RESEARCH ARTICLE

The C Allele of a Synonymous SNP (rs1805414, Ala284Ala) in PARP1 is a Risk Factor for Susceptibility to Breast Cancer in Saudi Patients

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Abstract

Background: Genetic aberrations of DNA repair enzymes are known to be common events associated with different cancer entities. The aim of the present study was to analyze genetic associations of rs1805404 (Asp81Asp) and rs1805414 (Ala284Ala) in the PARP1 gene with the risk of breast cancer in Saudi Arabia. Materials and Methods: These two SNP's were analyzed in a primary study group of breast cancer patients and healthy control subjects. Genotypes were determined by TaqMan SNP testing and analyzed using Chi-square or t test and logistic regression analysis with SPSS16.0 software. Results and Conclusions: Results showed that rs1805414 was associated with a significantly increased susceptibility to breast cancer, significant risk being observed for the TC, CC and TC+CC genotypes. In conclusion PARP1 rs1805414 SNP polymorphisms may be involved in the etiology of breast cancer in the Saudi population. In contrast, PARP1 rs1805404 did not show any significant association in overall in breast cancer samples when compared to healthy controls. Confirmation of our findings in larger populations of different ethnicities may provide evidence for a role of the PARP1 gene in breast carcinoma developnment.

Keywords: PARP1 - rs1805414 - breast cancer - genetic polymorphisms - Saudi Arabia

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Introduction

Breast cancer is the most common neoplasm and the second leading cause of cancer death in Saudi women. The recent increase in incidence has made breast cancer one of the most frequently recorded diseases among Saudi women (Ferlay et al., 2010). The age-adjusted death rate because of breast cancer in Saudi Arabia is also rising, with the most rapid increase in the world from 1985 to 2008 (Saudi National Cancer Registry, 2011). This malignancy represents a heterogeneous group of tumors with characteristic molecular features, prognosis and responses to available therapy.

DNA repair pathways exist in all cells for maintaining genome integrity (Hoeijmakers, 2001), and mutations within these pathways can result in cancer (Heinen et al., 2002). Interindividual variations in DNA damage and repair have been associated with an increased risk of breast cancer (Johnson et al., 2000; Tyrer et al., 2004). Poly (ADP-ribose) polymerase 1 (PARP-1) is a DNA double strand break-sensing protein, and its activation is one of the early responses to DNA damage (Schreiber et al., 2006). PARP-1 gene localizes to chromosome 1q41–42, consists of 23 exons and spans 47.3 kb (Schreiber et al., 2006). It encodes a multifunctional nuclear protein, which consists of an N-terminal DNA binding domain, a central auto-modification domain and a C-terminal catalytic domain (Cottet et al., 2000). PARP-1 catalyzes poly(ADP-ribosyl)ation, an immediate DNA-damage dependent post-translational modification of itself, histones and other nuclear proteins, which is believed to play a multifunctional role in various cellular processes, including DNA-damage detection and repair, cell death pathways and mitotic apparatus function (Kim et al., 2005). PARP-1 deficiency in mice resulted in spontaneous mammary carcinomas, and additional p53 mutations shorten the latency of mammary tumor formation suggesting a possible involvement of PARP-1 in breast carcinogenesis (Tong et al., 2007). PARP-1 has been implicated in tumorigenesis (Tong et al., 2001; Masutani et al., 2003). Few studies studies indicate that PARP-1 plays an important role in suppressing malignancy in mice. Interestingly, reduced PARP-1 activity in human peripheral blood lymphocytes has been linked with human breast, colon, lung (Pero et al., 1990) and laryngeal cancers (Rajaee-Behbahani et al., 2002).

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There are many identified single nucleotide polymorphism (SNP) in the PARP-1 gene, and some of which are reported to be implicated in carcinogenesis (Hao et al., 2004; Lockett et al., 2004; Shiokawa et al., 2005; Zhang et al., 2005). Several SNPs have been found in PARP1 gene (Figueroa et al., 2007). Although many studies have searched for the association between PARP-1 polymorphisms and the risk of malignancy, the results are inconsistent in different organs and in different ethnic groups. Few studies have reported positive associations between the SNP's rs1805404 and rs1805414 at position 81 and 284, have been reported to be significantly associated with an increased risk of Alzheimer's disease (Liu et al., 2010), Glioblastoma (Keller et al., 2011), protectively associated with Colorectal cancer (Berndt et al., 2007; Ogino et al., 2010). rs1805404 reported be strongly associated with Tourette syndrome risk (Wu et al., 2013).

To the best of our knowledge, till now there are no reports about the association between the SNPs of PARP-1 rs1805404 (Asp81Asp) and rs1805414 (Ala284Ala) and breast cancer in Saudi population. Therefore, in the current population based case-control study, we investigated the genotype distributions of the rs1805404 (Asp81Asp) and rs1805414 (Ala284Ala) in patients with breast cancer.

Materials and Methods

Study population

A total of 195 blood samples were obtained from King Khalid University Hospital. These encompassed 99 patients with breast cancer disease and 96 healthy controls. All controls were age-matched and recruited from physical examinations after diagnostic exclusion of cancer and cancer- related diseases. Blood samples of the experimental and control groups were obtained before treatment. Histopathology and medical records were reviewed to confirm diagnosis. Controls were frequency matched to cases on age/race and recruited from the clinic population receiving routine mammography at the breast screening and diagnostic center. Eligibility criteria for controls included normal mammography results and no prior cancer history. Written informed consent was obtained from all participants, and approval was received from the King Khalid University Hospital ethics review committee. Every study participant completed a self-administered baseline questionnaire, which included information on demographics, reproductive history, medical conditions and family history of cancer.

DNA extraction

Approximately 3 ml of blood samples were collected in sterile tubes containing ethylenediaminetetracetic acid (EDTA) from all subjects enrolled in the study. Genomic DNA was isolated from blood samples using QIAmp kit (QIAmp DNA blood Mini Kit, Qiagen, Valencia, CA) following the manufacturer's instructions. After extraction and purification, the DNA was quantitated on a NanoDrop 8000, to determine the concentration and its purity was examined using standard A260/A280 and A260/A230 ratios (NanoDrop 8000) (Sambrook et al., 1989).

Genotyping

Two SNPs (rs1805404 and rs1805414) in PARP1 gene were genotyped using TaqMan allelic discrimination assay (Livak, 1999). For each sample, 20 ng DNA per reaction was used with 5.6 μL of 2X Universal Master Mix and 200 nM primers (Applied Biosystems, Foster City, CA, USA). All genotypes were determined by endpoint reading on an ABI 7500 (Applied Biosystems, Foster City, CA, USA). Primers and probe mix were purchased directly through the assays-on-demand service of Applied Biosystems. Five percent of the samples were randomly selected and subjected to repeat analysis as a quality control measure for verification of genotyping procedures.

Statistical analysis

Genotype and allelic frequencies were computed and were checked for deviation from Hardy—Weinberg equilibrium (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl). Case-control and other genetic comparisons were performed using the chi-square test and allelic odds ratios (OR), and 95% confidence intervals (CI) were calculated by Fisher's exact test (two-tailed). Statistic analysis was done using SPSS 16.0 for Windows. We considered p-value of <0.05 as significant.

Results

A total of 99 BR cases and 96 healthy controls were included in this study. Clinical characteristics of breast cancer cases and healthy controls are given in Table 1. Out of 96 confirmed cases of breast cancer, 47 were ER positive and 43 ER negative, 49 were PR positive and 41 PR negative, 38 were HER positive and 52 HER negative (Table 1).

All the genotypic distributions were consistent with that expected in the Hardy–Weinberg model (Table 2). Homozygous ancestral allele was used as a reference to determine the odds of acquiring breast cancers in relation to the other two genotypes. The genotype distribution of

Table 1. Clinical Characteristics Of Study Subjects

Variable	Character	No of Samples
Age (Years)	Median age	48
Estrogen receptor	ER+/ER-	47/43
Progesterone receptor	PR+/PR-	49/41
HER Status	HER+/HER-	38/52

Table 2. Distribution of Genotypes and Allele Frequencies on PARP1 Gene Loci among Saudi Breast Cancer Patients and Controls

Genotype	Cases	HWE P-value	Controls	HWE P-value
rs1805404 (Asp81Asp)		0.087829		0.789714
CC (wild)	16 (0.0)		13 (0.13)	
CT	37 (0.0)		43 (0.45)	
TT (variant)	45 (0.0)		40 (0.42)	
rs1805414 (Ala284Ala)		0.365712		0.372279
TT (wild)	46 (0.47)		77 (0.8)	
CT	40 (0.4)		17 (0.18)	
CC (variant)	13 (0.13)		2 (0.02)	

Table 3. Genotype Frequencies of PARP1 Gene Polymorphism in Breast Cancer Cases and Controls

Genotype		Cases	Controls	OR	95% CI	X^2	p value
rs1805404 (Asp > Asp)	CC (wild)	16 (0.16)	13 (0.13)	Ref			
	CT	37 (0.38)	43 (0.45)	0.699	0.298-1.64	0.68	0.41018
	TT (variant)	45 (0.46)	40 (0.42)	0.914	0.392-2.13	0.04	0.83521
	CT+TT	82 (0.84)	83 (0.87)	0.803	.363-1.774	0.3	0.58652
	C	69 (0.35)	69 (0.36)	Ref			
	T	127 (0.65)	123 (0.64)	1.033	0.681-1.565	0.02	0.88007
rs1805414 (Ala > Ala)	TT (wild)	46 (0.47)	77 (0.8)	Ref			
	TC	40 (0.4)	17 (0.18)	3.939	2.006-7.734	16.77	0.00004
	CC (variant)	13 (0.13)	2 (0.02)	10.88	2.349-50.39	13.26	0.00027
	TC+TT	53 (0.53)	19 (0.20)	4.669	2.46-8.84	23.83	1.053×10^{-6}
	T	132 (0.67)	171 (0.89)	Ref			
	C	66 (0.33)	21 (0.11)	4.071	2.370-6.994	28.21	1.088×10^{-7}

*OR, Odds ration; C, Confidence Interval; X^2, Chi Square

Table 4. Genotype Frequencies of PARP1 Gene Polymorphism in Breast Cancer Cases below 48 and above 48 years

Genotype		< 48 Y	> 48 Y	OR	95% CI	X^2	p value
rs1805404 (Asp>Asp)	CC (wild)	9 (0.2)	7 (0.13)	Ref			
	CT	16 (0.35)	19 (0.37)	0.655	0.199-2.155	0.49	0.48494
	TT (variant)	21 (0.46)	26 (0.5)	0.628	0.200-1.97	0.64	0.42353
	CT+TT	25 (0.81)	45 (0.87)	0.64	0.217-1.882	0.67	0.41458
	C	34 (0.37)	33 (0.32)	Ref			
	T	60 (0.63)	71 (0.68)	0.793	0.439-1.432	0.59	0.44144
rs1805414 (Ala>Ala)	TT (wild)	20 (0.43)	26 (0.49)	Ref			
	TC	16 (0.35)	24 (0.45)	0.867	0.367-2.049	0.11	0.74433
	CC (variant)	10 (0.22)	3 (0.06)	4.333	1.052-17.85	4.54	0.03319
	CT+TT	26 (0.57)	27 (0.51)	1.252	0.566-2.768	0.31	0.57887
	T	56 (0.61)	76 (0.72)	Ref			
	C	36 (0.39)	30 (0.28)	1.629	0.898-2.952	2.6	0.10694

*OR, Odds ration; C, Confidence Interval; X^2, Chi Square

the analyzed SNPs along with the corresponding odds ratio and significance are shown in Table 3. We observed statistically significant association with one of the two SNPs (rs1805414) with breast cancer risk.

In the present study, we found no association with PARP1 rs1805405 and a significant difference in the distribution of PARP1 rs1805414 genotype between breast cancer cases and the matched healthy controls. The frequencies of rs1805404 (Asp81Asp) genotypes in breast cancer cases were 16 (0.16), 37 (0.38), and 45 (0.46) respectively, whereas as in healthy controls the frequencies were 13 (0.13), 43 (0.45), and 40 (0.42) respectively. Breast cancer patients didn't showed any risk when compared to healthy individuals (Table 3). As shown in Table 3, the frequency of the rs1805414, Ala284Ala T/T, T/C and C/C genotypes were 46 (0.47), 40 (0.40) and 10 (0.13) respectively in breast cancer patients and 77 (0. 80), 17 (0.18) and 2 (0.02) in controls. In SNP rs1805414 heterozygous allele (TC) and variant allele (CC) showed significantly higher risk in breast cancer patients when compared with controls (Table 3) (OR=3.939, χ^2=16.77, p=0.0004 and OR: 10.88, χ^2=13.26, p=0.00027). A significant risk in higher proportion of women with T/C + C/C were observed in breast cancer cases compared to healthy individuals (OR=4.669, χ^2=23.83, p<0.00001). The C allelic frequency of rs1805404 was higher in the breast cancer patients (0.33) than that in the control group (0.11) (OR=4.071, χ^2=28.21, p<0.00001).

In Saudi Arabian patients, the median age of onset of breast cancer is 47 years, substantially lower than 62 years observed in the American population (Anderson et al., 2006; Saudi National Cancer Registry, 2011). To evaluate the association of the analyzed SNPs with the young age at diagnosis of breast cancer, we stratified the patients as ≤48 (n=46) or >48 (n=53) years of age. The genotype distribution for the individual SNP along with the statistical analysis are shown in Table 4. Interestingly, only PARP1 rs1805414 which showed significant association in the overall study or in the patient group with age <48 years, indicated a significant risk on patients with ≤48 years (Table 4). It was noted that, women aged ≤48 years had slightly higher risk for developing breast cancer in Saudi population with this allelic change (OR=4.333, χ^2=4.54, p<0.03319).

We conducted the association of breast cancer risk with the individual SNPs based on the estrogen receptor (ER) status of the tumors. The genotype distribution in the ER+ (n=53) and ER- (n=43) groups were compared with each other (Tables 5). Interestingly, homozygosity of the minor allele (T) at SNP rs1805414 posed protective influence on ER+ breast cancer (OR=0.512; χ^2=4.42; p=0.0356). This association was not observed in the ER-ve category as well as in the overall study population. The rs1805404, that was significantly associated with increased risk of breast cancer in the overall study population doesn't exhibited any association for the ER+ as well as ER- group (Tables 5).

We performed the association of breast cancer risk with the individual SNPs based on the progesterone receptor (PR) status of the tumors. The genotype distribution in the

Table 5. Genotype Frequencies of PARP1 Gene Polymorphism in Breast Cancer Cases ER Positive and ER Negative

Genotype		ER+ve	ER -ve	OR	95% CI	X^2	p value
rs1805404 (Asp>Asp)	CC (wild)	7 (0.16)	9 (0.17)	Ref			
	CT	14 (0.33)	21 (0.4)	1.435	0.587-3.507	0.63	0.4279
	TT (variant)	22 (0.51)	23 (0.43)	1.23	0.390-3.875	0.12	0.72369
	CT+TT	36 (0.84)	44 (0.83)	1.366	0.609-3.065	0.58	0.44827
	C	34 (0.33)	39 (0.37)	Ref			
	T	58 (0.67)	67 (0.63)	1.206	0.662-2.196	0.37	0.54045
rs1805414 (Ala>Ala)	TT (wild)	25 (0.58)	21 (0.40)	Ref			
	TC	15 (0.35)	23 (0.43)	0.511	0.119-2.2	0.83	0.3625
	CC (variant)	3 (0.07)	9 (0.17)	0.28	0.067-1.170	3.28	0.07001
	CT+TT	18 (0.42)	32 (0.6)	0.367	0.093-1.450	2.17	0.14051
	T	65 (0.76)	65 (0.61)	Ref			
	C	21 (0.24)	41 (0.39)	0.512	0.273-0.960	4.42	0.0356

*OR, Odds ration; C, Confidence Interval; X^2, Chi Square

Table 6. Genotype Frequencies of PARP1 Gene Polymorphism in Breast Cancer Cases PR Positive and PR Negativee

Genotype		PR+	PR -ve	OR	95% CI	X^2	p value
rs1805404 (Asp>Asp)	CC (wild)	9 (0.16)	7 (0.17)	Ref			
	CT	25 (0.45)	10 (0.24)	0.352	0.139-0.893	4.97	0.02578
	TT (variant)	22 (0.39)	25 (0.6)	0.684	0.218-2.144	0.43	0.51409
	CT+TT	34 (0.84)	17 (0.84)	0.44	0.194-0.996	3.94	0.04719
	C	43 (0.38)	24 (0.29)	Ref			
	T	69 (0.62)	60 (0.71)	0.642	0.350-1.178	2.06	0.15141
rs1805414 (Ala>Ala)	TT (wild)	30 (0.53)	16 (0.38)	Ref			
	TC	18 (0.31)	22 (0.52)	2.75	0.726-10.42	2.31	0.12896
	CC (variant)	9 (0.16)	4 (0.10)	1.2	0.319-4.51	0.07	0.78722
	CT+TT	27 (0.47)	26 (0.62)	1.781	0.509-6.232	0.83	0.36162
	T	78 (0.68)	54 (0.64)	Ref			
	C	36 (0.32)	30 (0.36)	0.831	0.458-1.508	0.37	0.54182

*OR, Odds ration; C, Confidence Interval; X^2, Chi Square

Table 7. Genotype Frequencies of PARP1 Gene Polymorphism in Breast Cancer Cases HER Positive and HER Negative

Genotype		HER+ve	HER -ve	OR	95% CI	X^2	p value
rs1805404 (Asp>Asp)	CC (wild)	9 (0.22)	7 (.12)	Ref			
	CT	17 (0.41)	18 (0.32)	0.496	0.201-1.225	2.34	0.12617
	TT (variant)	15 (0.37)	32 (0.56)	0.365	0.114-1.166	3	0.0834
	CT+TT	26 (0.78)	25 (0.88)	0.451	0.198-1.027	3.65	0.05595
	C	35 (0.43)	32 (0.28)	Ref			
	T	47 (0.57)	82 (0.72)	0.524	0.288-0.954	4.53	0.03337
rs1805414 (Ala>Ala)	TT (wild)	19 (0.46)	27 (0.47)	Ref			
	TC	17 (0.42)	22 (0.39)	0.809	0.224-2.92	0.11	0.74585
	CC (variant)	5 (0.12)	8 (0.14)	0.888	0.251-3.138	0.03	0.85382
	CT+TT	22 (0.54)	30 (0.53)	0.851	0.257-2.817	0.07	0.79109
	T	55 (0.67)	76 (0.67)	Ref			
	C	27 (0.33)	38 (0.33)	0.982	0.537-1.795	0	0.95245

*OR, Odds ration; C, Confidence Interval; X^2, Chi Square

PR+ (n=57) and PR- (n=42) groups were compared with each other for disease association (Tables 6). Interestingly, any association was not observed in the PR category in the study population with rs1805414 (Tables 6). The heterozygous allele (CT) at SNP rs1805404 showed protective nature in breast cancer patients when compared with control samples (OR=0.352; χ^2=4.97; p=0.025). A slightly significant protective nature was observed in women with C/T+T/T in breast cancer cases compared to healthy individuals (OR=0.44, χ^2=3.94, p<0.047).

The association of breast cancer risk with the individual SNPs based on the Human Epidermal Growth Factor Receptor 2 (HER2) status of the tumors was also evaluated. The genotype distribution in the HER+ (n=41) and HER- (n=57) groups were compared with each other for disease risk. Interestingly, homozygosity of the minor allele (T) at SNP rs1805414 posed protective influence on HER2+ breast cancer (OR=0.524; χ^2=4.53; p=0.03337). This association was not observed in the ER-ve category as well as in the overall study population. The rs1805414, that was significantly associated with increased risk of breast cancer in the overall study population doesn't exhibited any association for the ER+ as well as ER- group (Table 7).

Discussion

DNA repair mechanisms play a major role in protecting against carcinogenesis and genetic defect in DNA repair can cause human cancer (Heinen et al., 2002). Through repairing DNA damage and maintaining genetic stability, PARP-1 has played an important role in prevention of carcinogenesis. Our results suggest that, despite the strong biological plausibility, PARP1 is a susceptibility locus for breast cancer in Saudi population. The human PARP1 gene consists of 23 exons spanning 43 kb and has been localized to chromosome 1q41-q42. The PARP-1 rs1805404 (Asp81Asp) and rs1805414 (Ala284Ala) polymorphism has been implicated in cancer susceptibility. In this study, we found for the first time that the PARP-1 rs1805414 (Ala284Ala) genotype significantly contributes to breast cancer susceptibility in Saudi population, which further extend the important role of PARP-1 in carcinogenesis.

Our findings suggests that, homozygotes at PARP1 locus (Ala284Ala) may be associated with an increased risk of Breast cancer (Table 3). Neither variant is likely to influence the activity of the protein itself: the T allele at Ala284Ala does not affect the amino acid sequence of the protein. Thus, our results supports Berndt et al. (2007) findings in which they suspected that the association that we observed with colorectal adenoma may be due to linkage disequilibrium with another variant in the region, possibly in the PARP1 promoter. Because PARP1 activity is highly regulated by its promoter, variants within transcription binding sites in the promoter may influence its expression (Berndt et al., 2007). In a similar way Milani et al. (2007) reported that PARP1 contains contain functional regulatory SNPs in their promoter regions and SNP rs1805414 expression level is effected by allelic imbalance in cancer cells. We have observed that rs1805414 is associated with the Age and breast cancer susceptibility (Table 4 and 5). Cao et al., (2007) reported that the Ala284Ala (T❑C) PARP-1 variant was likely associated with loss of estrogen- and progesterone-receptor expression. This implies that genetic variants of PARP-1 may contribute to breast cancerogenesis and that the PARP-1 Ala284Ala variant protein may influence hormonal therapy of breast cancer (Cao et al., 2007). But interestingly rs1805414 showed protective nature in ER positive samples in Saudi population when compared to ER negative samples.

Our study has some strengths: patients and controls came from the same geographical area; genotyping errors were avoided using duplicate samples; markers were tested to assure a true association. For the association testing, we also considered multiple genetic models, adjusted our analyses for possible confounders (age at study), and stratified our sample for multiple variables (age at study, ER, PR, HER status) to explore possible effect modifications.

In summary, our study firstly shows a significant association between the PARP-1 Ala284Ala (rs1805414) genotypes and increased risk of breast carcinoma in Saudi patients. These findings suggest that the SNP rs1805414 may modulate the occurrence of PARP1 mutations and contribute to breast carcinogenesis. Our findings suggest that PARP-1 dysfunction may play an important role in the development of breast carcinoma. Despite our data supports for a clear association between PARP1 and breast cancer in Saudi population and PARP1 gene plays a major role in the susceptibility to the disease. As the sample size of this study is not sufficiently large and is restricted to Saudi population, the present data should be validated in larger samples and in other ethnic groups. Additional functional as well as association studies investigating gene-gene interactions are required to elucidate this issue, and it remains possible that PARP1 variability may affect disease progression or the susceptibility to develop breast cancer. Additional studies are needed to confirm these findings and to further characterize the specific genetic variants that alter adenoma risk.

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References

Anderson WF, Chen BSE, Jatoi I, et al (2006). Effects of estrogen receptor expression and histopathology on annual hazard rates of death from breast cancer. *Breast Cancer Res Tr*, **100**, 121-6.

Berndt SI, Huang WY, Fallin MD, et al (2007). Genetic variation in base excision repair genes and the prevalence of advanced colorectal adenoma. *Cancer Res*, **67**, 1395-404.

Cao WH, Wang XG, Frappart L, et al (2007). Analysis of genetic variants of the poly (ADP-ribose) polymerase-1 gene in breast cancer in French patients. *Mutation Research-Genetic Toxicol Environm Mut*, **632**, 20-8.

Cottet F, Blanche H, Verasdonck P, et al (2000). New polymorphisms in the human poly(ADP-ribose) polymerase-1 coding sequence: lack of association with longevity or with increased cellular poly(ADP-ribosyl)ation capacity. *J Mol Med (Berl)*, **78**, 431-40.

Ferlay J, Shin HR, Bray F, et al (2010). GLOBOCAN 2008 v1.2, Cancer Incidence and Mortality Worldwide: IARC Cancer Base No. 10 [Internet]. Lyon, France: International Agency for Research on Cancer. Available from: http://globocan.iarc.fr, accessed on 11/03/2012.

Figueroa JD, Malats N, Real FX, et al (2007). Genetic variation in the base excision repair pathway and bladder cancer risk. *Human Genet*, **121**, 233-42.

Hao BT, Wang HJ, Zhou KX, et al (2004). Identification of genetic variants in base excision repair pathway and their associations with risk of esophageal squamous cell carcinoma. *Cancer Res*, **64**, 4378-84.

Heinen CD, Schmutte C, Fishel R (2002). DNA repair and tumorigenesis: lessons from hereditary cancer syndromes. *Cancer Biol Ther*, **1**, 477-485.

Hoeijmakers JHJ (2007). Genome maintenance mechanisms are critical for preventing cancer as well as other aging-associated diseases. *Mech Ageing Dev*, **128**, 460-62.

Johnson-Thompson MC, Guthrie J (2000). Ongoing research to identify environmental risk factors in breast carcinoma. *Cancer*, **88**, 1224-29.

Keller A, Harz C, Matzas M, et al (2011). identification of novel snps in glioblastoma using targeted resequencing. *Plos One*, **6**.

Kim MY, Zhang T, Kraus WL (2005). Poly(ADP-ribosyl)ation by PARP-1: 'PAR-laying' NAD(+) into a nuclear signal.

Gene Dev, **19**, 1951-67.
Liu HP, Lin WY, Wu BT, et al(2010). Evaluation of the poly(ADP-ribose) polymerase-1 gene variants in Alzheimer's disease. *J Clin Lab Anal*, **24**, 182-6.
Livak KJ (1999). Allelic discrimination using fluorogenic probes and the 5' nuclease assay. *Genet Anal-Biomol E*, **14**, 143-9.
Lockett KL, Hall MC, Xu J, et al(2004). The ADPRT V762A genetic variant contributes to prostate cancer susceptibility and deficient enzyme function. *Cancer Res*, **64**, 6344-8.
Masutani M, Nakagama H, Sugimura T (2003). Poly(ADP-ribose) and carcinogenesis. *Gene Chromosome Canc*, **38**, 339-48.
Milani L, Gupta M, Dhar S, et al (2007). Allelic imbalance in gene expression as a guide to cis-acting regulatory single nucleoticle polymorphisms. *Eur J Clin Invest*, **37**, 80-1.
Ogino H, Nakayama R, Sakamoto H, et al (2010). Analysis of poly(ADP-ribose) polymerase-1 (PARP1) gene alteration in human germ cell tumor cell lines. *Cancer Genet Cytogen*, **197**, 8-15.
Pero RW, Roush GC, Markowitz MM, et al (1990). Oxidative stress, DNA repair, and cancer susceptibility. *Cancer Detect Prev*, **14**, 555-61.
Rajaee-Behbahani N, Schmezer P, Ramroth H, et al (2002). Reduced poly(ADP-ribosyl)ation in lymphocytes of laryngeal cancer patients: results of a case-control study. *Int J Cancer*, **98**, 780-4.
Saudi National Cancer Registry (2011). Cancer Incidence and Survival Report Saudi Arabia 2007. Riyadh.
Schreiber V, Dantzer F, Ame JC, et al (2006). Poly(ADP-ribose): novel functions for an old molecule. *Nat Rev Mol Cell Biol*, **7**, 517-28.
Shiokawa M, Masutani M, Fujihara H, et al (2005). Genetic alteration of poly(ADP-ribose) polymerase-1 in human germ cell tumors. *Jpn J Clin Oncol*, **35**, 97-102.
Tong WM, Cortes U, Wang ZQ (2001). Poly(ADP-ribose) polymerase: a guardian angel protecting the genome and suppressing tumorigenesis. *Biochim Biophys Acta*, **1552**, 27-37.
Wu BT, Lin WY, Chou IC, et al (2013). Association of poly(ADP-ribose) polymerase-1 polymorphism with Tourette syndrome. *Neurol Sci*, (Inpress).