0.550 $4.6x10^{-7}$	0.017 0.105 0.0531 0.6263
CYP450 (rs1048943)	D D' *r* *P*-value			0.0184 0.514 0.143 0.1911

Notes: "*r*", correlation coefficient between pairs of loci; "*P*", is a statistical significance for $P < 0.05$. "Red-colored boxes" represent significant strong LD in GSTM1/GSTT1 and CYP450 rs4646903/rs1048943 SNP pairs (P = 0.0101, and 4.6x10-7, respectively). "Yellow-colored boxes" express insignificant weak LD among other genetic polymorphic loci.

Abbreviations: D, linkage equilibrium; D', coefficient of linkage equilibrium; LD, linkage disequilibrium.

homozygous genotype.[7] This genotype appears to be linked to a low detoxifying capacity of some xenobiotics and a reduced regulating oxidative stress caused by free radical activity.[41]

Moreover, the null *GSTM1* genotype has been associated with lung, hepatocellular, breast, and prostate cancers.[41–46] Studies conducted in different populations have reported a remarkable association between the *GSTM1* null genotype and CRC.[46–48] However, other studies could not find a significant relationship between the null *GSTM1* genotype and cancers.[49,50] Khabaz[51] revealed the *GSTM1* null genotype's effect on increasing the risk of CRC in the Saudi population, while Saeed et al[52] demonstrated that the *GSTM1* null genotype was found in 2% of CRC patients in the Saudi population. These contradictory results suggest the need to conduct genotyping studies of *GSTM1* in larger samples.

Despite much less is known about the properties, functions, and biological significance, long noncoding RNAs (lncRNAs) were later found to be associated with many human diseases, including cancers (http://bioinfo.life.hust.edu.cn/lncRNASNP2).[10] The mechanistic interactions between CYPs and ncRNAs are associated with environmental chemicals' toxicity and carcinogenicity.[53] A lncRNA RP11-108K3.2, mapped to a 15q21 chromosome locus, overlaps with a *CYP19A1* gene, changing the CRC risk inflammation-related mechanism.[34,54,55] In patients with CRC, the lncRNA CCAT1 and CCAT2 are highly expressed with a low survival rate and recurrence rate of the disease.[56]

Neither the noncoding rs4646903 nor the missense rs1048943 variant in *CYP450* was associated with the risk of CRC in the present study; hence, the relatively small sample size among the Saudi population might have made it difficult to detect associations with CRC. Previous studies have shown contradictory data regarding associations between *CYP1A1* SNPs and CRC susceptibility.[38,57] A meta-analysis study investigating the *CYP450* (rs1048943 A/G and rs4646903 T/C SNPs) has shown an increased risk of CRC with rs1048943, but not with the rs4646903.[58] Although our study did not examine gene-environmental correlations due to insufficient data, additional studies should evaluate potential gene-environmental interactions involving smoking, *CYP1A1* rs4646903, *CYP1A1*2C* rs1048943 SNPs, and CRC.[57,58]

Given the importance of p53 in multiple cellular functions, including gene transcription, DNA repair, and apoptosis, it is biologically reasonable that *TP53*

Table 4 In-silico Functional Predictions of Non-Synonymous SNPs

SNP ID	Allele Freq.	Biotype	Protein Position	cDNA Position	CDS Position	Codon	SIFT (Score)	PolyPhen-2 (Score)	Mutation-Taster	FATHMM	Mutation Assessor	LoFtool
rs1048943	A/G (0.133)	Protein-coding	I462V	1506	1384	ATT/GTT	0.35 (tolerate)	0.219 (benign)	1.9x10−6 (possible damage)	−0.44 (tolerate)	2.12 (moderate)	0.627 (possible damage)
rs1042522	G/C (0.46)	Protein-coding	P72R	348	215	CCC/CGC	0.37 (tolerate)	0.147 (possibly damaging)	1.355 (low)	−5.23 (damage)	0.338 (low)	0.00096 (probable damage
rs4646903	A/G (0.016)	lncRNA*	-	-	-	-	-	-	-	-	-	0.627* (possible damage)

Note: *The lncRNA, long coding RNA (the noncoding 3'untranslated region) (LoFtool, https://www.ensembl.org/vep).
Abbreviations: SIFT, sorting intolerant from tolerate; PolyPhen-2, polymorphism phenotyping-2; FATHMM, Functional Analysis Through Hidden Markov Models; LoFtool, loss-of-function tool.

polymorphisms could be associated with CRC risk.[59] Several studies have provided evidence that *TP53* rs1042522 (*c*.348G>C; *p*.P72R) could be associated with CRC, but results are still conflicting. Consistent with our study results, 72Arg is associated with CRC risk in populations in Greece,[60] Argentina,[61] Germany,[62] and Iran.[63] On the other hand, 72Pro is associated with a higher risk of CRC in Turkey[64] and Malaysia.[65] At the same time, other studies in different populations have failed to link *TP53* rs1042522 to CRC.[66,67]

The distribution of the *TP53* rs1042522 G/C polymorphism differs based on geographic regions and ethnicity. General populations from Latin America, the United States, and Europe show higher C-allele frequencies than the G-allele. On the other hand, the C-allele is less prevalent in African and Asian populations.[68,69] The present study showed that the *TP53* rs1042522 SNP was significantly associated with susceptibility to CRC. C/C homozygosity conferred a triple risk of CRC in cases compared with controls. Since the G/C genotype was more common in controls, the C-allele may be a protective moiety in the G/C heterozygous genotype. Thus, only one copy of the rs1042522 variant C-allele may not be sufficient to impact CRC development.

Our results revealed that CRC is more frequent in men than women (a ratio of 2.2: 1), with the most common diagnosis among 71–80 years. These outcomes are consistent with a 7849-case cohort study that reported the highest CRC diagnosis frequency in those ages 60–75 and older.[70] However, the mean age at diagnosis is still conflicting among different ethnic populations; our mean age at diagnosis was older than that reported in South-Eastern Asians (62.4 ± 13.5 years versus 59.3 ± 14.6 years).[71–73] Our finding of a relatively high frequency of CRC (28.6%) in those ages 40–50 years agrees with results from the United States that also showed a high percentage in those ages 35–49 years,[74] but disagrees with research from Japan.[75]

Esophageal, stomach, colorectal, hepatic, and pancreatic cancers are the major gastrointestinal cancers. Worldwide, CRC is the most common cancer with high age-standardized incidence rates (ASIRs) in both sexes,[76,77] but it is not among the top ten carcinomas in the Saudi population.[78] Recent studies have reported an increasing trend in ASIRs of CRC in Riyadh, Mecca, and the Eastern region in both sexes.[70] The ASIR and age-standardized mortality rate (ASMR) of CRC in the Saudi community are estimated to be 13.1 and 6.3 per 100,000

people, respectively. The highest ASIRs and ASMRs of CRC among both sexes were recorded in 2018 in Austria, New Zealand, Mongolia, and Hungary.[79]

The increasing CRC trend among young Saudi adults is alarming, signaling the need for significant lifestyle modifications. Siegel et al[80] have reported that CRC incidence in adults younger than 50 years old rose by 1.6% from 2000 to 2013–2014, and that mortality rose by 13% in the same period. In addition to tobacco-smoking habits being implicated in the development of various cancers, several popular foods and other dietary and lifestyle products and habits are well known as serious risk factors for CRC.[81] Lifestyle modifications could prevent about 50–60% of incident CRC cases in the United States.[82,83] Smoking, high levels of body fat, and consumption of red and processed meat have been established to increase CRC risk,[71] whereas physical activity and consumption of dietary fiber, whole grains, dairy products, calcium supplements, vitamin D, and marine omega-3 fatty acid may lower disease risk (https://www.wcrf.org/sites/default/files/Colorectal-Cancer-2017-Report.pdf). Under the environmental pressure of malnutrition or insufficient nutrition, cancer cells grow better than normal cells.[84,85]

Study Limitations

Conflicting results in establishing genetic associations with CRC are common. Poor replication of results could be due to several factors. First, previous studies that did not separate smokers and nonsmokers suggested positive associations between CRC and *GST, CYP*, and *TP53* genes. Excluding Saudi smokers from the present study resulted in a much smaller sample size, making it more difficult to detect associations. However, to improve accuracy, we used nonsmoker conditions to exclude 39 individuals (those who have ulcerative colitis, having other malignancies, or cases subjected to radio- or chemotherapy) with CRC (approximately 50%) originally recruited. Second, some studies have been conducted in populations with admixed ethnicities, while we confined our criteria to only individuals from the Western region. Third, some studies used multiple sources for CRC cases, which would decrease the overall results' power. Fourth, environmental and lifestyle factors (eg, fatty/red meat consumption, vegetable consumption, physical exercise) should be addressed in CRC risk studies.

Conclusion

The present study represents unreeled investigation of *GST, CYP450*, and *TP53* with colorectal carcinoma (CRC) among the nonsmoking Saudi community. The null *GSTM1* allele and the *TP53* rs1042522 G/C polymorphism were targeting risk factors in CRC nonsmokers. Even though the *GSTT1* and *CYP450* (rs4646903 and rs1048943) SNPs may not be individual risk factors for CRC, the LD of the polymorphic *GSTM1/GSTT1* and rs4646903/rs1048943 pairs showed potential significant effects on CRC risk. However, heterozygosity of *CYP450* rs4646903T/C, *CYP450* rs1048943A/G, and *TP53* rs1042522 G/C cannot be considered risk factors for CRC in this cohort. So far, these outcomes should be taken with caution, as the examined SNPs do not act alone to explain such complex multifactorial malignancies.

Moreover, mutations in non-coding regions may play an important role in the development and progression of tumors. Thus, an in-depth prospective study of the molecular mechanism and clinical application of lncRNAs helps explain the mechanistic binding with the CYP and p53 for CRC development and provides new prognosis and management targets. More linkage outcomes based on a next-generation sequencing approach instead of a single gene or a few candidate genes may help discover new genes associated with susceptibility to CRC. An ongoing large-scale whole-exome analysis may identify further predictive factors and help explain a predisposition to the disease. The increasing trend of CRC among young Saudi adults suggests that significant lifestyle modifications are necessary. Unfortunately, malnutrition or insufficient food will also inhibit cell apoptosis and thus enhance tumor cells' growth.

Data Sharing Statement

Data sets analyzed during this study are provided by the corresponding authors.

Ethics Approval and Consent to Participate

Written informed consent was obtained from all study participants enrolled in this project, approved by the Institutional Biomedical Ethics Committee of Umm Al-Qura University (reference #HAPO-02-K-012) licensed from the National Committee Medical and Bioethics, KACST (http://bioethics.kacst.edu.sa/About.aspx?lang=en-US).

Consent for Publication

Written informed consent was taken from all study participants to publish the results.

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Author Contributions

NAE, and IAS designed the research (Corresponding authors); SNE, NAE, MTT, and MA made the clinical investigations, surgery, and managements; IAS, AOB, AHM, HMN, ENE, MA, and NAE performed the practical work; IAS, NAE, AHM, and ENE work for in-silico predictions and statistical analysis. All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

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