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ORIGINAL ARTICLE

The hOGG1 Ser326Cys Gene Polymorphism and Breast Cancer Risk in Saudi Population

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Abstract The purpose of this study was to test the association between human 8-oxoguanine glycosylase 1 (*hOGG1*) gene polymorphisms and susceptibility to breast cancer in Saudi population. We have also aimed to screen the *hOGG1* Ser326Cys polymorphism effect on structural and functional properties of the *hOGG1* protein using in silico tools. We have analyzed four SNPs of *hOGG1* gene among Saudi breast cancer patients along with healthy controls. Genotypes were screened using TaqMan SNP genotype analysis method. Experimental data was analyzed using Chi-square, t test and logistic regression analysis using SPSS software (v.16). In silco analysis was conducted using discovery studio and HOPE program. Genotypic analysis showed that *hOGG1* rs1052133 (Ser326Cys) is significantly associated with breast cancer samples in Saudi population, however rs293795 (T >C), rs2072668 (C>G) and rs2075747 (G >A) did not show any association with breast cancer. The *hOGG1* SNP rs1052133 (Ser326Cys) minor allele T showed a significant association with breast cancer samples (OR = 1.78, $\chi 2 = 7.86$, p = 0.02024). In silico structural analysis was carried out to compare the wild type (Ser326) and mutant (Cys326) protein structures. The structural prediction studies revealed that Ser326Cys variant may destabilize the protein structure and it may disturb the *hOGG1* function. Taken together this is the first In silico study report to confirm Ser326Cys variant effect on structural and functional properties of *hOGG1* gene and Ser326Cys role in breast cancer susceptibility in Saudi population.

Keywords OGG1 · Saudi population · Breast cancer · OGG1 Ser326Cys

Introduction

Various mutations in gene sequences may result in either no effect at all, cause slight variation in the gene product or may result in the inhibition of the gene function. Most of these variations which affect the gene function may result in several diseases including cancer. Majority of the disease-causing variants are usually found in the exons i.e. the protein coding regions mainly as single nucleotide polymorphisms (SNPs) [1]. However, a large number of these mutations are repaired by DNA repair pathways to maintain the genome integrity. The cellular DNA repair process stabilizes the genome by reducing the number of mutations caused by the carcinogens [2]. Recent studies on the association of the genetic variants in the DNA repair genes and breast as well as colon cancer susceptibility have been focused primarily on the base excision repair pathway (BER pathway) genes including *XRCC1, PARP1, Pol-β* and *OGG1* among various populations worldwide

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[3]. We have recently reported the association of *XRCC1*, *PARP1* and *APE1* polymorphisms with breast cancer in Saudi population [4–6]. The base excision repair (BER) mechanism is a frontline repair pathway responsible for maintaining the genome stability, and consequently protecting humans from several deadly diseases such as cancer. The mechanism is responsible for an efficient repair system by fixing millions of lesions and breaks in the DNA strand that are frequently triggered by various mutagens [7].

The human 8-oxoguanine glycosylase 1 (*hOGG1*), located at chromosome 3p26.2, is synthesized by the 8-oxoguanine DNA glycosylase gene (*hOgg1*) [8, 9]. The *hOgg1* gene belongs to the BER pathway and has a DNA glycosylase/AP-lyase activity, catalyzing the excision of 8-oxoG. Minowa et al. [10] reported that knockout mice with inactive *hOgg1* gene have greater levels of 8-oxoG abrasions compared to the normal mice. The *hOgg1* defective mice showed increased number of spontaneous mutations when exposed to oxidative stress suggesting that the *hOgg1* plays a key role in DNA repair process [11]. The *h*OGG1 gene is highly polymorphic and several polymorphisms associated with cancer and other diseases have previously been reported in this gene [12–14]. The *hOgg1* Ser326Cys variant is one of the key polymorphisms caused due to a substitution of Serine to Cysteine residue at codon 326 of exon 7 (C > G, rs1052133). The *hOgg1* Cys326 polymorphism has been reported to display reduced efficiency in controlling the G:C to T:A transversions in the *Escherichia coli* mutants compared to the Ser326 form. Previous studies have shown that the *hOgg1* Cys326 may have a lower capability to excise 8-OH-G from the DNA [15, 16]. Yamane et al. [17], have observed similar results of hOgg1 Cys326 polymorphism in human cells under in vivo conditions. The reduction in DNA repair capability due to 326Cys allele has also been reported to be associated with an increased risk of cancer [8, 18] such as colorectal [16], orolaryngeal [19] and lung cancers [20]. Along with rs1052133 few other *hOgg1* SNP's such as rs2072668 [13, 21], rs293795 [22] have been reported to be associated with increased cancer in different populations. In our recent study [9] we have compared the frequencies of SNP rs1052133 in Saudi population with other HapMap populations, we observed that Saudi population genotypic frequencies were similar to only central European population [9].

The present study was conducted to examine the association of SNPs rs1052133 (Ser326Cys), rs293795 (T 12486C), rs2072668 (C 11513G) and rs2075747 (G 11096A) with breast cancer in Saudi Arabian population. To the best of our knowledge, this is the first study to screen the association of these SNPs in *hOgg1* among Saudi breast cancer patients. Additionally, this is the first report on the structural prediction of *hOgg1* Ser326Cys.

Materials and Methods

Study Population

A total of 210 blood samples were obtained from the King Fahd Medical City Hospital and King Khalid University Hospital, Riyadh, Saudi Arabia. The samples comprised of 100 breast cancer patients along with 110 gender matched controls. Disease free and age matched controls were recruited following standard diagnosis and physical examination procedures. Blood samples from cancer patients were collected prior to treatment. Histopathology, clinical data and medical records were also reviewed to confirm the diagnosis. For all the breast cancer samples, demographic and clinical details are shown in supplementary Table 1. A written informed consent was obtained from all participants, and ethical approval was obtained from the ethics review committee of King Fahad Medical City Hospital, Riyadh, Saudi Arabia.

Genotyping

The genomic DNA was extracted from the blood samples of breast cancer patients and controls using DNA extraction kit (Qiagen, Valencia, CA). The SNPs were selected from LD TAG SNP Selection (TagSNP) and based on the previously conducted published studies. The SNPs rs1052133 (Ser326Cys), rs293795 (T>C), rs2072668 (C>G) and rs2075747 (G>A) in the *hOgg1* gene were genotyped using TaqMan assays and determined by endpoint reading on an ABI 7500 real-time PCR machine (Applied Biosystems, Foster City, CA, USA). The genotyping experiments were conducted as previously described by Alanazi et al. [4].

In Silico Structural Modeling

The *hOgg1* exonic SNP Ser326Cys was studied using in silico tools to evaluate the mutation effect. Due to the unavailability of a PDB structure for the selected variant region (*hOgg1* Se326Cys), we used I-TASSER prediction program [15] to generate the 3D model of hOGG1. The multiple templates were generated (RCSB PDB Codes: 1LWW, 2XHI, 2NOH, 1KO9). The model generated for hOGG1 Ser326 (wild type) protein was evaluated using ProSA-web [23]. Using Rampage server, a Ramachandran plot was designed [24] for further evaluation of the predicted structure. The hOGG1 variant 326Cys structure was created via Discovery studio 2.5 (MODELER) based on the homology modeling using the predicted wild type structure [25]. The quality of the refined obtained hOGG1 structure (Fig. 2) was checked with Verify_3D. The stability of the mutant protein was checked using I-mutant v2.0 [26]. The effect of the variant allele 326cys on the structure and protein activity were carried out using Have yOur Protein Defined (HOPE) program [27].

Statistical Analysis

The allele frequencies and genotype distributions of the patient and control samples were computed and compared using the Fisher's exact χ2 test for Hardy-Weinberg equilibrium [28]. The odds ratios (ORs) and 95 % confidence intervals (95 % CIs) were calculated by two-sided chi-squared (χ2) tests to evaluate the association between the genotype frequencies and breast cancer risk. The SPSS 22.0 program for Windows was used to carry out all the statistical analyses. The linkage disequilibrium (LD) analysis was performed using the Haploview (v5.0) program.

Results

To evaluate the risk of susceptibility to breast cancer with the *hOgg1* gene variants, four different loci comprising of SNPs i.e., rs1052133 [Ser326Cys (C>T)] from exonic region, rs293795 (T>C) near 3′ region, rs2072668 (C>G) in the intronic region, and rs2075747 (G>A) in the intronic region were examined among Saudi breast cancer patients.

The frequencies of *hOgg1* SNPs were estimated in the studied breast cancer samples, and compared with matching controls. The genotypic distributions for all alleles were in accordance with Hardy–Weinberg equilibrium (HWE). A total of 100 breast cancer samples along with 110 age matched control samples were studied in the present study. The clinical and demographic characteristics of breast cancer and control samples are given in the Supplementary Table 1. Out of 100 breast cancer samples, 58 were estrogen-receptor-positive (ER+) and 42 estrogen-receptor-negative (ER-), 56 were progesterone-receptor-positive (PR+) and 44 progesterone-receptor-negative (PR-), 40 were human epidermal growth factor receptor 2 positive (HER2+) and 57 human epidermal growth factor receptor 2 negative (HER2-) (Supplementary Table 1). The HER2 status for three samples were not known.

Table 1 Genotype frequencies of OGG1 gene polymorphism in breast cancer cases and controls

Genotype	Cases	Controls	OR	95 % CI	X^2	*p*- value	*p*- value*
rs1052133							
CC	37 (0.37)	58 (0.53)	Ref				
CT	40 (0.40)	37 (0.34)	1.69	0.92–3.11	2.91	0.08819	0.35276
TT	23 (0.23)	14 (0.13)	2.57	1.18–5.63	5.79	0.01613	0.06452
CT+TT	63 (0.63)	51 (0.47)	1.94	1.11–3.37	5.53	0.01871	0.07484
C	114 (0.57)	153 (0.70)	Ref				
T	86 (0.43)	65 (0.30)	1.78	1.19–2.66	7.86	0.00506	**0.02024**
rs293795							
TT	81 (0.82)	89 (0.81)	Ref				
TC	15 (0.15)	18 (0.16)	0.92	0.43–1.93	0.05	0.81743	1
CC	3 (0.03)	3 (0.03)	1.10	0.22–5.60	0.01	0.90971	1
TC+CC	18 ()	21 (0.19)	0.94	0.47–1.89	0.03	0.86624	1
T	177 (0.89)	196 (0.89)	Ref				
C	21 (0.11)	24 (0.11)	0.97	0.52–1.80	0.01	0.9205	1
rs2072668							
CC	44 (0.44)	54 (0.49)	Ref				
CG	44 (0.44)	48 (0.44)	1.12	0.64–1.99	0.16	0.68584	1
GG	12 (0.12)	8 (0.07)	1.84	0.69–4.90	1.52	0.21773	0.87092
CG + GG	56 (0.56)	56 (0.51)	1.23	0.71–2.11	0.55	0.46018	1
C	132 (0.66)	156 (0.71)	Ref				
G	68 (0.34)	64 (0.29)	1.26	0.83–1.90	1.17	0.2791	1
rs2075747							
GG	12 (0.12)	8 (0.07)	Ref				
GA	43 (0.43)	43 (0.40)	0.67	0.25–1.79	0.65	0.42012	1
AA	45 (0.45)	57 (0.53)	0.53	0.20–1.40	1.69	0.19301	0.77204
GA + AA	88 (0.88)	100 (0.93)	0.59	0.23–1.501	1.26	0.26163	1
G	67 (0.34)	59 (0.27)	Ref				
A	133 (0.67)	157 (0.73)	0.75	0.49–1.13	1.88	0.17016	0.68064

The *p*-values in bold are significant

**p* value: Bonferroni corrected *P* value

After applying Bonferroni correction method in the overall analysis, a significant association was observed only with the T allele of rs1052133 (Ser326Cys (C>T)) which showed about 2 fold increased risk associated with developing breast cancer compared to the individuals with A allele (OR = 1.78, $\chi2 = 7.86$, $p = 0.02024$) (Table 1). The genotype frequencies of rs1052133 as CC, CT, and TT in breast cancer patients were 37 (0.37), 40 (0.40), and 23 (0.23), whereas in healthy controls the frequencies were 58 (0.53), 37 (0.34), and 14 (0.13), respectively. The frequency of rs1052133 SNP T allele was significantly higher 86 (0.43) in breast cancer cases when compared to the controls 65 (0.30) (Table 1). The TT genotype (OR = 2.57, $\chi2 = 5.79$) and CT+TT genotypes (OR = 1.94, $\chi2 = 5.33$ showed significant risk before Bonferroni's correction.

The genotype frequencies of rs293795, rs2072668 and rs2075747 were not statistically significant between the breast cancer and control samples (Table 1). In linkage disequilibrium analysis, out of four SNPs analyzed, all the four SNP's were in LD in both cases ($r^2 = >0.80$) and controls ($r^2 = >0.80$) (Fig. 1).

Association of *hOgg1* SNPs with Breast Cancer Risk Based on Age

To check the association of OGG1 SNPs with the age at the time of disease diagnosis, the breast cancer patients were classified based on the median age ≤48 years ($n = 47$) and >48 years ($n = 53$). The genotype frequencies were compared with the age matched healthy individuals. Interestingly, the rs2072668 variant which did not show any association in the overall comparison exhibited a significant risk association whereas the variant rs2075747 showed a protective association among the younger aged (≤48 years) patients before Bonferroni correction (Table 2). The genotypes of rs1052133 and rs293795 did not show any association in younger aged (≤48 years) breast cancer patients. However, in older (>48 years) breast cancer patients variant rs1052133 showed a significant association with CT + TT genotype (OR = 2.47, $\chi2 = 5.07$) as well as for the T allele (OR = 2.10, $\chi2 = 6.17$) before Bonferroni correction (Table 3). The genotypes of rs2072668, rs2075747 and rs293795 did not show any association in older (>48 years) breast cancer patients (Table 3).

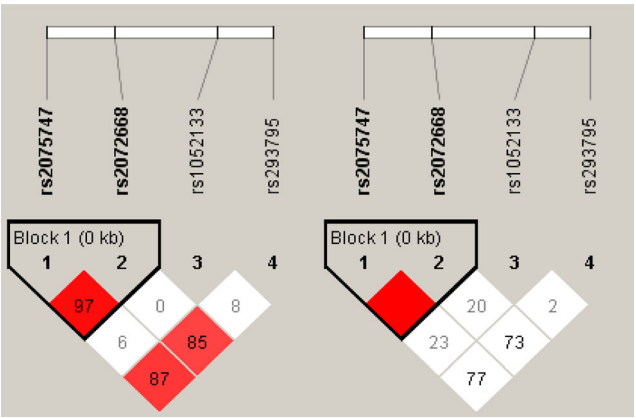

Fig. 1 Linkage disequilibrium (LD) plot was constructed using four SNPs from *hOgg1* gene. Each rs numbers correspond to a SNP, whereas the level of pairwise D' indicates the degree of LD between two SNPs

Effect of ER Status on the Association of *hOgg1*SNPs with Breast Cancer

In the present study the association of breast cancer risk with *hOgg1* SNPs based on the ER status of patients was examined. None of the SNPs showed any association with the ER+ breast cancer samples after Bonferroni's correction. The genotypic frequencies of ER+ and ER- breast cancer samples were also compared with overall control samples (Tables 4 and 5). Interestingly, except the SNP rs293795 all the three SNPs showed significant risk association with ER- breast cancer samples even after applying the Bonferroni's correction. The frequency of CT heterozygosity of rs1052133 was higher in breast cancer cases 24 (0.57) compared to the healthy controls (0.34) (OR = 3.40, $\chi2 = 9.01$, p =0.01072) (Table 5). A significantly high risk was also observed in the ER-ve group of breast cancer patients for the rs1052133 CT+TT genotype compared to the control samples (OR = 3.20, $\chi2 = 8.92$, p =0.01128) (Table 5). Additionally, the minor allele T of variant rs1052133 showed significantly higher risk association with the breast cancer among ER- patients (OR = 1.94, $\chi2 = 6.42$, p =0.04524). For the SNP rs2072668, individuals with GG genotype have significantly higher risk of developing ER- breast cancers compared to the CC homozygote genotypes (OR = 4.50, $\chi2 = 7.97$, $p = 0.019$). Furthermore, the intronic region SNP rs2075747 which indicated a protective effect among younger (≤48 years) patients also showed strong protection against the ER- breast cancers individuals with AA (OR = 0.20, $\chi2 = 9.36$, $p = 0.00884$) and GA+AA genotypes (OR = 0.26, $\chi2 = 7.7$, p =0.02204) as well as for those having minor allele A (OR = 0.45, $\chi2 = 8.88$, $p = 0.01152$).

In Silico Structure Prediction of h*OGG1 Ser326Cys*

The structures for the wild-type hOGG1 from 1 to 345 amino acids and the mutant structure with the Cys 326 amino acid were predicted (Fig. 2). The predicted structural model of hOGG1 Ser326 protein was selected based on the C-score (−0.50). The selected model was also assessed using various validation tools. The Z-score and local Energy plots were within the expected region, therefore the overall predicted model quality was good. The Ramachandran plot exhibited a very good quality plot and displayed 98.5 % of the residues in the favorable regions. The 98.63 % value for the wild type and a 95 % value for the mutant were within the acceptable

Table 2 Genotype frequencies of OGG1 gene polymorphism in breast cancer cases below 48 years

Genotype	Case	Controls	OR	95 % CI	X^2	*p*- value	*p*- value*
rs1052133	<48 Y						
CC	18 (0.38)	29 (0.49)	Ref				
CT	17 (0.36)	21 (0.36)	1.30	0.545–3.11	0.36	0.54869	1
TT	12 (0.26)	9 (0.15)	2.15	0.75–6.11	2.09	0.14819	0.59276
CT+TT	29 (0.62)	30 (0.51)	1.56	0.71–3.39	1.25	0.26376	1
C	53 (0.56)	79 (0.67)	Ref				
T	41 (0.44)	39 (0.33)	1.57	0.89–2.74	2.49	0.11486	0.45944
rs293795							
TT	37 (0.81)	49 (0.82)	Ref				
TC	8 (0.17)	8 (0.13)	1.32	0.45–3.86	0.27	0.60580	1
CC	1 (0.02)	3 (0.05)	0.44	0.04–4.42	0.51	0.47559	1
TC+CC	9 (0.19)	11 (0.18)	1.09	0.41–2.88	0.03	0.87236	1
T	82 (0.89)	106 (0.88)	Ref				
C	10 (0.11)	14 (0.12)	0.92	0.39–2.18	0.03	0.85594	1
rs2072668							
CC	22 (0.47)	28 (0.47)	Ref				
CG	17 (0.36)	30 (0.50)	0.72	0.32–1.63	0.62	0.43187	1
GG	8 (0.17)	2 (0.03)	5.09	0.98–26.43	4.32	0.03767	0.15068
CG+GG	25 (0.53)	32 (0.53)	0.99	0.46–2.14	0.001	0.98836	1
C	61 (0.65)	86 (0.72)	Ref				
G	33 (0.35)	34 (0.28)	1.37	0.77–2.44	1.12	0.28898	1
rs2075747							
GG	8 (0.17)	2 (0.03)	Ref				
GA	16 (0.34)	27 (0.47)	0.15	0.03–0.79	6.00	0.01434	0.05736
AA	23 (0.49)	29 (0.50)	0.20	0.04–1.03	4.29	0.03829	0.15316
GA+AA	39 (0.83)	56 (0.97)	0.17	0.03–0.86	5.55	0.01847	0.07388
G	32 (0.34)	31 (0.27)	Ref				
A	62 (0.66)	85 (0.73)	0.71	0.39–1.28	1.32	0.24982	0.99928

**p* value: Bonferroni corrected *P* value

range limit for the predicted structures. Both the wild-type Ser326 (Fig. 2a) and the variant 326Cys structures (Fig. 2b) of the hOGG1 were also superimposed on each other using PyMOL to estimate the possible effects of the 326Cys variant (Fig. 2c). The RMSD value was 0.24Å indicating that the variant (326Cys) structure was comparable to the wild-type structure (Ser326).

In Silico Prediction of the Native and Mutant Residues The generated 3D-structure was used to examine the functional effects of the Ser326Cys substitutions in the hOGG1. Figure 3 shows the predicted models of the wild type and mutant amino acids. The backbone with the same amino acid residue was colored in red. The side chain that was unique for each amino acid, was colored in black. Because each residue has its own specific size, charge, and hydrophobicity-value, the mutant residue (Cysteine) was smaller than the wild-type (Serine) residue. The wild-type amino acid (Serine) at codon 326 is positively charged while the variant amino acid (Cysteine) is more hydrophobic than the wild-type amino acid. Consequently, this mutation may cause an alteration in the hOGG1 structure and will have a deleterious effect on the hOGG1 protein function.

Impact of the Ser326Cys Mutation on the Protein Structure and Function

The mutant residue 326Cys was more hydrophobic than the wild-type Ser326 residue. The variant was located in a region with known splice variants, described as: “In isoform 2E.”, “In isoform 2D.”, “In isoform 2C.”, “In isoform 2B.”, “In isoform 2A.”, “In isoform 1C.”, and “In isoform 1B.”.

Domains This amino acid was shown to be part of an interpro domain named “8-oxoguanine DNA-glycosylase” (IPR004577) and annotated with the following Gene-Ontology (GO) terms which indicated its function (oxidized purine nucleotide base lesion DNA N-glycosylase activity GO: 0008534). In general,

Table 3 Genotype frequencies of OGG1 gene polymorphism in breast cancer cases above 48 years

Genotype	Case parameter	Control	OR	95 % CI	X^2	p- value	p- value*
rs1052133	>48 Y						
CC	19 (0.36)	29 (0.58)	Ref				
CT	23 (0.43)	16 (0.32)	2.19	0.93–5.19	3.24	0.07185	0.2874
TT	11 (0.21)	5 (0.10)	3.36	1.01–11.20	4.10	0.04290	0.1716
CT+TT	34 (0.64)	21 (0.42)	2.47	1.12–5.47	5.07	0.02430	0.0972
C	61 (0.58)	74 (0.74)	Ref				
T	45 (0.42)	26 (0.26)	2.10	1.16–3.79	6.17	0.01302	0.05208
rs293795							
TT	44 (0.83)	40 (0.80)	Ref				
TC	7 (0.13)	10 (0.20)	0.64	0.22–1.83	0.71	0.39943	1
CC	2 (0.04)	0 (0)	4.55	0.21–97.64	1.78	0.18208	0.72832
TC+CC	9 (0.17)	10 (0.20)	0.82	0.30–2.22	0.16	0.69300	1
T	95 (0.90)	90 (0.90)	Ref				
C	11 (0.10)	10 (0.10)	1.04	0.42–2.57	0.01	0.92871	1
rs2072668							
CC	22 (0.41)	26 (0.52)	Ref				
CG	27 (0.51)	18 (0.36)	1.77	0.78–4.04	1.87	0.17148	0.68592
GG	4 (0.08)	6 (0.12)	0.79	0.20–3.15	0.11	0.73579	1
CG+GG	31 (0.59)	24 (0.48)	1.53	0.70–3.33	1.14	0.28611	1
C	71 (0.67)	70 (0.70)	Ref				
G	35 (0.33)	30 (0.30)	1.15	0.64–2.07	0.22	0.64123	1
rs2075747							
GG	4 (0.08)	6 (0.12)	Ref				
GA	27 (0.51)	16 (0.32)	2.53	0.62–10.35	1.74	0.18768	0.75072
AA	22 (0.42)	28 (0.56)	1.18	0.30–4.70	0.05	0.81575	1
GA+AA	49 (0.92)	44 (0.88)	1.67	0.44–6.31	0.58	0.44556	1
G	35 (0.33)	28 (0.28)	Ref				
A	71 (0.67)	72 (0.72)	0.79	0.43–1.43	0.61	0.43459	1

*p value: Bonferroni corrected P value

Gene-Ontology interpretations specified the domain with lyase activity function (GO: 0016829) and hydrolase activity (GO: 0016787). The mutated residue was located in a domain that is important for the main protein activity and the mutation of the residue may probably disturb this function.

Amino Acid Properties The hydrophobicity of the wild-type Ser326 and variant 326Cys amino acids differed significantly. The mutation introduced a more hydrophobic residue at this position which may cause the loss of hydrogen bonds and/or disturb the correct folding.

Alterations in Protein Stability Upon Amino Acid Substitution

The I-mutant v2.0 analysis for the thermodynamic protein stability changes suggested that the residual alteration at this position may consequently result in low levels of folding free energy ($\Delta\Delta G = -0.38$ kcal/mol) and will have destabilizing effects on the hOGG1 protein structure.

Discussion

During the ATP production cells produce reactive oxygen species, such as superoxide, H_2O_2, hydroxyl radicals etc., damage DNA, amino acids, and membrane lipids [29]. Cells with damaged DNA are eliminated by the activation of apoptotic pathway. However, when these cells survive and propagate, the mutated DNA passes on to the daughter cells which may develop to be cancerous. One of the products of oxidative DNA damage is 8-hydroxy-2′-deoxyguanosine. Elevated levels of 8-hydroxy-2′-deoxyguanosine have been reported among cancer patients. Normally, the BER pathway repairs the oxidative DNA damages through the excision of damaged bases; especially *hOgg1* gene plays a key role in

Table 4 Genotype Frequencies of OGG1 gene polymorphism in ER positive breast cancer cases

Genotype	Case Parameter	Control	OR	95 % CI	X^2	p- value	p- value*
ER status	ER +ve						
rs1052133							
CC	26 (0.44)	58 (0.53)	Ref				
CT	16 (0.28)	37 (0.34)	0.96	0.46–2.04	0.01	0.92477	1
TT	16 (0.28)	14 (0.13)	2.55	1.09–5.99	4.76	0.02915	0.1166
CT+TT	32 (0.56)	51 (0.47)	1.40	0.74–2.65	1.06	0.30224	1
C	68 (0.59)	153 (0.70)	Ref				
T	48 (0.41)	65 (0.30)	1.66	1.04–2.66	4.52	0.03346	0.13384
rs293795							
TT	43 (0.75)	89 (0.81)	Ref				
TC	11 (0.19)	18 (0.16)	1.26	0.55–2.91	0.31	0.58021	1
CC	3 (0.06)	3 (0.03)	2.07	0.40–10.68	0.78	0.37589	1
TC+CC	14 (0.25)	21 (0.19)	1.38	0.64–2.97	0.68	0.41019	1
T	97 (0.85)	196 (0.89)	Ref				
C	17 (0.15)	24 (0.11)	1.43	0.73–2.79	1.12	0.29046	1
rs2072668							
CC	29 (0.50)	54 (0.49)	Ref				
CG	27 (0.47)	48 (0.44)	1.05	0.54–2.01	0.02	0.88935	1
GG	2 (0.03)	8 (0.07)	0.47	0.09–2.34	0.90	0.34375	1
CG+GG	29 (0.50)	56 (0.51)	0.96	0.51–1.82	0.01	0.91078	1
C	85 (0.73)	156 (0.71)	Ref				
G	31 (0.27)	64 (0.29)	0.89	0.54–1.47	0.21	0.64693	1
rs2075747							
GG	2 (0.03)	8 (0.07)	Ref				
GA	25 (0.43)	43 (0.40)	2.33	0.46–11.82	1.08	0.29812	1
AA	31 (0.53)	57 (0.53)	2.17	0.43–10.88	0.93	0.33428	1
GA+AA	56 (0.97)	100 (0.93)	2.24	0.46–10.91	1.04	0.30671	1
G	29 (0.25)	59 (0.27)	Ref				
A	87 (0.75)	157 (0.73)	1.13	0.67–1.89	0.21	0.64866	1

*p value: Bonferroni corrected P value

repairing oxidative DNA damage caused by reactive oxygen species.

In this case control study, we genotyped four different SNPs rs1052133, rs293795, rs2072668 and rs2075747 of *hOgg1* gene in breast cancer patients in Saudi population. Out of the four *hOgg1* SNPs only rs1052133 showed a significant risk association with breast cancer in the combined analysis. The remaining three SNPs rs293795, rs2072668 and rs2075747 did not show strong association.

A significantly low frequency of the minor allele T of *hOgg1* SNP rs1052133 in the normal control samples (0.30), compared to the breast cancer samples (0.43), revealed that this genotype could be a risk factor in the Saudi population. This significant risk association was observed even after Bonferroni's correction. Recent studies have revealed that variation at *hOgg1* Ser326Cys (C.977C>G) in exon 7 plays a key role in carcinogenesis. Several reports have suggested that *hOgg1* Ser326Cys is associated with cancer in a few populations with a distinct genetic background of certain ethnicity [30, 31]. Regarding the *hOgg1* Ser326Cys SNP, conflicting results have previous been reported. Our results were in agreement with the results reported by Rodrigues et al. [32] in Spanish population which showed that the *hOgg1* Ser326Cys polymorphism is associated with high risk of breast cancer. Sangrajrang et al. [33] have also reported that Thai women with variant allele of *hOgg1* Ser326Cys were likely to have an increased risk to breast cancer. Korean population *hOgg1* Ser326Cys was shown to have strong association with breast cancer risk along with APEX1 variant Asp148Glu [21]. Contrary to this, Synowieca et al. [34] have shown protective association of Cys allele in Polish population, while other studies showed no association between *hOgg1* Ser326Cys (rs1052133) and breast cancer risk [35–37].

We also observed that the *hOgg1* 326 Cys/Cys polymorphism in *hOgg1* showed a trend to an increased breast cancer

Table 5 Genotype frequencies of OGG1 gene polymorphism in ER negative breast cancer cases

Genotype	Case	Control	OR	95 % CI	X^2	*p*- value	cP value*
ER status	ER –ve						
rs1052133							
CC	11 (0.26)	58 (0.53)	Ref				
CT	24 (0.57)	37 (0.34)	3.42	1.50–7.80	9.01	0.00268	**0.01072**
TT	7 (0.17)	14 (0.13)	2.64	0.87–8.02	3.04	0.08106	0.32424
CT + TT	31 (0.74)	51 (0.47)	3.20	1.46–7.02	8.92	0.00282	**0.01128**
C	46 (0.55)	153 (0.70)	Ref				
T	38 (0.45)	65 (0.30)	1.94	1.16–3.27	6.42	0.01131	**0.04524**
rs293795							
TT	38 (0.90)	89 (0.81)	Ref				
TC	4 (0.10)	18 (0.16)	0.52	0.16–1.64	1.28	0.25852	1
CC	0 (0)	3 (0.03)	0.33	0.02–6.58	1.27	0.26007	1
TC+CC	4 (0.10)	21 (0.19)	0.45	0.14–1.39	2.02	0.15479	0.61916
T	80 (0.95)	196 (0.89)	Ref				
C	4 (0.05)	24 (0.11)	0.41	0.14–1.21	2.75	0.09744	0.38976
rs2072668							
CC	15 (0.36)	54 (0.49)	Ref				
CG	17 (0.40)	48 (0.44)	1.27	0.57–2.83	0.36	0.54914	1
GG	10 (0.24)	8 (0.07)	4.50	1.51–13.40	7.97	0.00475	**0.019**
CG+GG	27 (0.64)	56 (0.51)	1.74	0.83–3.61	2.19	0.13854	0.55416
C	47 (0.56)	156 (0.71)	Ref				
G	37 (0.44)	64 (0.29)	1.92	1.14–3.23	6.13	0.01329	0.05316
rs2075747							
GG	10 (0.24)	8 (0.07)	Ref				
GA	18 (0.43)	43 (0.40)	0.33	0.11–0.99	4.12	0.04235	0.1694
AA	14 (0.33)	57 (0.53)	0.20	0.7–0.9	9.36	0.00221	**0.00884**
GA+AA	32 (0.76)	100 (0.93)	0.26	0.09–0.70	7.7	0.00551	**0.02204**
G	38 (0.45)	59 (0.27)	Ref				
A	46 (0.55)	157 (0.73)	0.45	0.27–0.77	8.88	0.00288	**0.01152**

The *p*-values in bold are significant

**p*- value: Bonferroni corrected *P* value

risk among premenopausal Saudi women (age <48 years) however, the association was found to be statistically not significant. A recent meta-analysis on *hOgg1 hOgg1* Ser326Cys polymorphism also did not find a significant association with breast cancer risk [38]. A protective effect was observed for the SNP variant rs2075747 in heterozygous and variant genotypes among younger breast cancer patients, but this association became non-significant when tested for multiplicity. This is the first report on rs2075747 association with cancer.

The *hOgg1* 326 Cys/Cys, Ser/Cys + Cys/Cys genotypes and Cys allele showed increased risk among ER positive breast cancer samples, but again the association was not statistically significant. Interestingly, *hOgg1* 326 hetrozygous Ser/Cys genotype, Ser/Cys + Cys/Cys genotype and minor allele Cys showed significantly higher risk in ER negative breast cancer samples. The SNP variant rs2072668 which did not show any association in overall analysis showed a significantly increased risk for ER- breast cancers with homozygous variant genotype GG. Thus, it is plausible that the ER- breast cancer patients with 326Cys and GG homozygosity for the variant rs2072668 might have deficient *hOgg1* activity. It would be interesting to examine these assumptions in future functional studies. The *hOgg1* variant rs2075747 has shown to confer significant protection against ER negative breast cancers.

In the present study, we also examined the Ser326Cys variant's influence on the functional and structural properties of the hOGG1 protein by means of in silico computational biology tools. We have observed that *hOgg1* 326Cys may cause structural destabilization in the protein. The structural and functional studies on native (Ser326) and variant (326Cys) structures showed that the mutant residue was more hydrophobic than native type amino acid, which may alter the structure and will have deleterious effect on the function of hOGG1 protein. The *hOgg1* Ser326Cys variant was located in a region with splice sites, whereas the variant residue was present in an

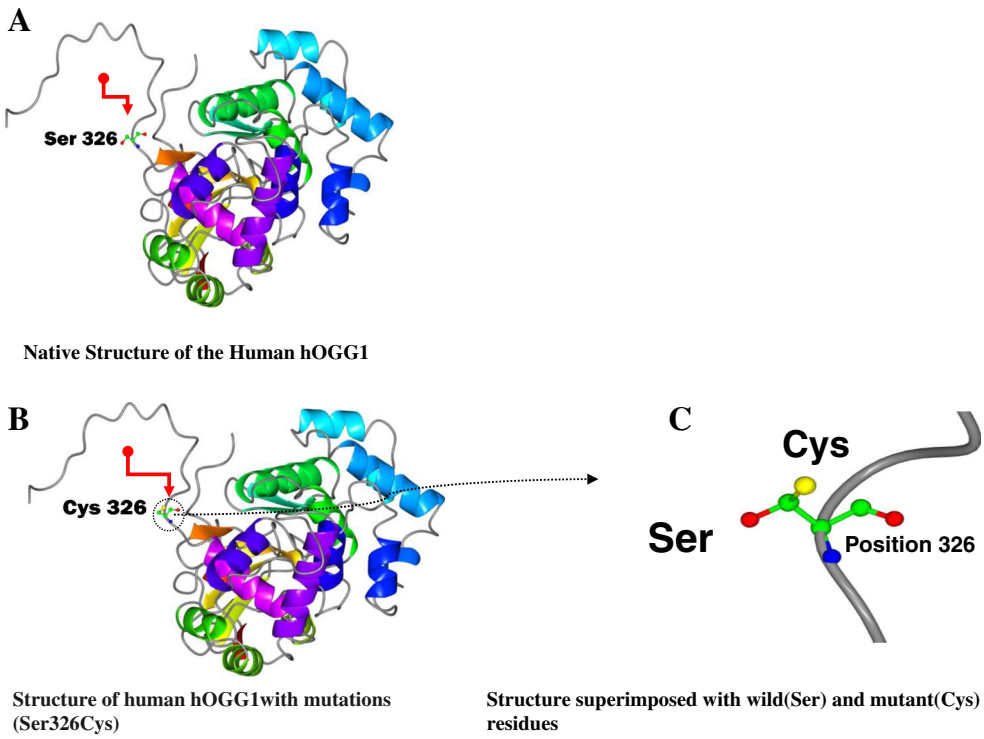

Fig. 2 **a** Ribbon diagram of human hOGG1 protein showing all the secondary structures **b** Location of mutations (Cys 326) identified in the hOGG1 protein of Breast Cancer patients, C. Structure of Human 8-oxoguanine DNA Glycosylase superimposed with wild and mutant residues with mutation Ser326Cys enlarged

important domain which has DNA glycosylase activity (GO: 0008534), lyase activity (GO: 0016829) and hydrolyase activity (GO: 0016829). Hence mutated site may affect the hOGG1 protein function.

Our results are in agreement with the previous in vivo studies conducted by Hill and Evans et al. [39]. The study showed that the functional studies with deletion mutants suggested that this domain is important for the DNA binding and enzymatic activity [Hill and Evans, [39]. Simonelli et al. [40] reported that cells with homozygous Cys326 showed less hOGG activity compared to the homozygous Ser326 allele carrier cells. They also reported that the *hOgg1*Cys326 allele affected the efficiency of h*Ogg1* gene function and base excision repair pathway activities. In in vitro reconstruction of the 8-OH-Gua repair pathway, when the repair was initiated by the variant protein, showed a significant decrease in the yield of the repaired DNA products. The purified Cys326Cys showed significantly lower enzymatic activity whereas the mutant protein showed low capacity to complete the repair synthesis in the BER reaction. They also proposed that this variant may alter the protein

Fig. 3 Schematic structure of the wild type (Ser) and mutant (Cys) amino acids

conformation and stability. In the present study, we have verified and confirmed this notion through computational and bioinformatics in silico tools. Hill and Evans have reported that Cys326Cys allele of *hOgg1* has approximately 30 % lower activity than the (Ser326Ser) wild-type enzyme [39], [41] and was relatively resistant to displacement from a basic site by APE1 [39]. Additionally, regional LD plot was also generated using SNAP (http://www.broadinstitute.org/mpg/snap/ldplot.php) for hOGG1 326Ser. As Saudi Population showed similarity in genotypic frequencies with Central European Population [9], the LD plot was generated using Central European Population (CEU) data. The LD plot indicated that there were multiple loci near the SNP rs1052133 with high LD ($r^2 > 0.8$), which suggested that fine mapping is necessary to evaluate the genetic effect of the *hOgg1* as well as functional effects on the cancer (Supplementary Figures S1 and S2). The present study has shown some strengths and limitations. One of the strengths of this study was that this was the first report to evaluate the *hOgg1* variant Ser326Cys polymorphism among the patients belonging to the central region of Saudi Arabia. Furthermore, this is also the first report to examine the in silico effects of the *hOgg1* Ser326Cys polymorphism. Additionally, the cases and control individuals evaluated in this study were from the same ethnicity along with matching age and gender. On the other hand, our study sample was relatively modest and hence the output from the SNPs results must also be validated in large population samples.

In conclusion, the present study is the first report that showed a significant association between the *hOgg1* variant

Ser326Cys genotype and elevated risk of breast cancer among Saudi patients. Additionally, our findings suggest a protective role for *hOgg1* variant rs2075747 against ER- breast cancers. This is also the first report that deals with the structural implications of *hOgg1* variant Ser326Cys followed by structural prediction and In silico computational analysis.

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