RESEARCH ARTICLE

Novel Mutations of the *PARP-1* Gene Associated with Colorectal Cancer in the Saudi Population

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Abstract

Background: colorectal cancer (CRC) is the third most common type of cancers and the fourth leading cause of death worldwide. In Saudi Arabia, CRC accounts for 8.5% of all tumors; it ranks first among all cancers in males and third among females. The aim of this study was to link between different PARP-1 mutations and risk of CRC in Saudi population and to determine common variants of *PARP-1* in Saudi CRC patients and normal individuals. Materials and Methods: DNA samples were isolated from fifty CRC patients and from a comparable number of control subjects then sequenced to detect different variations present in exons 3, 17, and 21 of the *PARP-1* gene. Results and Conclusions: When comparing the genotype and allele frequencies of all detected SNPs in CRC patients with those in controls, we found none were significantly different for all variants even the most common SNP in *PARP-1* gene (Val762Ala). However, two novel alterations in exon 21 were found to be associated with increased risk of CRC. The variants identified as (1) Lys933Asn [p-value 0.0318] and (2) Lys945Asn [p-value 0.0257]. Our results suggest that *PARP-1* Lys933Asn and Lys945Asn alterations could be associated with increased risk of CRC in the Saudi population.

Keywords: *PARP-1* - colorectal cancer - DNA repair enzymes - single nucleotide polymorphism - Saudi Arabia

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Introduction

The incidence and related mortality rates of CRC in Saudi Arabia have been on a constant rise over the past twenty years (Mosli and Al-Ahwal, 2012) In 2010, Al-Huzaim and his colleagues reported that CRC accounts for about 8.5% of all tumors in Saudi Arabia (Al-Huzaim et al., 2010) and now it is considered as the first type of cancers among males and the third among females (Sibiani et al., 2011).

Poly(ADP-ribose) polymerase-1 (PARP-1) (**EC 2.4.2.30**) is a nuclear zinc finger DNA-binding enzyme catalyzes the conversion of nicotinamide adenine dinucleotide (NAD^+) to long branched adenosine diphosphate ribose polymers (PARs) and the addition of these PARs to target proteins as a post translational modification of these proteins (Schreiber et al., 2006). PARP-1 is responsible for at least 90% of total cellular PARP activity and it is considered, along with its close homologue PARP-2, the DNA damage response arm of the PARP family (Canto and Auwerx, 2011).

PARP-1 has a crucial functional role in DNA damage repair also it involves in many cellular processes including maintaining genomic stability, cell cycle regulation, regulation of chromatin structure, differentiation, proliferation, cell death, controlling gene expression, inflammation, and malignancy (D'Amours et al., 1999; Luo and Kraus, 2012). In 2012, Abd Elmageed and his colleagues revealed a significant role of this enzyme in cell transportation system and cellular trafficking in both physiological and patho-physiological situations (Abd Elmageed et al., 2012).

PARP-1 is a multi-domain enzyme consists of three functional domains: an amino-terminal DNA-binding domain (DBD) that contains three zinc finger motifs; the first two homologues Cyc-Cys-His-Cys zinc fingers (ZnF I & ZnF II) mediate the binding of PARP-1 to DNA-strand breaks. The third zinc finger (ZnF III) is important for coupling damage-induced changes in the DBD to alterations in PARP-1 catalytic activity (Rouleau et al., 2010). This domain also contains a nuclear localization signal (NLS) that targets PARP-1 to the nucleus and a caspase3 cleavage site (Hakme et al., 2008). Central auto-modification domain (AMD) that functions as the target of covalent auto-poly(ADP-ribosyl)ation (Ame et al., 2004). It contains a BRCA1 carboxyl-terminal (BRCT) domain which mediates protein-protein interaction (Schreiber et al., 2006). Specific glutamate and lysine

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residues in AMD serve as acceptor sites for poly(ADP-ribosyl)ation (Yelamos et al., 2011). PARP-1 contains a weak leucine-zipper motif in the amino-terminal of the AMD that might be involved in homo- and/or hetero-dimerization (D'Amours et al., 1999). It also contains unknown functional motif, named WGR, that is rich with tryptophan (W), glycine (G) and arginine (R) residues that may allow the AMD to serve as a nucleic acid binding domain. This motif is found in the carboxylic-terminal of AMD of only three human PARP-like proteins: PARP-1, PARP-2, and PARP-3 (Hassa et al., 2006; Krishnakumar and Kraus, 2010). The third domain is the carboxyl-terminal catalytic domain (CD) that catalyzes three different enzymatic reactions: the attachment of the first ADP-ribose moiety onto an acceptor amino acid (initiation reaction), the addition of further ADP-ribose units onto already existing ones (elongation reaction), and the generation of branching points (branching reaction) (Ruf et al., 1996). It is composed of a PARP signature motif; a highly evolutionally conservative sequence that contains key amino acid residues involve in the formation of both the active site and NAD^+ acceptor site (Rouleau et al., 2010).

The *PARP-1* gene polymorphisms have been associated with the risk of various carcinomas, including colon cancer (Brevik et al., 2010). Polymorphisms of *PARP-1*, as well as differences in the levels of PARP-1 and poly-(ADP-ribosyl)ation of proteins may influence germ cell tumor development and responses to chemo/radio-therapies (Hideki et al., 2010). Studying the *PARP-1* gene polymorphisms can aid in elucidating the role of PARP-1 in the development of different types of cancer and could potentially provide highly effective therapies for cancer that have increased sensitivity to PARP-1 inhibition (Bryant et al., 2005).

In this study we attempted to link between inherited *PARP-1* mutations and susceptibility to develop CRC in Saudi population. We explored whether this gene was or was not hereditarily mutated in CRC. This study aims to (1) Study the genotype and allele frequencies of *PARP-1* gene polymorphisms in patients with CRC and compare it with healthy subjects. (2) Evaluate the role of *PARP-1* polymorphisms as predisposing factors for CRC in Saudi population.

Materials and Methods

Study population

Blood samples were collected from fifty consented CRC patients attended various clinics at King Khalid University Hospital, Riyadh, Saudi Arabia. The diagnosis of CRC was based on standard clinical, endoscopic, radiological, and histological criteria. Clinical and demographic characteristics were recorded including; age at diagnosis, gender, and disease location. Blood were also collected from another fifty consented, age and sex-matched healthy individuals with no family history of CRC who attended the hospital for minor illnesses. The study was conducted after review and approval of the Institutional Review Board of the Ethics Committee at King Khalid University Hospital, Riyadh, Saudi Arabia.

DNA extraction

Peripheral blood samples from all participants were collected in EDTA-containing tubes and genomic DNA was isolated from peripheral blood lymphocytes using DNA extraction kit (Gentra Systems Inc., USA) according to the manufacturer's instructions. The concentration of extracted DNA was quantitated on a NanoDrop 8000 (Thermo Scientific, USA) and its purity was examined using standard A260/A280 and A260/A230 ratios.

Polymerase chain reaction

Polymerase chain reactions (PCRs) were used to detect *PARP-1* gene alterations in DNA samples extracted from 5 mL of each blood sample. All PCR assays were performed in 50 μL volume reaction containing 10 mmol/L Tris-HCl, PH 8.3, 50 mmol/L KCl, 2 mmol/L $MgCl_2$, 250 μmol/L of dNTPs (Invitrogen, USA), 0.20 μmol/L concentration of each primer (Integrated DNA Technologies, USA), 2.5U of Taq DNA polymerase (Qiagen, USA), and 50 ng of genomic DNA. All PCRs involved an initial denaturing step at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 45 sec., annealing at 60°C for 1 min. (see Table 1), and extension at 72°C for 55 sec. All PCR reactions were followed by terminal extension step at 72°C for 7 min. Finally, the reactions were then held at 4°C until analysis. All PCR products were electrophoresed in agarose gel and visualized by ethidium bromide staining.

Sequencing reaction

In the present study we conducted a full sequence analysis of *PARP-1* exons 3, 17, and 21. The mutant sites were screened and validated using Sanger sequencing method. For cycle sequencing in 96-well; 8 μl of sequencing reagent (ETT) premix (GE Healthcare Amersham, Sweden) and 2.5 μl of forward primers (with final concentration of 5 pmol/μl) were added to 2.5 μl of purified PCR product and 7 μl of water to give a total reaction volume of 20 μl. Cycle sequencing was carried out using veriti® thermocycler (Applied Biosystem, USA) for 25 cycles of 95°C for 20 sec., 52°C for 15 sec., and 60°C for 1 min.

The post sequencing reaction products were purified using ethanol precipitation protocol. Following this step the plate was ready to be subjected for sequencing by using automated MegaBACE-1000 DNA analysis system (American Laboratory Trading, USA). All samples with novel SNPs were subjected to sequencing for both strands to confirm the presence of SNPs on both strands.

Prediction of PARP-1 protein structure

Prediction of protein structure were done by

Table 1. Primers Used for Amplifying Different *PARP-1* SNPs

Gene mutation	Primers sequence (5`↦3`)	Product size (bp)	Ta (°C)
Missense mutation Met129Thr	GGGAGGAGATGGAATGTCAG CTGCTGGGTCCAAAAGAGAC	656	60
Missense mutation Val762Ala	CCCAAATGTCAGCATGTACG GGTTCTCAAAGGACCACCAG	567	60
Missense mutation Lys940Arg	GCACAAACTATGTTCAGCCG GTTTGACAGCCAGCCACAAG	500	60

determining the QMEAN6 scoring function in order to discriminate the native from non-native models of the PARP-1 enzyme. The torsion angle potential over three consecutive amino acids was applied to analyze the local back-bone geometry of the PARP-1 protein structure and to recognize the native structure. The solvation free energy was calculated to discriminate the native protein structure from decoy ones, to describe the burial status of amino acids residues, and to study the effective energy surface of proteins in the solution that has a major determinant of protein stability. All-atom energy enables the scoring function to select a good protein model by measuring the protein's energy in a vacuum (Benkert et al, 2009). We constructed and analyzed the protein models of the mutant PARP-1 enzyme on Lys933Asn and Lys945Asn. The PARP-1 protein models were predicted by SWISS-MODEL and were viewed in Swiss-Pdb Viewer.

Statistical analysis

Statistical Package for the Social Sciences (SPSS) (IBM Corporation, New York, U.S.) version 20.0 was used for this study. Genotype and allele frequencies were tested using the chi-square (χ^2) goodness of fit; a p-value ≤0.05 was considered significant. Odds ratio (OR) and corresponding 95% confidence interval (CI) were also estimated to study the association between different *PARP-1* gene mutations and CRC risk.

Results

The demographic and clinical characteristics of the CRC patients are shown in Table 2. The demographic data of controls were similar to those of the patients. Among the CRC patients, 34 (68%) were males and 16 (32%) were females and 76% of this population were above 50 years of age. The majority of the CRC patients have lower colorectal mass; 32% of patients with sigmoid mass and 28% of patients with rectum mass.

Frequency of PARP-1 Met129Thr polymorphism

The *PARP-1* Met129Thr T>C polymorphism in DBD was assayed in fifty CRC patients and a comparable number of control subjects. All the samples were homozygous for the wild type T allele and we could not find the mutant non-heterozygous C allele in neither the CRC samples nor the controls (data not shown). We were able to detect a heterozygous T/A allele alteration that converts methionine to lysine (ATG/AAG) at the same position (129), which was only found in a single CRC patient. The analyses of *PARP-1* Met129Lys genotypes in Saudi CRC patients are presented in Table 3. The OR for patients with the Met/Lys genotype versus the Met/Met genotype was 3.0606 (p-value=0.4966, and 95% CI=0.121-76.953). These results show no significant association between *PARP-1* Met129Lys variant and CRC risk in Saudi patients.

Frequency of PARP-1 Val762Ala polymorphism

Allele and genotype frequencies of *PARP-1* Val762Ala (GTG/GCG) SNP in exon 17 are presented in Table 4. The homozygous mutant allele for Val762Ala SNP was found in a single patient and was not detected in any of the controls. Two patients and one control subject were carried the heterozygote Val/Ala genotype. Our results (p-value 0.5540 for the heterozygous Val/Ala genotype and p-value 0.4885 for the mutant homozygous Ala/Ala genotype) show no association between *PARP-1* Val762Ala SNP and overall risk of CRC among Saudis.

Table 2. Distribution of CRC Patients According to Demographic Data

		No.	%
Sex	Male	34	68
	Female	16	32
Age	>50	38	76
	<50	12	24
Disease location	Rectum mass	14	28
	Sigmoid mass	16	32
	Transverse	4	8
	Splenic flexure mass	4	8
	Right colon	6	12
	Cecum	6	12

Table 3. Genotype Frequencies of the *PARP-1* Met129Lys Variant in Saudi CRC Cases and Controls

Genotype	Cases	Controls	OR	95% CI	p-value
Met/Met	49 (0.98)	50 (1.00)	Ref.		
Met/Lys	1 (0.02)	0 (0.00)	3.0606	0.1217-76.953	0.4966
Lys/Lys	0 (0.00)	0 (0.00)	1.0202	0.0198-52.433	0.9921
Met/Lys+Lys/Lys	1 (0.02)	0 (0.00)	3.0606	0.1217-76.953	0.4966
Met	99 (0.99)	100 (1.00)	Ref.		
Lys	1 (0.01)	0 (0.00)	3.0302	0.1220-75.283	0.4988

Table 4. Genotype Frequencies of the *PARP-1* Val762Ala Variant in Saudi CRC Cases and Controls

Genotype	Cases	Controls	OR	95% CI	p-value
Val/Val	47 (0.94)	49 (0.98)	Ref.		
Val/Ala	2 (0.04)	1 (0.02)	2.0851	0.1829-23.770	0.554
Ala/Ala	1 (0.02)	0 (0.00)	3.1263	0.1242-78.663	0.4885
Val/Ala+Ala/Ala	3 (0.06)	1 (0.02)	3.1277	0.3141-31.143	0.3308
Val	96 (0.96)	99 (0.99)	Ref.		
Ala	4 (0.04)	1 (0.01)	4.125	0.4529-37.574	0.2087

Frequency of PARP-1 Lys940Arg polymorphism

The mutant genotype for Lys940Arg A>G was not detected in any of our samples, but we found another alteration, with a heterozygous A/C allele, that converts the lys940 to glutamine, in 16% of CRC patients and 12% of the control subjects. The genotype and allele frequencies of the Lys940Gln (AAG/CAG) were calculated for CRC patients and controls (see Table 5). However, we did not observe any statistical significance for the Lys/Gln genotype versus the Lys/Lys genotype, OR for patients with the heterozygous Lys/Gln genotype was 1.3968 (p-value=0.5655, and 95% CI=0.4468-4.3669). According to our data no association was found between *PARP-1* Lys940Gln and increased risk of CRC in Saudi population.

Novel mutations found in PARP-1 exon 21

While we were screening exon 21 for the Lys940Arg A>G SNP, we detected four new sequence alterations in

both normal and CRC samples that were not reported before. These novel variations encode amino acids located within the highly conservative PARP-1 CD; Lys933Asn, Ser939Cys, Lys943Met, and Lys945Asn. Only two of these alterations, namely Lys933Asn and Lys945Asn were significantly associated with CRC risk in Saudi population (p-value=0.0318, OR=3.1429, and 95% CI=1.104-8.942 for the Lys933Asn heterozygous G/T genotype and p-value=0.0257, OR=2.5714, and 95% CI=1.121-5.895 for the Lys945Asn heterozygous G/T genotype). Although we found that Lys943 was altered in many CRC patient to three different amino acids; glutamine, asparagine, and methionine (data not shown), none of these alterations show a significant association with increased risk of CRC in Saudi population.

Table 6 shows the comparisons of different allele frequencies. The statistical analysis identified a significant difference between Val762Ala and Lys945Asn (p<0.0001). Comparing the significance of each single mutation against the other mutations studied showed that the *PARP-1* Lys933Asn and Lys945Asn were more effective than Val762Ala mutation with significant differences of p=0.0033 and p<0.0001, respectively. The *PARP-1* Lys945Asn mutation was more effective than Ser939Cys, and the difference was highly significant (p=0.0003). Moreover; Lys943Met and Lys945Asn mutations were more effective than Lys933Asn mutation with significant differences of p=0.0166 and p=0.0430, respectively. The *PARP-1* Lys945Asn mutation was more effective than both Lys940Gln and Lys943Met mutations, and the differences were highly significant (p=0.0005 and 0.0001, respectively). From this comparison we can conclude that the Lys945Asn is the most significant variation compared to others.

Table 5. Genotype Frequencies of the *PARP-1* Lys940Gln Variant in Saudi CRC Cases and Controls

Genotype	Cases	Controls	OR	95% CI	p-value
Lys/Lys	42 (0. 84)	44 (0.88)	Ref.		
Lys/Gln	8 (0.16)	6 (0.12)	1.3968	0.4468-4.3669	0.5655
Gln/Gln	0 (0.00)	0 (0.00)	1.0471	0.0203-53.971	0.9818
Lys/Gln+Gln/Gln	8 (0.16)	6 (0.12)	1.3968	0.4468-4.3669	0.5655
Lys	92 (0.92)	94 (0.94)	Ref.		
Gln	8 (0.08)	6 (0.06)	1.3623	0.4549-4.0798	0.5806

Table 6. Comparison Between Val762Ala, Lys933Asn, Ser939Cys, Lys940Gln, Lys943Met and Lys945Asn Variations in Saudi CRC Patients

	Val762 Ala No. (%)	Lys933 Asn No. (%)	Ser939 Cys No. (%)	Lys940 Gln No. (%)	Lys943 Met No. (%)	Lys945 Asn No. (%)
Wild	47 (94)	35 (70)	43 (86)	42 (84)	45 (90)	25 (50)
Hetero	2 (4)	15 (30)	7 (14)	8 (16)	5 (10)	25 (50)
p1		p=0.0033*	p=0.1056	p=0.0671	p=0.2657	p<0.0001*
p2			p=0.0583	p=0.1007	p=0.0166*	p=0.0430*
p3				p=0.7796	p=0.5399	p=0.0003*
p4					p=0.3762	p=0.0005*
p5						p=0.0001*

*Statistically significant at p≤0.05; p1: p value between Val762Ala and each other mutation; p2: p value between Lys933Asn and each other mutation; p3: p value between Ser939Cys and each other mutation; p4: p value between Lys940Gln and each other mutation; p5: p value between Lys943Met and Lys945Asn

Table 7. Haplotype Analysis of Val762Ala, Lys933Asn, Ser939Cys, Lys940Gln, Lys943Met and Lys945Asn Variations in CRC Patients and Controls

Val762 Ala	Lys933 Asn	Ser939 Cys	Lys940 Gln	Lys943 Met	Lys945 Asn	Case No.	Case %	Controls No.	Controls %	OR[a]	95% CI	p-value
1	1	1	1	1	1	49	(98)	50	(100)	3.0606	0.1217-76.953	0.4966
1	1	1	1	1	2	24	(48)	14	(28)	2.3736	1.0350-5.4436	0.0412*
2	1	1	1	1	1	3	(6)	1	(2)	3.1277	0.3141-31.143	0.3308
2	1	1	1	1	2	1	(2)	1	(2)	1	0.0608-16.444	1
2	2	1	1	1	1	0	(0)	1	(2)	0.3267	0.0130-8.2151	0.4966
2	2	1	1	1	2	0	(0)	1	(2)	0.3267	0.0130-8.2151	0.4966
1	2	1	1	1	1	15	(30)	6	(12)	3.1429	1.1046-8.9425	0.0318
1	2	1	1	1	2	10	(20)	4	(8)	2.875	0.8365-9.8809	0.0936
1	2	1	2	1	1	1	(2)	2	(4)	0.4898	0.0430-5.5818	0.5653
1	2	1	2	1	2	1	(2)	1	(2)	1	0.0608-16.444	1
1	2	1	1	2	1	1	(2)	0	(0)	3.0606	0.1217-76.953	0.4966
1	2	1	1	2	2	1	(2)	0	(0)	3.0606	0.1217-76.953	0.4966
1	2	2	1	1	1	1	(2)	2	(4)	0.4898	0.0430-5.5818	0.5653
1	2	2	1	1	2	1	(2)	1	(2)	1	0.0608-16.444	1
1	2	2	2	1	1	1	(2)	2	(4)	0.4898	0.0430-5.5818	0.5653
1	2	2	2	1	2	1	(2)	1	(2)	1	0.0608-16.444	1
1	1	2	1	1	1	7	(14)	5	(10)	1.4651	0.4319-4.9696	0.5399
1	1	2	1	1	2	7	(14)	4	(8)	1.8721	0.5118-6.8483	0.3433
1	1	2	1	2	1	3	(6)	0	(0)	7.4421	0.3744-147.93	0.1882
1	1	2	1	2	2	3	(6)	0	(0)	7.4421	0.3744-147.93	0.1882
1	1	2	2	1	1	6	(12)	5	(10)	1.2273	0.3490-4.3158	0.7496
1	1	2	2	1	2	6	(12)	4	(8)	1.5682	0.4143-5.9351	0.5076
1	1	2	2	2	1	2	(4)	0	(0)	5.2062	0.2436-111.24	0.2909
1	1	2	2	2	2	2	(4)	0	(0)	5.2062	0.2436-111.24	0.2909
1	1	1	2	1	1	8	(16)	6	(12)	1.3968	0.4468-4.3669	0.5655
1	1	1	2	1	2	6	(12)	5	(10)	1.2273	0.3490-4.3158	0.7496
1	1	1	2	2	1	2	(4)	0	(0)	5.2062	0.2436-111.24	0.2909
1	1	1	2	2	2	2	(4)	0	(0)	5.2062	0.2436-111.24	0.2909
1	1	1	1	2	1	5	(10)	0	(0)	12.2088	0.6567-226.97	0.0934
1	1	1	1	2	2	5	(10)	0	(0)	12.2088	0.6567-226.97	0.0934

*statistically significant at p≤0.05; [a] Odds ratio of disease status of each haplotype (1-1-1-1-1-1 reference haplotype);1 wild-type; 2, mutant

Table 8. Scoring Functions of Native and Mutated forms of PARP-1 Protein

	PARP-1		Lys933Asn		Lys943Asn		Lys943Met		Lys943Gln		Lys945Asn	
Scoring function term	Raw	Z	-Raw	Z	Raw	Z	Raw	Z	Raw	Z	Raw	Z
C-beta interaction energy	-115.76	-0.45	-113.82	-0.5	-106.39	-0.64	-110.25	-0.51	-122.92	-0.29	-123.17	-0.29
All-atom pairwise energy	-8811.4	-0.48	-8724.1	-0.51	-8628.8	-0.53	-8823.3	-0.43	-9155	-0.3	-8991.9	-0.36
Solvation energy	-29.37	-0.74	-37.69	0.16	-35.7	-0.04	-32.92	-0.4	-32.25	-0.46	-33.42	-0.35
Torsion angle energy	-57.14	-2.17	-61.89	-1.83	-57.91	-2.01	-46.72	-2.65	-62.08	-1.95	-62.18	-1.95
Secondary structure agreement	0.793	-0.36	0.828	0.33	0.815	0.06	0.795	-0.31	0.803	-0.15	0.803	-0.15
Solvent accessibility agreement	0.858	0.95	0.869	1.18	0.847	0.76	0.864	1.06	0.856	0.9	0.853	0.85
QMEAN6 score	0.776	0.07	0.806	0.41	0.765	-0.07	0.77	0	0.781	0.13	0.776	0.07

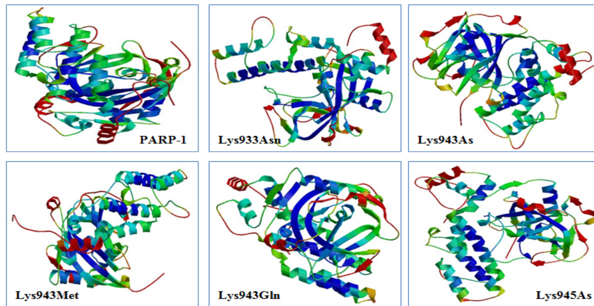

Figure 1. Three Dimensional Structure of Native PARP-1 Protein and Mutated Models as Predicted by Using SWISS-MODEL

Haplotype analysis of variations detected in PARP-1 gene

The haplotype frequencies for the Val762Ala, Lys933Asn, Ser939Cys, Lys940Gln, Lys943Met and Lys945Asn alleles were estimated for the CRC patients and controls (see Table 7). A score for each haplotype (Hap-score) was calculated and the p-value was obtained for the significance of each Hap-score. The haplotype 1-1-1-1-1-2, which only differed from the protective 1-1-1-1-1-1 haplotype at Lys945Asn, was significantly associated with CRC (OR 2.3736, 95% CI 1.0350-5.4436, and P 0.0412). All haplotypes containing more than one mutation were not significantly associated with the disease in the Saudi population.

Prediction of PARP-1 protein structure

Computer construction and analysis of protein models of the mutant *PARP-1* enzyme (Figure 1) revealed that the mutant Lys933Asn *PARP-1* protein had various changes on protein's secondary structure, increased torsion angle energy different protein surface solvent-accessibility, and decreased C-beta interaction energy that might affect the binding ability of the enzyme to its substrate. The mutant Lys945Asn *PARP-1* showed variations in protein's secondary structure, different protein surface solvent-accessibility, and increased C-beta interaction energy that might increase the affinity of *PARP-1* to NAD^+.

Discussion

Over the past years, at least 439 SNPs have been reported in *PARP-1* gene including 17 non-synonymous SNPs (nsSNPs). The Val762Ala SNP is the most studied and the only SNP found in *PARP-1* gene that was associated with overall risk of cancer (Yu et al., 2012). The Ala762 variant results from a base substitution from thymine to cytosine at codon 762 in exon 17 causing amino acid change from valine to alanine in the sixth helix of the most conserved coding region (PARP signature motif) of the CD in which the NAD^+binds to the enzyme (Cottet et al., 2000). The loss of a methyl group from valine to alanine moves the 762 residue further away from its closest neighbor glutamine 888 (from 4.01A° for valine to 5.19A° for alanine), which in turn affects the PARP-1 enzymatic activity (Cottet et al., 2000) and thereby contributes to cancer susceptibility (Zaremba et al., 2009).

It has been reported that *PARP-1* Val762Ala polymorphism significantly contributes to lowering the PARP-1 catalytic activity by 30-40%, in a dosage-dependent manner, which might diminishes the BER ability to repair damaged DNA and thus increases cancer risk (Wang et al., 2007). Interestingly, the frequencies of the *PARP-1* Ala762 allele are known to be different among ethnic populations. However, previous studies have reviled that Val762Ala SNP was associated with increased overall cancer risk only in Chinese population and was associated with decreased risk among Caucasians (Yu et al., 2012). In our study we found no significant association between *PARP-1* Val762Ala SNP and risk of CRC in Saudi population, the same result was also found in Singapore Chinese population (Stern et al., 2007). Although *PARP-1* Val762Ala SNP has been suggested to contribute in cancer development (Wang et al., 2007), to our knowledge, no previous studies have investigated the association between *PARP-1* Val762Ala SNP and risk of CRC in Middle Eastern countries, particularly in Saudi Arabia.

The mutant genotype for *PARP-1* Met129Thr (ATG/ACG) was first detected by Shiokawa and his colleagues (2005) as alteration in exon 3 of the *PARP-1* gene in both tumor and normal tissues of a single patient that causes amino acid substitution from methionine to thrionine at position 129 located in the second zing finger motif of the PARP-1 enzyme (Shiokawa et al., 2005). Recent study showed that the recognition and binding of PARP-1 to DNA strand breaks occur only after the interaction and cooperation between the first two zing fingers present in DBD of PARP-1 protein. It also suggested that dimerization of DBD on DNA strand breaks involves specific in-trans interaction between ZnF I and ZnF II, which in turn leads to PARP-1 activation (Ali et al., 2012). Another study proposed that recognition of damaged DNA ends occurs directly by ZnF I that leads to conformational changes in PARP-1 structure therefore enhances its activity (Langelier et al., 2012). Exchange of a hydrophobic amino acid to polar one in DBD, namely Met129Thr was suggested to affect the DNA binding ability of PARP-1 enzyme (Shiokawa et al., 2005). In

addition to that, a decreased in auto-poly(ADP-ribosyl)ation activity was observed in PARP-1 protein with Met129Thr (ATG/ACG) substitution, which may cause germ-line tumor development and it has been proven to affect the response to chemotherapy (Ogino et al., 2010). This particular SNP was not found in any of our samples, but we were able to detect a heterozygous T/A allele alteration that converted the same amino acid to lysine (ATG/AAG) at the same position (129), which was only found in one CRC sample while none of the controls carried the T/A allele.

The lysine residue at codon 940 is a highly conserved amino acid located in the CD of the PARP-1 enzyme in which this residue may play a critical role in the enzyme structure. The substitution of lysine to arginine at position 940 results from a base transition from adenosine to guanine. The definite biological significance of this SNP is still unknown; but because of its location, it may affect the PARP-1 catalytic activity. Previous study suggested that Lys940Arg SNP may affect the response to chemotherapeutic agents in particular to DNA-damaging agents in patients with lung cancer (Shiraishi et al., 2010). However, this SNP was associated with increased risk of gastric cancer in Chinese population (He et al., 2012) and CRC in Singapore Chinese (Stern et al., 2007). Also it can contribute in the development of human germ cell tumors (Shiokawa et al., 2005).

As we mentioned above this polymorphism was associated with increased risk of CRC only in Singapore Chinese population (Stern et al., 2007) and that was the only study done to associate between the *PARP-1* Lys940Arg SNP and CRC susceptibility; therefore, it is unknown whether this SNP is or is not associated with CRC risk in other populations. Instead of Lys940Arg polymorphism, we found another alteration, with a heterozygous A/C allele, in 16% of the CRC patients and 12% of the control subjects. This alteration converts the lysine residue in position 940 to glutamine. This variation, which has not been reported before, may reduce the PAPR-1 enzymatic activity or it may affect the affinity of the enzyme to bind to its substrate (NAD^+). Substitution of lysine reside with glutamate in the putative ATP-binding sequence of the RecD subunit of the RecBCD enzyme from Escherichia coli resulted in a reduction of the RecD protein's catalytic activities including: DNA-dependent ATPase, ATP-dependent nuclease, and DNA helicase activity. In addition, the mutant enzyme RecBCD has impaired ability to unwind DNA double helix and has lower affinity to its substrate (ATP) compared to the wild-type enzyme (Korangy and Julin, 1992).

Four novel mutations were detected that are located within the highly conservative CD, Only two of these alterations, namely Lys933Asn and Lys945Asn were significantly associated with CRC risk in Saudi population (p-value=0.0318, OR=3.1429, and 95% CI=1.104-8.942 for the Lys933Asn heterozygous G/T genotype and p-value=0.0257, OR=2.5714, and 95% CI=1.121-5.895 for the Lys945Asn heterozygous G/T genotype). Since they both are located in PARP-1 CD, thus their substitution could affect the PARP-1 catalytic activity. Previous study showed that several lysine residues of PARP-1 protein can function as acceptor sites for enzymatic covalent auto-poly(ADP-ribosyl)ation which can be present in AMD or outside this domain (Altmeyer et al., 2009). Mutations of these crucial lysine residues may be responsible for PARP-1 enzyme inactivation which may play a key role in carcinogenesis.

Protein models of the mutant PARP-1 protein show significant alterations on the protein's secondary structure based on computer construction and analysis technology. This study provides further evidence for the important effect of the decoy PARP-1 on the pathogenesis of CRC.

Our results showed limited association of *PARP-1* polymorphisms with CRC which could be attributed to the limited number of samples that needs to be expanded to confirm the results. Scanning the whole gene of *PARP-1* in Saudi CRC patients is required to gather the full picture behind this protein and its full impact on CRC development. The novel detected alterations require full biochemical analysis to determine their actual effect on PARP-1 structure and function which can help in discovering its role in carcinogenesis. Association studies and expression analysis are required to link the alterations of *PARP-1* gene with other types of cancer and to assess the role of different variations detected in *PARP-1* gene with the development of CRC in Saudi population.

In conclusion, this study indicates that the previously reported SNPs in *PARP-1* gene namely, Met129Thr, Val762Ala, and Lys940Arg did not show any association with increased risk of CRC in Saudi population. However, novel alterations located in *PARP-1* exon 21 were identified in both CRC and control DNA samples, these alterations encoded for amino acids located within the highly conservative PARP-1 CD. They are; Lys933Asn, Ser939Cys, Lys940Gln, Lys943Met, and Lys945Asn. Among these novel alterations, only Lys933Asn and Lys945Asn show highly significant association with CRC among Saudis. Further studies are required to determine the effect of the new alterations detected on PARP-1 enzymatic activity.

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