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Potential genetic biomarker of Saudi Arabian patients with colorectal cancer

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ABSTRACT. – Colorectal cancer (CRC) is one of the leading causes of cancer deaths globally. We implemented a comprehensive literature review regarding CRC genetics studies to offer a perception into the genes associated with CRC recognized in Saudi patients. Definite genetic variants in *ABCB1, ADIPOQ, CTNNB1, SFRP3, LRP6, CYP19A1, PARP*-1, *TDG* genes exhibited significant protection against CRC development in Saudi population. Whereas, other gene mutations in *ABCB1, ABCC1, CASR,* IL-17F, *NOTCH1, NOTCH4, PRNCR1, TDG, TLR2, TLR4, TLR-9, TSLP, TSLPR* and *TNF-α* genes showed irrelevant correlation with CRC risk in Saudi Arabia. On the other hand, specific mutations in *ABCC1, ADIPOQ, CYP1A1, KIR, IL-17A, MMP2, NOTCH3, PRNCR1, RETN, TDG, TLR2, BRAF, PARP-1, TLR4, TLR*-9, *TNF*-α, *TSLP* and *XRCC1* genes demonstrated a substantial augmented CRC risk development in Saudi patients. Furthermore, *ATR, ATM, BMI1, CCAT1, Chk1, Chk2, COX-2, FoxM1, FSCN1, Ki67, MALAT1, miR-29, miR-34a, miR-92, miR-182-5, PANDAR, PIK3CA, TIGAR* over-expression revealed a robust association with CRC in Saudi Arabia (KSA). Moreover, gene alterations in *APC, EGFR, FBXW7, TP53, PTEN, K-ras* genes were concomitant in CRC. As well as, lower expression of *MLH1, MSH2, MSH6, PMS2, EPCAM and MUTYH* genes were recognized in LS patients and future CRC Saudi patients. These gene mutations may be used as diagnostic and/or prognostic genetic markers in CRC Saudi patients and could offer a potential therapeutic target for CRC management.

Key Words:
Colorectal cancer, Gene, Saudi Arabia, Risk, Over-expression, Mutation, SNP.

Introduction

Cancer is the second utmost cause of death all around the world. Colorectal cancer (CRC), which includes colon cancer and rectal cancer, is the third most common cancer worldwide after lung and breast cancers[1]. CRC arises due to a series of epigenetic and genetic variations with subsequent high mortality rate. The etiological and risk influences of CRC development are inconsistently emulating the multifactorial nature of the disease. Numerous risk factors are linked to the CRC development risk, for instance age (older than 50 years), inflammatory bowel diseases[2], history of adenomatous polyps, high-red meat diet, smoking and limited physical activity lifestyle[3]. At the molecular level, CRC is very intricate and necessitates establishing comprehensive patient stratification models through the identification of patients who will benefit or will not benefit from specific targeted therapy. Thus, understanding the personalized genetic map of each patient will help in developing the utmost appropriate management of his/her case.

Incidence Rate of CRC in Saudi Arabia

Colorectal cancer is the second most common cancer after breast cancer in Kingdom of Saudi Arabia (KSA)[4]. In KSA, CRC incidence is about half of the incidence in the US, nevertheless the greatest incidence is at a lower age group (40-60 years in the KSA *vs.* 60-80 years in the US)[5]. Saudi Cancer Registry stated that CRC is the second most common malignancy among Saudis for all ages (10.3%), the first cancer in Saudi males (11.8%)[6], and the third one among Saudi females[4]. Also, between 1994 and 2003 age-standardized rates for CRC in KSA nearly doubled[6]. Between 2001 and 2003, whereas the annual percent change (APC) of CRC incidence among Saudi females displayed an insignificant rise, Saudi males showed extremely rising incidence, with an APC reaching 20.5%. Furthermore, it is anticipated that

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by the year 2030, the CRC incidence in KSA may upsurge four-fold[7]. Almatroudi (2020)[8] preformed a retrospective observational population-based epidemiological study of CRC established on the Saudi Cancer Registry information, which included all CRC patients January 2006 to December 2016. The results verified that the highest mean age-standardized incidence rates (ASIRs) of the CRC were in Riyadh, Makka, and Eastern Province regions, whereas the lowest mean ASIRs were stated Jazan and Najran regions[8].

Risk Factors

In KSA, there is no nationwide policy for CRC screening despite the escalation incidence[9]. Notably, most of CRC cases were identified throughout clinical assessments rather than through screening programs. Aljumah and Aljebreen (2017)[9] recommended that the developing and applying CRC screening policy would be cost-effective application, which would eventually decline the financial burden on government spending, as well as improve the populations' health status. Saudi Centre for Evidence-Based Healthcare panel meeting in 2015 agreed on the existence of a lack of nationwide incidence data concerning adenomatous polyps or the age groups in which the CRC incidence surges. Furthermore, there were no national clinical trials assessing the effectiveness of the diverse modalities of screening for CRC and their influence on mortality[10]. It is crucial to recognize the risk factors connected to CRC development. Al-Zalabani (2020)[11] recognized some of the population attributable fractions (PAFs) of CRC Saudi cases. The study results displayed that the largest fraction of attributable CRC cases among men and women was triggered by physical inactivity (16.13% and 16.45%), followed by extra weight (obesity: 9.71% and 6.93%; overweight 6.05% and 1.9%); and smoking (present smoker: 3.04% and 0.18%; prior smoker: 3.29% and 0.12%)[11]. Moreover, harmful actions such as consanguinity among the Saudi population, which were and still are detrimental factors for CRC increasing risk among KSA population. Subsequently, the risks for transporting genetic variations to the next generations in KSA are higher than in Europe and America, where there are less consanguineous marriages, and the family size is generally smaller[12]. Although many advances have been made to diagnose and treat CRC in KSA, the latest cancer incidence report from the National Cancer Registry (NCR) indicated a worrying escalation in CRC patients. Accordingly, additional studies are still indispensable to conclude the diverse genotype biomarkers that can be used to predict the Saudi patients who are at risk of developing CRC.

The aim of the existing review is to offer an understanding into the genes recognized in CRC Saudi patients. These gene mutations may be used as diagnostic and/or prognostic genetic markers in CRC Saudi patients and could offer a potential therapeutic target for CRC management.

To achieve the current review goal, we accomplished a comprehensive state-of-art literature review regarding the genetics of CRC in KSA. A literature search was executed using online available databases like PubMed, ClinVar, Online Mendelian Inheritance in Man (OMIM), Phenotype-Genotype Integrator (PheGenI), DisGeNET and GWAS Catalog to retrieve all genetic studies executed on Saudi CRC patients or samples until December 2021. The search was done using the following strings: (colorectal), (Saudi), (gene), (colon), (rectal) and (cancer). All published articles including Saudi CRC patients were included in this review.

Genes Associated with Colorectal Cancer

Several genes' mutations were included in this review, some of which were associated with increased risk of colorectal cancer, whereas others genetic mutations, were not associated with the development of CRC. Additionally, some genetic mutations showed a protective role against CRC development in Saudi patients. More than 40 genes mutations were included and for each of these we tried to explain a little about the gene associated proteins and their roles in CRC development as shown in the Supplementary Table I.

ABC

Adenosine Triphosphate (ATP) binding cassette (ABC) transporters play an indispensable part in the development of numerous disorders, including cancers, through drug resistance mechanism[13].

ABCB1

ABCB1 gene is positioned on chromosome 7 and encodes a P-glycoprotein (Pgp) which is responsible for the active efflux of drugs from cells[14]. Al Qahtani et al[15] (2019) studied the genotype distributions and the allele frequencies of two major variants in *ABCB1* gene, *C3435T* and *T129C*, and linked them with CRC in Saudi Arabia. They recognized no significant association between *ABCB1,* 3435C>T and 129T>C polymorphisms

with CRC risk. Furthermore, patients with 3435 homozygous (TT) genotypes had lower risk of developing CRC risks[15]. The same group of research[16] implemented another study on 62 CRC patients to determine the genotypic distribution and allele frequency of another two *ABCB1* Single nucleotide polymorphisms (SNPs), T1236C and G2677T, in Saudi CRC patients. The outcomes displayed no noteworthy variations in T1236C in CRC patients than controls. However, G2677T showed a protecting action against CRC progression.

ABCC1

Abdulkhaleq et al[17] (2019) conducted a case-control study on 51 colon cancer patients to recognize the effect of two SNPs; G128C and C218T in the *ABCC1* gene on CRC development. The results showed an association between heterozygous (CT) genotype for variant C218T and an increased risk of colon cancer (3 times over) and high-grade stages (III and IV). They concluded that the CT genotype of variant C218T in *ABCC1* gene might intensify the risk of developing colon cancer among Saudi population, suggesting that this variant can be used as a prognostic marker for colon cancer. In contrast, the variant G128C exhibited no association with colon cancer.

ADIPOQ

Adiponectin is an adipose-specific protein, which has anti-atherogenic, anti-inflammatory and anti-diabetic actions. In order to appraise the influence of the adiponectin gene *ADIPOQ* polymorphisms to the risk of colon cancer, Al-Harithy and Al-Zahrani (2012)[18] conducted a case-control study on 60 colon cancer patients. They examined the link between two SNPs, rs1501299 (G276T) and rs2241766 (T45G), in the *ADIPOQ* gene and CRC risk. The results showed that carriers of the heterozygous (GT) genotype of G276T displayed a higher risk of colon cancer than carriers of (GG) genotype. By contrast, the G allele in position 45 of the *ADIPOQ* gene had a lower risk of colon cancer than carriers of the normal (TT) genotype. The results suggested that the G276T SNP contributes to the genetic risk of colon cancer, while the presence of the G allele in position 45 of the *ADIPOQ* gene acted as a defensive factor against colon cancer.

ALK

Anaplastic lymphoma kinase (ALK) is a tyrosine kinase that was acknowledged as part of chromosomal rearrangement as a fusion partner of nucleophosmin[19]. Bavi et al[20] (2013) identified the frequency and nature of *ALK* alterations via recruiting 770 Saudi CRC patients. CRC prognosis was poor in patients with *ALK* gene amplification and gain in copy number as compared to CRC patients with standard *ALK* gene copy number. *ALK* gene amplification and gain in copy number were considered as an independent prognostic marker for poor survival in CRC across all stages[20].

APC

The adenomatous polyposis coli (*APC*) gene mutation is one of the primary events in CRC. Ninety-five tumor samples were retrospectively recruited in a study accomplished by Almuzzaini et al[21] (2021), and 96% of the samples exhibited at least one confirmed *APC* gene pathogenic variant[21]. Five novel variants, at the time, out of total 38 variants were detected in the *APC* gene. These variants included c.1696G>A (p.V566I) missense mutation at exon 14, c.1697delT (p.V566X) frame shift mutation at exon 14, c.2680_2681delGTinsTA (p.Val894Ter) stop gain mutation at exon 16, c.3917delA (p.E1306X), frame shift mutation at exon 16, and c.4320-4341del ACCACCTCCTCAAACAGCTCAA (p. PPPPQTAQ1440-1447X). Additional study investigated 99 CRC cases *via* targeted sequencing, which led to the identification of frequent mutations in *APC*. *APC* gene was the second most commonly mutated gene in that cohort, with 36.4% of the cases examined demonstrating missense, nonsense or frameshift mutations in the hotspot regions of this gene. The most common mutation identified was the p. Arg1450Ter change, resulting in the expression of truncated APC and thus loss of control on nuclear β-catenin mediated gene expression and poorly regulated WNT pathway[22].

ATR and ATM

The expression of four telomere-associated proteins, hTERT, TRF1, TRF2, POT1 were studied by Aljarbou et al (2018)[23]. There are six individual proteins associated with telomeric DNA, collectively called shelterin complex. They are essential in preventing the recognition of the telomere as single or double strand breaks *via* the inhibition of Ataxia telangiectasia mutated (ATM) and Ataxia telangiectasia and Rad3 related (ATR) dependent by DNA damage response (DDR) pathway. The expression of *ATR*, *ATM* and *Chk1*, *Chk2* were significantly escalated in cancer tissues. Thus, the expression of *ATR*/*Chk1* and *ATM*/*Chk2* pathways, may serve as a therapeutic re-

Table I. Summary of the genes mentioned in this review and mutations occurred in them, as well as their association with CRC development risk.

Gene of interest	Mutation occurred and association with CRC development risk	Ref.
ABCB1	No significant association between *ABCB1* 3435C > T and 129T > C polymorphisms with CRC risk. Patients with 3435 homozygous (TT) genotype had lower risk of developing CRC risk.	15
	No noteworthy variations in T1236C in CRC patients. G2677T showed a protecting against CRC progression.	16
ABCC1	An association between heterozygous (CT) genotype for variant C218T and increased risk of colon cancer and high-grade stages (III and IV).	17
ADIPOQ	Carriers of the heterozygous (GT) genotype of G276T displayed a higher risk of colon cancer than (GG) genotype. By cont rast, the G allele of T45G had a lower risk of colon cancer than the normal (TT) genotype.	18
ALK	CRC prognosis was poor in patients with *ALK* gene amplification and gain in copy number as compared with CRC patients with standard *ALK* gene copy number.	20
APC	96% of the samples exhibited at least one confirmed *APC* gene pathogenic variant.	21
	APC gene was the second most commonly mutated gene. Most common mutation identified was the p. Arg1450Ter change, resulting in the expression of truncated APC.	22
ATR & ATM	The expressions of *ATR*, *ATM* and *Chk1*, *Chk2* were escalated in cancer tissues.	23
BCAR4	No significant expression of *BCAR4* in CRC samples.	24
BRAF	V600E mutation was detected in female patients, deletion of A at c.1758 bp, causing a frameshift and truncated protein, insertion of A/C at c.1860 bp and an insertion of A, leading to frameshift and stop codon. Mutation at c.1780G > A (D594N), and c.1826insT, resulted in frame shift and truncated protein. *BRAF* frameshift mutations E586E, Q609L, and M620I lead to truncated defective *BRAF* proteins.	28
CASR	Intron 4 variant (rs3804594) in *CASR* gene was not correlated to CRC risk.	30
CCAT1&C-CAT2	The expression level of *CCAT1* was augmented in CRC patients. No noteworthy *CCAT2* expression in CRC samples.	24
COX-2	*COX-2* over expression in CRC cases and associated with shorter survival.	32
CTNNB1	Genetic variants in *CTNNB1* (β-catenin) (rs4135385), *SFRP3* (rs7775), and *LRP6* (rs2284396) genes were associated with a defense against CRC development. GG genotype was associated with lower risk CRC developing relative to AA genotype at rs4135385 of *CTNNB1* signifying an association of the rs4135385 in *CTNNB1* gene with a reduced CRC risk.	34
CYP1A1	High distribution of *CYP1A1wt/*2A* genotype in CRC patients, reflecting a significant rise of cancer risk associated with *CYP1A1wt/*2A* genotype.	36
CYP2E1	*CYP2E1 wt/*6* was not related to CRC risk in Saudi populations.	36
CYP19A1	Three SNPs rs4774585, an A > G transition, rs936308, C > G transversion, and rs4775936 C > T transition. AA genotype of rs4774585, GG state of rs936308 and TT state of rs4775936 exhibited a negative association with CRC development in Saudi patients.	38
EGFR	Frequent mutations in *EGFR* were associated with young age of onset and poor disease-specific survival in CRC.	22
FBXW7	Frequent mutations in *FBXW7* were identified	22
FOXM1	*FoxM1* protein overexpression was elevated in CRC tissues and was related with poorly differentiated and highly proliferative tumors.	40
FSCN1	*FSCN1* intensified in CRC patients and was accompanied by reduced OS and DFS. Furthermore, high *BMI1*/*FSCN1* patients experienced the worst OS and DFS.	26
GSTM1	Majority of CRC cases harbored the null genotype (*GSTM1*0/*0*).	43
GSTP1	None of the genotypes of *GSTP1* studied were associated with an increased risk of CRC.	44
hBD	*hBD-1* mutations and mutation 1 of *hBD-3* lead to truncated pre-proteins. *hBD-3* mutation 2 protein was greatly destabilized. These outcomes established a significant reduction of hBDs in cancer tissues.	46
hTERT	All CRC samples expressed *hTERT*; however, there was no difference between tumor and adjacent mucosa.	23

Table continued

Table I. ***(Continued).*** Summary of the genes mentioned in this review and mutations occurred in them, as well as their association with CRC development risk.

Gene of interest	Mutation occurred and association with CRC development risk	Ref
IL-17	Males harboring the A allele of *IL-17A* G197A SNP exhibited higher risk of developing CRC. No connection between *IL-17F* (rs763780) polymorphism and CRC susceptibility.	48
KIR	Five activating *KIR* genes (*2DS1*, *2DS2*, *2DS3*, *2DS5*, and *3DS1*) were significantly more prevalent in the CRC patients. The highest risk was associated with the *3DS1* gene, followed by the *2DS1* gene. Additionally, no associations were found between *3DL1* and *2DS4* and CRC, whereas *2DS2* was inversely associated with CRC risk.	50
K-ras	No mutations were identified in codon 61 of exon 3. Exon 4 aberrations harbored p.Ala146Thr (a missense mutation), p.Ala134Val, p.Arg135Lys, p.Gln150Stop, p.Lys147Lys, p.Gln150Stop, p.Gln150Stop, p.Gly-138Gly, p.Arg149Gly, p.Gly138Gly, and p.Gly138Gly mutations. Missense *K-ras* mutations altering the amino acid sequence of the protein (A134V, R135K, E143K and R149G) were distinguished. Moreover, two synonymous *K-ras* mutations (G138G and K147K) and a nonsense truncating mutation (Q150X) were detected. E143K mutation is predicted to have a damaging effect on the protein. R149G mutation is predicted to be neutral, but molecular modeling showed that this mutation caused changes in the helix and loop chains near the GTP binding pocket. Q150X mutation introduced a premature stop codon.	5
	Most mutations detected were at codon 12 and were associated with metastasis. *K-ras* mutations were concomitant with advanced stage of CRC, shorter RFS and OS. Mutations in codons 12/13 of *K-ras* exon 2 were associated with reduced benefit from EGFR antibody treatment for metastatic CRC.	53
	K-ras mutations were in codon 12, most commonly p.G12D, and codon 13. *K-ras* mutations were higher in young patients and in the right-sided tumors.	54
	Mutations in *K-ras* were acknowledged in codon 12; G12D, G12V, G12R, G12A, and G12C, in codon 13 included G13D and G13C, 17, S17R and in codon 31, c.91 G > A.	55
	K-ras mutations were associated with poor disease-specific survival in cases with wild-type TP53.	22
LRP6	Decreased risk of developing CRC in CC genotype compared to TT genotype at SNP rs2284396 in *LRP6* gene.	34
MALAT1	The expression levels of *MALAT1* were significantly high in CRC patients.	24
MED12	Three *MED12* somatic mutations in CRC patients were found.	57
MEG3	No significant expression of *MEG3* in CRC was detected.	24
miRNAs	Significant rises in *miR-29* and *miR-92* and their expression levels in CRC, while *miR-145* and *miR-195* decreased in CRC tissues.	59
	A noteworthy escalation in *miR-34a* in CRC colon specimens was identified. *miR-34a* rs2666433 AA and AG genotype carriers were found to be more likely to develop cancer than GG carriers.	60
	miR-182-5p gene was amplified in CRC patients.	61
MLH1	Structural loss in the genomic regions of *MLH1* (3p23-p14.2), *MSH2*, *MSH6*, *EPCAM* (2p21-p16.3), *PMS2* (7p22.1) and *MUTYH* (1P34.1-p33) in LS patients. This structural loss resulted in lower expression of *MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM* and *MUTYH* genes.	12
	Positive *MLH1* correlation was found in around 27% and that MSI was present in more than 90% of CRC patients with Lynch syndrome.	64
MMP2	C1306 T mutation was significantly more common in colon Saudi patients.	65,66
NOTCH1	SNPs rs3124591 in *NOTCH1* and rs3820041 in *NOTCH4* did not exhibit any association with CRC. rs1043994 in *NOTCH3* displayed a significant association with CRC in males.	68
PANDAR	The expression levels of *PANDAR* were significantly enlarged in CRC patients.	24
PARP-1	SNPs in *PARP-1* gene, including Met129Thr, Val762Ala, and Lys940Arg, did not show any association with CRC risk in Saudi population. Lys933Asn and Lys945Asn showed significant association with CRC among Saudis.	69
	SNP rs8679 diminished susceptibility to colorectal cancer at heterozygous TC allele and at minor allele C.	70
PCAT6	No significant expression of *PCAT6* was detected in CRC patients.	24

Table continued

Table I. ***(Continued).*** Summary of the genes mentioned in this review and mutations occurred in them, as well as their association with CRC development risk.

Gene of interest	Mutation occurred and association with CRC development risk	Ref.
POLD1 and *POLE*	Numerous variants in *POLE* gene were associated with an increased risk for CRC. Low *POLE* protein expression exhibited a substantial link with lymph node involvement and grade III tumors. Whereas for *POLD1*, low expression was related with adenocarcinoma histology, larger tumor size and stage III tumors.	72
PRNCR1	One SNPs, rs1456315, in *PRNCR1* gene showed an association with CRC with the homozygous CC variant allele, among younger age patients (≤ 57) and in female patients. Three other SNPs, rs1016343 (C > T), rs13252298 (A > G), and rs16901946 (A > G), in *PRNCR1* gene did not display any association with CRC.	74
P35 and *PTEN*	*P53* positive expression was established in 25.4% CRC, whereas loss of *PTEN* expression was recognized in 32.3% CRC patients.	64
RETN	SNPs in *RETN* gene rs1862513 (C-420G) and rs3745367 (G+299A) had increased the risk of colon cancer. Carriers of the heterozygous (GA) genotype of SNP 299 had a significantly higher colon cancer risk than carriers of the wild (GG) genotype.	77
SFRP3	Genetic variant (rs7775) was correlated to considerable protection against CRC progression. Women having Gly at codon 324 (rs7775) of SFRP3 have 2.5-fold lower risk of developing CRC compared to those having Arg at this locus.	34
SMAD4	*SMAD4* gene was identified and correlated to CRC development.	22
TDG	SNP rs4135113 showed a significant risk association of its genotype AA and of the minor allele A in CRC Saudi patients. SNP rs1866074 presented a protective association with the GG allele and the additive (AG+GG) allele in CRC patients. Other four SNPs (rs4135050, rs4135066, rs3751209, and rs1882018) showed no association with CRC patients in the Saudi population.	79
TIGAR	*TIGAR* expression was found in 68% of the tumor samples with nuclear localization and was significantly amplified in early stage (stage I and II) and late stage (stage III and IV) of CRC.	81
TLR2	*TLR2* rs3804099 and *TLR2* rs4696480 SNP were associated with CRC susceptibility, while *TLR2* (rs3804100 C > T) disclosed no association with CRC susceptibility in Saudi patients.	83
TLR4	A clear association between *TLR4* rs10759931 polymorphism, the G allele, and susceptibility to CRC development risk was detected. *TLR4* rs2770150 is associated with CRC in women aged over 50 years. Whereas SNP rs10759932 and rs4986790 appeared not to have any association with colon cancer.	84
TLR6	rs3796508 is a protective factor against CRC in the older male Saudi population. Two other non-synonymous SNP S249P and V327M were common in a few patients and were predicted as damaging.	85
TLR 9	An association between the rs187084 SNP and CRC risk was found in female patients. T allele exhibited lower frequency in female cancer patients. Additionally, rs352139 and rs352144 SNPs were found to be correlated with colon cancer development. SNPs rs352144, rs187084 and rs5743839 were not associated with colorectal cancer in males.	87
TNF-α	SNPs -308 and -857 were not associated with CRC, while -238 (G/A) genotype was significantly concomitant with high risk of CRC. AA genotype of -238 G/A SNP was higher proportion in CRCs.	88
PTEN	Loss of *PTEN* has been reported in CRC.	89
TP53	*KRAS* or *PIK3CA* mutations were significantly associated with poor disease-specific survival in cases with wild-type *TP53*.	22
TSLP	SNP rs10043985 presented a strong correlation with CRC Saudi patients, whereas rs2289276 SNP did not show any relation with CRC.	93
UCA1	No significant expression of *UCA1* detected in 63 CRC.	24
VDR	No association between the four *VDR* polymorphisms with CRC risk was found in the overall analysis. *ApaI* and *BsmI* loci were associated with CRC in elderly and female patients.	95
	ApaI SNP (rs7975232), only the heterozygous (AA) genotype increased the risk of CRC. *TaqI* SNP (rs731236) carriers with either the heterozygous (TT) or homozygous (TT) genotype displayed an increased risk for the disease. Heterozygous (Bb) and homozygous (bb) carriers of the *BsmI* SNP (rs1544410) had significantly lower risk for CRC. *FokI* SNP (rs2228570) showed no association with CRC risk.	96
XPD and XRCC1	No significant difference in *XPD* Lys751Gln polymorphism in CRC. An association between the GG genotype of *XRCC1* polymorphism and the increased risk of CRC was detected . Also, *XRCC1* (AG + GG) polymorphism may be associated with increased clinic pathological parameters of CRC.	98

sponse biomarker to certain therapies that induce these DNA damages[23].

BCAR4

Siddique et al[24] (2019) designed a study to measure the expression of several oncogenic lncRNAs, including *BCAR4*. The study revealed that there was no significant expression of *BCAR4* in CRC blood samples. However, a small number of subjects limited the statistical power in some comparisons.

BMI1

BMI1 polycomb gene promotes cancer cell survival *via* p53-dependant cell death suppression[25]. *BMI1* expression was related to cancer progression and poor clinical outcome. Alajez (2016)[26] demonstrated a significant escalation of *BMI1* in CRC Saudi patients. Furthermore, high *BMI1* expression was concomitant with reduced overall survival (OS) and reduced disease-free survival (DFS).

BRAF

BRAF (B-Raf proto-oncogene, serine/threonine kinase) is a member of the rapidly accelerated fibrosarcoma (RAF) family. *BRAF* regulates the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) signaling pathway, thus affecting cell division, differentiation, and secretion[27]. Rasool et al[28] (2021) recognized numerous *BRAF* mutations in 14% of CRC Saudi patients studied. Of these, 5% of these mutations were in the V600E position, especially in female patients. The second most common mutation was the deletion of A at c.1758 bp, causing a frameshift and truncated protein of 589 amino acids only. Thirdly, heterozygous insertion of A/C at c.1860 bp and an insertion of A, leading to frameshift and stop codon after 629aa. Additionally, a mutation in at c.1780G > A (D594N), and c.1826insT, that resulted in frame shift and truncated protein of 610 amino acids. *BRAF* frameshift mutations E586E, Q609L, and M620I were detrimental, leading to truncated defective BRAF proteins. Accordingly, these frameshift mutations predict the worst clinical prognosis, as well as impaired response to therapy.

CASR

CASR (calcium sensing receptor) expression exhibits a protective role in CRC patients *via* several signaling transductions, such as stimulating cell differentiation, prompting apoptosis and constraining proliferation[29]. However, Al-Ghafari (2019)[30] verified that intron 4 variant (rs3804594) in *CASR* gene did not correlate with CRC risk.

CCAT1 and CCAT2

Siddique et al[24] (2019) showed that the expression level of *CCAT1* was significantly augmented in CRC patients, concluding that *CCAT1* expression could be used as possible biomarkers for CRC prognosis. Alternatively, no noteworthy *CCAT2* expression has been found in CRC samples when related to control[24].

COX-2

Numerous inflammatory mediators and cytokines are elaborated in the cancer pathogenesis, one of them belonging to the family of cyclooxygenases[31]. Albasri et al[32] (2018) conducted a retrospective study including 324 CRC diagnosed cases. They observed *COX-2* over expression in 40% of normal colonic mucosa, 65% of colorectal adenoma and 84.6% of CRC cases. Furthermore, elevated patients showing an elevated *COX-2* expression were found to suffer from shorter survival.

CTNNB1 (β-catenin)

The N-terminus of β-catenin harbors highly conserved residues, S33, S37, S45, and T41 encoded by exon 3 of the human *CTNNB1* gene. Alteration in any of these amino acid residues in *CTNNB1* gene exon 3 produces a stabilized β-catenin. Stabilized β-catenin could not be phosphorylated, causing constitutively active transactivation complexes, with subsequent loss of cell growth control[33]. Parine et al[34] (2019) studied thirteen SNPs in 8 genes including *CTNNB1* (β-catenin) (rs4135385, rs13072632), *SFRP3* (rs7775), *APC* (rs454886, rs459552), *LRP6* (rs2075241, rs2284396), *DKK4* (rs3763511), *DKK3* (rs6485350), *TCF4* (rs12255372) and *AXIN2* (rs3923086, rs3923087, rs4791171) in CRC patients. From the 13 SNPs, only genetic variants in *CTNNB1* (β-catenin) (rs4135385), *SFRP3* (rs7775), and *LRP6* (rs2284396) genes were associated with considerable defense against CRC development. GG genotype was associated with a lower risk of CRC developing relative to AA genotype at rs4135385 of *CTNNB1*, translating into an association of the rs4135385 in *CTNNB1* gene with a reduced CRC risk[34].

CYP

CYP1A1

CYP1A1 is important for the conversion of carcinogenic polycyclic aromatic hydrocarbons[35].

Saeed et al[36] (2013) demonstrated that a high distribution of *CYP1A1wt/*2A* genotype in CRC patients, reflecting a significant rise of cancer risk associated with *CYP1A1wt/*2A* genotype.

CYP2E1

CYP2E1 enzyme is responsible for the metabolic oxidation of low molecular weight carcinogens[37]. A nucleotide replacement (7632T>A) in intron 6 resulted in the absence of a DraI restriction enzyme site (*CYP2E1*6* allele, rs. 6413432). However, *CYP2E1 wt/*6* was not related to CRC risk in Saudi population[36].

CYP19A1

Al-Mukaynizi et al[38] (2017) designed a case-control study to explore the effect of three SNPs in the *CYP19A1*, on CRC risk. Two of the SNPs, rs4774585, an A > G transition and rs936308, C > G transversion, are positioned in the promoter region. The third SNP rs4775936 is situated in the intronic region and is a C > T transition. AA genotype of rs4774585, GG state of rs936308 and TT state of rs4775936 exhibited a negative association with CRC development in Saudi patients[38].

EGFR

A study[22] has investigated 99 CRC cases, leading to the recognition of frequent mutations in *EGFR* (11%). *EGFR* mutations were relatively frequent and significantly associated with young age of onset and poor disease-specific survival in CRC.

FBXW7

Dallol et al[22] (2016) identified frequent mutations in FBXW7 in 99 CRC cases via targeted sequencing.

FOX

Forkhead box protein M1 (FoxM1) is a member of the FoxM family, which initiates cell cycle progression and evasion of growth arrest. Consequently, FoxM1 deregulation was found to be involved in cancer pathogenesis[39]. FoxM1 protein overexpression was revealed in 66% of 448 CRC tissues and was related with poorly differentiated and highly proliferative tumors. Thus, *FOXM1* gene may serve as a valuable molecular biomarker, as well as a possible therapeutic target[40].

FSCN1

FSCN1, an actin-binding protein, stimulates cancer cell relocation, invasion and metastasis[41]. Alajez (2016)[26] distinguished *FSCN1* intensification in CRC patients, which was accompanied by reduced OS and DFS. Furthermore, high *BMI1*/*FSCN1* patients experienced the worst OS and DFS. Therefore, these two genetic markers (*BMI1* and *FSCN1*) in combination may represent superior prognostic markers than either one alone. Also, *BMI1* and *FSCN1* may offer potential therapeutic chances for CRC.

GST

GSTM1

Glutathione S-transferases (GSTs) are family of phase II enzymes vital in carcinogen detoxification. One of these GSTs is the *GSTM*, a protective enzyme that detoxifies several carcinogens. *GSTM*0/*0* genotype exhibits deficiency in enzyme activity with subsequent reduced carcinogen-detoxification ability[42]. A study[43] implemented using 83 CRC patients, showed that the majority (83%) of CRC cases harbored the null genotype (*GSTM1*0/*0*). The remaining (17%) cases had either the *GSTM1wt/wt* or the *GSTM1wt/*0* genotype. Therefore, among the control cases, 65% had the null genotype (*GSTM1*0/*0*) and 35% had either the *GSTM1wt/wt* or the *GSTM1wt/*0* genotype[43]. Another study[36] found *GSTM1*0/*0* genotype in only 2% of CRC patients, suggesting that *GSTM1*0/*0* is not a risk factor in CRC Saudi patients.

GSTP1

In the fifth exon at the codon 105 of Glutathione S-transferase pi (*GSTP1*), A changes to G which triggers substitution of isoleucine (Ile) with valine (Val) (Ile105Val), with a subsequent low enzyme activity. A study[44] tried to examine the potential influence of *GSTP1* (Ilel05Val) polymorphism in CRC risk in Saudi patients. None of the genotypes of *GSTP1* was associated with an increased risk of CRC development.

hBD

Human β-defensins (hBDs) belong to a family of antimicrobial peptides that constitute an important part of the innate immune defense. To date, four hBDs (1-4) have been identified in human tissues[45]. Semlali et al[46] (2015) designed a study to analyze the expression and genetic variations in hBDs (*hBD-1*, *hBD-2*, *hBD-3* and *hBD-4*) and their putative association with CRC in Saudi population. Numerous mutations, generally insertions, were recognized in different exons (1/2).

These mutations contributed to significant changes in the protein structure of hBDs (i.e., *hBD-1*, *hBD-3* and *hBD-4*). *hBD-1* mutations and mutation 1 of *hBD-3* lead to truncated pre-proteins with no predicted mature *hBD-1* protein synthesis. *hBD-3* mutation 2 protein is greatly destabilized because of the absence of a disulfide bridge caused by the substitution of cysteine to leucine (Cys63Leu). In addition, in the same mutant, lysine to glutamic acid (Lys67→Glu) substitution introduces a negatively charged, acidic residue in a positively charged, hydrophobic C-terminal section. These outcomes established a significant reduction of hBDs in cancer tissues related to normal tissues.

hTERT

The expression of four telomere-associated proteins, *hTERT*, *TRF1*, *TRF2*, POT1 were studied by Aljarbou et al[23] (2018). All CRC samples expressed *hTERT*; however, there was no difference between tumor and adjacent mucosa. Tissues adjacent to tumors showed detectable *hTERT* mRNA levels, while normal tissues do not express *hTERT*. Thus, Aljarbou et al[23] (2018) findings can be attributed to the presence of cancer-associated genetic modifications. Additionally, a positive correlation between the age of the patients and *hTERT* expression was identified[23].

IL-17

IL-17 is a major cytokine created by Th17 cells to prompt inflammatory cytokines and chemokines production by neutrophils and macrophages, thus playing a crucial role in human malignancies[47]. Al Obeed et al[48] (2018) detected that males harboring the A allele of *IL-17A* G197A SNP exhibited higher risk of developing CRC. No connection between *IL-17F* (rs763780) polymorphism and CRC susceptibility[48] has been found.

KIR

Natural killer (NK) cells play a fundamental role in the immunity regulation against infected and malignantly transformed cells through their killer cell immunoglobulin-like receptors (KIRs). *KIRs* interacts with human leukocyte antigen (HLA) molecules[49]. Al Omar et al[50] (2015) verified five activating *KIR* genes (*2DS1*, *2DS2*, *2DS3*, *2DS5*, and *3DS1*), which were significantly more prevalent in CRC patients. The highest risk was associated with the *3DS1* gene, followed by the *2DS1* gene. Additionally, no association was found between *3DL1* and *2DS4* and CRC, whereas *2DS2* was inversely associated with CRC risk.

K-ras

In *K-ras* gene, the majority of somatic mutations occur at codons 12 and 13 (situated in exon 2). Other less frequent mutations occur in exon 3 (codons 59/61) and exon 4 (codons 117/146)[51]. Wild type *K-ras* protein resides in the GDP-bound state on the plasma membrane in inactive cells, whereas mutated *K-ras* is in a continuous stimulation. Subsequently, mutant *K-ras* proteins are driving tumor formation and progression[52]. Additionally, activated *K-ras* is associated with increased aggressiveness of CRC and reduced responsiveness to targeted therapies[27]. Numerous studies[5,22,53,54] explored *K-ras* mutations in Saudi Arabia. These studies[5,22,53,54] showed different prevalence of *K-ras* gene mutations including 35%, 42.85%, 42% and 56%. It is not clear why there is a wide range difference in the percentage of *K-ras* mutations in Saudi Arabia. A study[5] was performed on *K-ras* mutations in cancerous tissue obtained from 56 Saudi sporadic CRC patients from the Eastern Province. *K-ras* gene mutations were detected in the cancer tissue of 24 out of the cases studied. Of these, 11 had exon 4 mutations localized between codons 134 and 150, while 13 had mutations in exon 2, affecting codons 12 and 13. No mutations were identified in codon 61 of exon 3. The 11 cases with exon 4 aberrations harbored p. Ala146Thr (a missense mutation), p. Ala134Val, p. Arg135Lys, p. Gln150Stop, p. Lys147Lys, p. Gln150Stop, p. Gln150Stop, p. Gly138Gly, p. Arg149Gly, p. Gly138Gly, and p. Gly138Gly mutations. Missense *K-ras* mutations which altered the amino acid sequence of the protein (A134V, R135K, E143K and R149G) were distinguished. Moreover, two synonymous *K-ras* mutations (G138G and K147K) and, a nonsense truncating mutation (Q150X) were detected. E143K mutation is predicted to have a damaging effect on the protein. R149G mutation is predicted to be neutral, but molecular modeling showed that this mutation caused changes in the helix and loop chains near the GTP binding pocket. Q150X mutation introduced a premature stop codon[5].

Another study[53], one of the largest studies investigated *K-ras* mutations, was accomplished via examining 300 CRC patients in KSA. Most mutations detected were at codon 12 (89%) and were associated with metastasis. The prevalence of mutated *K-ras* was 42% in patients and mostly in stages II-IV, suggesting that *K-ras* mutations were concomitant with advanced stage of CRC, shorter RFS and OS. Additionally, mutations in

codons 12/13 of *K-ras* exon 2 are associated with reduced benefit from EGFR antibody treatment for metastatic CRC[53].

A third study[54] investigated and analyzed retrospectively the frequency of *K-ras* mutation and its correlation with patients' characteristics and clinicopathological features in CRC patients. *K-ras* mutations were in codon 12 (75%), most commonly p. G12D, codon 13 (20%). *K-ras* mutations were higher in young patients (≤ 50, 54.5%) and in the right-sided tumors (57.1%). A fourth study[55] collected 80 CRC tumor tissues and sequenced the *K-ras* gene. Mutations in four different codons (12, 13, 17, and 31) were recognized in 26 patients. Several mutations in *K-ras* were acknowledged in codon 12 (61.5% of all mutations), glycine was substituted by aspartate (G12D), glycine substituted by valine (G12V), glycine substituted by arginine (G12R), glycine substituted by alanine (G12A), and glycine substituted by cysteine (G12C). The presence of glycine at position 12 seemed to be imperative for appropriate *K-ras* gene functioning and disruption, or the replacement of this amino acid led to failure in function efficiency. Other mutations in *K-ras* recognized in codon 13 included glycine was replaced by aspartate (G13D) and glycine substituted by cysteine (G13C). As for codon 17, mutations in which serine was substituted with arginine (S17R) were detected. Whereas in codon 31, a very rare mutation was identified, c.91 G > A, in which glutamic acid was replaced by lysine[55].

Another study[22] has investigated 99 CRC cases, identifying frequent mutations in *K-ras* (35%) which were associated with poor disease-specific survival in cases with wild-type TP53.

LRP6

Parine et al[34] (2019) verified a fourfold decreased risk of developing CRC in CC genotype, compared to TT genotype at SNP rs2284396 in *LRP6* gene[34].

MALAT1

Siddique et al[24] Alsufiani (2019) displayed that the expression levels of metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1)* were significantly escalated in CRC patients.

MED12

MED12 encodes a member of mediator, a multiprotein complex involved in the transcriptional regulation of many genes by mediating the interaction of RNA polymerase with various transcriptional factors[56]. Siraj et al[57] (2018) identified three *MED12* somatic mutations in 27 CRC patients, which expand the role of *MED12* as a tumor suppressor in CRC.

MEG3

Siddique et al[24] (2019) revealed no significant expression of *MEG3* in CRC when compared with control.

miRNAs

Several classes of non-coding RNAs (*ncRNAs*), including microRNAs (*miRNAs*), exhibit differential expression in many types of cancer, including CRC, and their dysregulation promote carcinogenesis[58]. Al-Sheikh et al[59] (2016) showed significant rises in *miR-29* and *miR-92* and their expression levels. Whereas *miR-145* and *miR-195* decreased in CRC tissues compared with adjacent neoplasm-free mucosal tissues[59]. Another study performed by Fawzy et al[60] (2020) identified noteworthy escalation in *miR-34a* in CRC colon specimens, specifying that *miR-34a* rs2666433 AA and AG genotype carriers were more likely to develop cancer than GG carriers[60]. Additionally, Al-Sheikh et al[61] (2019) demonstrated that *miR-182-5p* gene was amplified in CRC patients.

MLH1

Lynch syndrome (LS) is associated with early onset of CRC and enhanced risk of many extra colonic malignancies[62]. LS is mainly caused by germline pathogenic mutations in DNA mismatch repair (MMR) genes, mostly in four of the genes, MutL Homolog 1 (*MLH1*), MutS Homolog 2 (*MSH2*), MutS Homolog 6 (*MSH6*) and PMS1 Homolog 2 (*PMS2*)[63]. Rasool et al[12] (2020) detected structural loss in the genomic regions of *MLH1* (3p23-p14.2), *MSH2*, *MSH6*, *EPCAM* (2p21-p16.3), *PMS2* (7p22.1) and *MUTYH* (1P34.1-p33) in LS patients. This structural loss resulted in lower expression of MLH1, MSH2, MSH6, PMS2, EPCAM and MUTYH genes. Moreover, Ahmed (2017)[64] detected a positive *MLH1* correlation in around 27% and that MSI was present in more than 90% of CRC patients with Lynch syndrome.

MMP-2

MMPs overexpression is associated with tumor invasion, metastasis, and a worse prognosis. Two studies[65,66] confirmed that *MMP2* C1306 T mutation was significantly more common in colon Saudi patients.

NOTCH1

The *NOTCH* gene family consists of four receptors (*NOTCH1, NOTCH2*, *NOTCH3*, and *NOTCH4*). Notch signaling plays an important role in several cellular processes, including proliferation, epithelial cell polarity/adhesion and apoptosis[67]. Alanazi et al[68] (2021) demonstrated that SNPs rs3124591 in *NOTCH1* and rs3820041 in *NOTCH4* did not exhibit any association with CRC. Although rs1043994 in *NOTCH3* was not associated with CRC in the overall analysis, it displayed a significant association with CRC in males. The GA heterozygote males of this SNP were two-fold higher risk of CRC development compared to GG homozygotes.

PANDAR

Siddique et al[24] (2019) showed that the expression levels of the promoter of CDKN1A antisense DNA damage-activated RNA (*PANDAR*) were significantly enlarged in CRC patients.

PARP-1

Poly (ADP-ribose) polymerase-1 (*PARP-1*) has a crucial role in DNA damage repair and is involved in many cellular processes. Thus, *PARP-1* gene polymorphisms are associated with the risk of various carcinomas, including colon cancer. Alshammari et al[69] (2014) demonstrated that SNPs in *PARP-1* gene, including Met129Thr, Val762Ala, and Lys940Arg, did not show any association with CRC risk, while Lys933Asn and Lys945Asn showed significant association with CRC among Saudis[69]. Another study formulated by Alhadheq et al[70] (2016) demonstrated that SNP rs8679 diminished the susceptibility to colorectal cancer at heterozygous TC allele and at minor allele C.

PCAT6

Siddique et al[24] (2019) revealed no significant expression of *PCAT6* in CRC patients.

PIK3CA

PIK3CA mutations were significantly associated with poor disease-specific survival in cases with wild-type *TP53* in CRC Saudi patients[22].

POLD1 and POLE

POLD1 and *POLE* encode the catalytic subunit of the polymerase enzyme complexes Epsilon (ε) and Delta (δ), which play an imperative part in DNA replication and repair[71]. Siraj et al[72] (2020) demonstrated that four variants in *POLE* gene were associated to an increased risk of CRC, besides the three *POLE* variants p. His342Tyr, p. Gly395Glu, and p. Thr457Met, which were found in early onset CRC patients. Furthermore, *POLD1* variant of c.932G > A: p. Arg311His was established in a late onset patient, causing loss of function of POLD1. Generally, low *POLE* protein expression exhibited a substantial link with lymph node involvement and grade III tumors. Whereas for *POLD1*, low expression was related to adenocarcinoma histology, larger tumor size and stage III tumors.

PRNCR1

Prostate cancer non-coding RNA (*PRNCR1*) propagates colon cancer from epithelial cells, causing an increase the tumor size in CRC patients[73]. One SNPs, rs1456315, in *PRNCR1* gene revealed an association with CRC with the homozygous CC variant allele. This risk association was observed among younger age patients (≤ 57) and in female patients. Three other SNPs rs1016343 (C > T), rs13252298 (A > G), and rs16901946 (A > G) in *PRNCR1* gene did not display any association with CRC[74].

P35 and PTEN

Mutation of *p53* frequently happens in almost half of all human malignancies and contributes to tumor progression[75]. A retrospective cohort study[64] was performed out over a five-year period in which 130 samples were recruited. The study revealed that a *P53* positive expression was found in 25.4%, whereas loss of *PTEN* expression was recognized in 32.3% CRC patients.

RETN

Resistin gene (*RETN*) codes a peptide hormone called resistin, which is secreted predominantly by adipose tissue, in particular from adipocytes and macrophages[76]. A study accomplished by Alharithy (2014)[77] indicated that SNPs in *RETN* gene rs1862513 (C-420G) and rs3745367 (G+299A) had increased the risk of colon cancer. Additionally, carriers of the heterozygous (GA) genotype of SNP 299 had a significantly higher colon cancer risk than carriers of the wild (GG) genotype.

SFRP3

As already mentioned, Parine et al (2019)[34] verified that genetic variants in *SFRP3* (rs7775) gene were correlated with considerable protection against CRC progression. Women having Gly at codon 324 (rs7775) of *SFRP3* have 2.5-fold lower risk of developing CRC compared to those having

Arg at this locus. Thus, *SFRP3* gene may serve as a protective marker in female patients harboring the minor allele G[34].

SMAD4

Frequent mutations in *SMAD4* were identified and correlated with CRC development, as showed by a study done by Dallol et al[22] (2016).

TDG

In addition to its DNA repair function, thymine DNA glycosylase (*TDG*) is also involved in other critical cellular processes[78]. SNP rs4135113 in *TDG* gene showed a significant risk association between its genotype AA and the minor allele A in CRC Saudi patients in general, and in patients aged more than 57 years. On the other hand, SNP rs1866074 in *TDG* gene presented a protective association between the GG allele and the additive (AG+GG) allele in CRC patients. Other four SNPs (rs4135050, rs4135066, rs3751209, and rs1882018) in *TDG* gene showed no association with CRC patients in the Saudi population[79].

TIGAR

The TP53-induced glycolysis and apoptosis regulator (*TIGAR*) regulates glycolysis by acting as fructose bis-phosphatase (FBPase) and modulate reactive oxygen species[80]. Al-Khayal et al[81] (2016) revealed that *TIGAR* expression was found in 68% of the tumor samples with nuclear localization and was significantly amplified in early stages (stage I and II) and late stages (stage III and IV) of CRC. Thus, *TIGAR* expression may be used as a biomarker for CRC recognition and even as a target for developing therapeutics for CRC treatment[81].

TLRs

Toll-like receptors (*TLRs*) represent the first line of defense against invading pathogens, initiating inflammatory responses; thus, they play a key role in immune cell regulation, survival, and proliferation[82].

TLR2

A study[83] goal was to determine the association of *TLR2* SNPs (rs3804099, rs3804100, and rs4696480) and the risk of colon cancer development in a Saudi Arabia population[83]. *TLR2* rs3804099 and *TLR2* rs4696480 SNP were closely associated with CRC susceptibility. However, *TLR2* (rs3804100 C > T) disclosed no association with CRC susceptibility in Saudi patients.

TLR4

Semlali et al[84] (2016) revealed a clear association between *TLR4* rs10759931polymorphism, the G allele, and susceptibility to CRC development risk in the Saudi Arabian population. Also, the *TLR4* rs2770150 is associated with CRC in women aged over 50 years and is linked to the decreased levels of female sex hormones during the post-menopausal period. Whereas *TLR4* SNPs rs10759932 and rs4986790 appeared not to have any association with colon cancer[84].

TLR6

Semlali et al[85] (2019) illustrated that Val/Met genotype of rs3796508 of *TLR6* gene had a significantly higher frequency in the control group than in the CRC male cases, suggesting that *TLR6* rs3796508 is a protective factor against CRC in the older male Saudi population. Two other non-synonymous SNPs S249P and V327M were common in a few patients and were predicted as being damaging[85].

TLR 9

TLR 9 is the only *TLR* which is administered systemically and has shown substantial evidence of anticancer activity in human clinical trials[86]. Semlali et al[87] (2016) clarified a significant association between the *TLR-9* rs187084 SNP and CRC risk in female patients. T allele exhibited lower frequency (2.8 times) in female cancer patients. Additionally, *TLR-9* rs352139 and rs352144 SNPs were found to be suggestively correlated with colon cancer development when the tumor was located in the rectal area, but not in the colon area localization. On the other hand, all three *TLR-9* SNPs rs352144, rs187084 and rs5743839 were not associated with colorectal cancer in males.

TNF-α

Hamadien et al[88] (2016) verified that *TNF-α* SNPs, -308 and -857, were not associated with CRC. *TNF-α* -238 (G/A) genotype was significantly concomitant with high risk of CRC. This is because AA genotype of -238 G/A SNP was observed at considerably higher proportion in CRCs.

PTEN

Loss of *PTEN* has been reported[89] in many types of cancers, including CRC. The overall loss of *PTEN* expression (negative) was identified in 32.3% of the CRC patients[64]. In another study in KSA, *PTEN* was inactivated in 66.1% of the 51 CRC cases, and *PTEN*loss was more frequent in

CRC[90]. A third study[22] identified *PTEN* mutations in 13% of CRC cases.

TP53

Interestingly, *KRAS* or *PIK3CA* mutations were significantly associated with poor disease-specific survival in cases with wild-type *TP53*[22]. Mutant *TP53* may serve as an emerging target for cancer treatment using small molecule therapeutics that restores wild-type *TP53* function, inducing cell cycle arrest and apoptosis[91].

TSLP

TSLP, *IL-7* like cytokine, triggers *STAT1*, *STAT3*, *STAT4*, and *STAT5,* stimulating the proliferation, development, differentiation, migration, and death of apoptotic cells, depending on the type of stimuli and cells[92]. Semlali et al[93] (2021) established that *TSLP* rs10043985 presented a strong correlation with CRC Saudi patients, indicating that this mutation in the promoter region of *TSLP* gene might play a detrimental role in CRC. However, rs2289276 SNP of *TSLP* gene did not show any relation with CRC. On the other hand, *IL-7R* rs1053496 SNP showed no association with CRC in female subjects or in CRC patients who are more than 57 years of age[93].

UCA1

Siddique et al[24] (2019) revealed no significant expression of lncRNA urothelial carcinoma-associated 1 (*UCA1*) in 63 CRC cases when compared with control.

VDR

More than sixty SNPs of the *VDR* gene, located in the promoter region, have been related to cancer occurrence and prognosis. *ApaI* (rs7975232), *TaqI* (rs731236), *BsmI* (rs1544410) and *FokI* (rs10735810) are *VDR* SNPs that affect *VDR* gene expression and mRNA stability[94]. Alkhayal et al[95] (2016) did not observe any association of the four *VDR* polymorphisms with CRC risk in the overall analysis. However, *ApaI* and *BsmI* loci were associated with CRC in elderly and female patients, respectively[95]. In contrast, Al-Ghafari et al (2020)[96] demonstrated that for the *ApaI* SNP (rs7975232), only the heterozygous (AA) genotype increased the risk of CRC. Moreover, they showed that *TaqI* SNP (rs731236) carriers with either the heterozygous (TT) or homozygous (TT) genotype displayed an increased risk for the disease. In contrast, heterozygous (Bb) and homozygous (bb) carriers of the *BsmI* SNP (rs1544410) had significantly lower risk for CRC. Finally, for the *FokI* SNP (rs2228570), there was no association with CRC risk. Another study performed by Al-Ghafari et al[96] (2020 found that the *VDR* SNPs *ApaI* and *TaqI* upsurge the risk of CRC, whereas *BsmI* lessens the risk of CRC in the selected Saudi population.

XPD and XRCC1

The xeroderma pigmentosium group D (*XPD*) protein participates in nucleotide excision repair (NER), one of DNA repair pathways. X-ray repair cross-complementing group 1 (*XRCC1*) is acknowledged to participate in base excision repair (BER)[97]. Karam et al[98] (2016) demonstrated no significant difference in *XPD* Lys751Gln polymorphism in CRC. Regarding *XRCC1* polymorphism, they demonstrated there was an association between the GG genotype of *XRCC1* polymorphism and the increased risk of CRC. Moreover, *XRCC1* (AG + GG) polymorphism may be associated with increased clinic pathological parameters of CRC.

Genetic Variation Irrelevant to CRC

ABCB1 (C3435T, T129C and T1236C), *ABCC1* (G128C), *CASR* (rs3804594), *IL-17F* (rs763780), *NOTCH1* (rs3124591), *NOTCH4* (rs3820041), *PRNCR1* (rs1016343, rs13252298, and rs16901946), *TDG* SNPs (rs4135050, rs4135066, rs3751209, rs1882018), *TLR2* (rs3804100), *TLR4* (rs10759932, rs49867 90), *TSLP* (rs2289276), *TSLPR* (rs36139698, rs36177645, rs361334 95), and *TNF-α* (-308 and -857) polymorphisms were unrelated to CRC risk in KSA. Additionally, Met129Thr, Val762Ala, and Lys940Arg and *XPD* (Lys751Gln) polymorphisms in *PARP-1* were unconnected to CRC risk in Saudi population. Moreover, genotypes *CYP2E1*6*, *GSTM1*0/*0* were not associated with the CRC development. Besides, no significant expressions of *BCAR4*, *CCAT2*, *MEG3*, *PCAT6*, *UCA1* were found in CRC samples, nor did any genotypes of GSTP1 showed association with CRC development. Finally, three *TLR-9* SNPs (rs352144, rs187084 and rs5743839) were unrelated to colorectal cancer in males, whereas *IL-7R* rs1053496 SNP showed insignificant association with CRC in female subjects or in CRC patients who were more than 57 years of age.

Genetic Variation Protected Against CRC

Genetic variants in *ABCB* (rs3435, TT genotype, G2677T, female), *ADIPOQ* (T45G, G allele), *CTNNB1* (rs4135385, GG genotype), *SFRP3*

(rs7775, GG genotype), *LRP6* (rs2284396, CC allele), *CYP19A1* (rs4775936, TT allele in females; rs4774585, AA allele in males; rs936308, GG allele), *PARP-1* (rs8679, TC allele), *TDG* (rs1866074, GG allele) genes allied with a significant protection against CRC in Saudi population. In addition, *MED12* somatic mutations, *2DS2* in *KIR* genes, *TLR6* rs3796508 have been found to have a crucial role as protective factors against CRC.

Genetic Variation Strongly Associated with CRC

ABCC1 (C218T, CT genotype), *ADIPOQ* (G276T, T allele), *CYP1A1* (*wt/*2A* genotype), *KIR* (3DS1, 2DS1), *IL-17A* (G197A, A allele in males), *MMP2* (C1306 T), *NOTCH3* (rs1043994, GA genotype, males), *PRNCR1* (rs1456315, CC, young age, female), *RETN* (rs1862513 and rs3745367), *TDG* (rs41351130, AA genotype), *TLR2* (rs3804099, rs4696480) polymorphisms exhibited a substantial augmented CRC risk of development in CRC Saudi patients. Additionally, *TLR4* (rs1075993, G allele; rs2770150 women aged over 50 years), *TLR-9* (rs187084, female), *TNF-α* (-238 (G/A) genotype), *TSLP* (rs10043985) and *XRCC1* (GG genotype) mutations displayed a significant amplified CRC development. Furthermore *ATR*, *ATM*, *BMI1*, *CCAT1*, *Chk1*, *Chk2*, *COX-2*, *FoxM1*, *FSCN1*, *Ki67*, *MALAT1*, *miR-29*, *miR-34a*, *miR-92*, *miR-182-5*, *PANDAR*, *PIK3CA*, *TIGAR* over-expression showed a correlation with CRC Saudi inhabitants. Besides, significant associations between *BRAF* (E586E, Q609L, and M620I) and *PARP-1* (Lys933Asn and Lys945Asn) and CRC risk have been detected. Moreover, *ALK* gene amplification and gain in copy number and gene mutations in *APC*, *EGFR*, *FBXW7*, *TP53*, *PTEN*, *K-ras* were concomitant in CRC Saudi population. Structural loss of *MLH1* (3p23-p14.2), *MSH2*, *MSH6*, *EPCAM* (2p21-p16.3), *PMS2* (7p22.1) and *MUTYH* (1P34.1-p33), with subsequent lower expression of *MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM* and *MUTYH* genes, were recognized in LS patients and future CRC patients.

Conclusions

In this review, we performed a comprehensive literature review concerning CRC genetics to offer an insight into the CRC genes in Saudi Arabia patients. All these gene mutations may be used as diagnostic and/or prognostic genetic marker in CRC Saudi patients and could offer a potential therapeutic target for CRC management. For each of these genes, we tried to explain a little about the gene and its role in cancer development and clinical phenotype on the Saudi patients and the mutation occurring in these genes. Several genes' mutations were included in this review, from which some genetic variations were either associated or strongly related to, nor even protected against the CRC development.

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Conflicts of Interest

The authors declare no conflicts of interest.

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Authors' Contributions

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