Cellular & Molecular Biology
Cell. Mol. Biol. 2015; 61 (4): 70-74
Published online August 28, 2015 (http://www.cellmolbiol.com)
Received on June 28, 2015, Accepted on August 25, 2015.
doi : 10.14715/cmb/2015.61.4.12

The association of *ApE1* -656T>G and 1349T>G polymorphisms with breast cancer susceptibility in northern Iran

F. Mashayekhi[1]✉, M. Yousefi[1], Z. Salehi[1], H. S. Saedi[2] and M. Pournourali[1]

[1] Department of Biology, Faculty of Sciences, University of Guilan, Rasht, Iran
[2] Department of Radiotherapy, Faculty of Medicine, Guilan University of Medical Sciences, Rasht, Iran

Corresponding author: Farhad Mashayekhi, Department of Biology, Faculty of Sciences, University of Guilan, Namjoo St., P.O.Box 1914, Rasht, Iran. E-mail: mashayekhi@guilan.ac.ir

Abstract

Breast cancer is the most common cancer and one of the main causes of cancer-related death in the world and has become a major public health concern. Many genes have been shown to be involved in the progress of the breast cancer. Human apurinic/apyrimidinic endonuclease 1 (*APE1*) is a multifunctional protein that has an important role in the base excision repair (BER) pathway. The aim of this study was to evaluate the association between *ApE1* -656T>G and 1349T>G polymorphisms and the susceptibility to breast cancer in northern Iran population. Samples were obtained from 150 patients diagnosed with breast cancer and 150 control subjects and genotyped by tetra – primer amplification refractory mutation system PCR (T–ARMS-PCR). We observed a significant difference in genotype distributions of -656T>G polymorphism between patients and controls (P= 0.03). Findings indicated that individuals with the variant TG genotypes had a significant decreased risk of breast cancer (OR= 0.55, 95% CI= 0.33 – 0.91, P= 0.019). However, the significant association between 1349T>G polymorphism and breast cancer risk was not observed (P = 0.89). Larger studies are needed to confirm our results.

Key words: *ApE1*, breast cancer, polymorphism.

Introduction

Breast cancer is the most common form of malignancy experienced by women worldwide. This cancer accounts for 23 % (1.38 million) of the total new cancer cases and 14 % (458,400) of the total cancer deaths in 2008. There are evidences to show a consequent increase in breast cancer prevalence at recent years. So, it has become a major public health concern (1). The mechanism of breast carcinogenesis is not fully understood, but it is a multifactorial disease caused by complex genetic and environmental factors (2). There are relations between breast cancer and inter-individual variations in DNA damage and repair process (3,4). Altered DNA repair function, resulting in aggregation of DNA damage, followed by cell apoptosis or unregulated cell growth and cancer (5).

DNA repair gene system is one of the most important mechanisms in human body and has a key role in protecting against gene mutations (6). The human apurinic/apyriminic endonuclease 1 (*APE1*), also known as ApE, APEX and Ref-1, is underlying enzyme in the base excision repair (BER) pathway. BER protects cells against the effect of endogenous and exogenous agents and also is responsible for the repair of DNA brought about oxidation / alkylation damage (7). *ApE1* is a multifunctional protein that located on chromosome 14q11.2-q12, consists of five exons and four introns spanning 2.21 kb. By hydrolyzing 3'-blocking fragments from oxidized and alkylated DNA, *ApE1* comes out with normal 3'- hydroxyl nucleotide termini that are necessary for DNA repair synthesis and ligation at single – or double – strand breaks (8 and 9). Also, *ApE1* has an important role in redox signaling (Ref-1). Ref-1 was characterized as a redox activator for a number of transcription factors such as NF-κB, HIF-1α and p53 which are involve in cellular growth and differentiation, cell cycle control, apoptosis, and angiogenesis, that all of them have implications for the development of cancer therapeutics. *APE1*/Ref-1 expression is altered in many cancers including breast, prostate, colon, cervical, ovarian, and germ cell tumors (10).

Recent studies have shown that single nucleotide polymorphisms (SNP_s) in DNA repair genes may be the critical molecular mechanism of the individual variation of DNA repair capacity and influence both cancer progression and clinical responses to chemotherapy and radiotherapy (11). Eighteen polymorphisms in *ApE1* have been recognized but transversion of T to G is the most extensively studies polymorphism (12,13). *ApE1* SNP T>G found in exon 5 led to substitution of Asp>Glu at codon 148 which is located within the endonuclease domain of the protein. The *ApE1* Asp148Glu (1349T>G) polymorphism is the only known common non-synonymous *ApE1* coding region variant (14). The other important *ApE1* SNP -656T>G (rs1760944), is located in the promoter region (15). *APE1* Asp148Glu and -656T>G polymorphisms exhibited unfavorable changes in torsion which influenced the overall stability and folding of the protein that lead to increased redox activity of APE1/Ref-1 in cancer cells and upregulated further in response to certain chemotherapeutics and radiation damage (16). The aim of this study was to evaluate the association between *ApE1* (-656T>G and 1349T>G) polymorphisms and susceptibility to breast cancer in northern Iran population.

Table 1. The primers sequences that used for target sequences of *ApE1*.

Polymorphism	Allele	Length (bp)	sequence of primers (bp) 5′ ⟶ 3′
-656T>G	T	384	F: 5′-TGTTTTTTTCCCTCTTGCACAT-3′ R: 5′ -TTAAGGGTCCTGACTCAAGC-3′
	G	446	F: 5′ -TGTTTTTTTCCCTCTTGCACAG-3′ R: 5′ -CATTTCATAGTGCCTTGTACC-3′
1349T>G	T	152	F: 5′-GACCCTATTGATGCCTAATGCC-3′ R: 5′- GCCTTCCTGATCATGCTCCACA-3′
	G	450	F: 5′- GACTGTTTAACCCGTGCGTA-3′ R: 5′- GCCTTCCTGATCATGCTCCACC-3′

Materials and methods

Patients and Controls

The current study included a total of 150 patients with breast cancer and 150 disease-free control subjects. Controls and patients were selected from same population living in the Guilan province, north of Iran, which were recruited between 2013 and 2014. Data on patient characteristics at the study entry for each subject were collected from the oncology clinic of Razi Hospital (Rasht, Iran). Control subjects were selected among individuals getting health examinations at the Health Examination Center of hospital during the same period and any subjects who had a history of cancer were excluded from the study. The control subjects were frequency-matched with the cancer patients based on mean age and age at menarche. Each subject donated 2 ml blood and drawn into EDTA-Coated tubes (Venoject, Belgium), which was used for genomic DNA extraction. This project has been approved by the local licensing committee and informed consent was obtained from all subjects and has been performed according the Helsinki Declaration of 1975, as revised in 1983.

Genotyping

Genomic DNA was extracted from whole-blood samples using the Gpp solution kit (Gen pajoohan, Iran) according to the manufacturer's instructions. DNA purity and concentration were determined by spectrophotometer at 260 and 280 nm. Each DNA sample was stored in TE buffer (5mμ Tris, HCl, 0.1 mμ EDTA, pH 8.5) at -20˚C until analysis. Polymorphism spanning fragments were amplified by the polymerase chain reaction (PCR) and performed using tetra– primer amplification refractory mutation system PCR (T–ARMS-PCR). Oligonucleotide primers used for amplification of the target sequences of *ApE1* were designed by means of Oligo primer analysis software (version 7.54, Molecular Biology Insights Inc., Cascade, Co, USA). The primers sequences are shown in Table 1. Similarly, the PCR conditions for both polymorphisms of the *ApE1*- were as follow: initial denaturation at 95˚C for 5 min, 35 cycles of denaturation at 95˚C for 45 s, annealing at 58˚C (-656T>G) and 53˚C (1349T>G) for 40 s, with a final step at 72˚C for 5 min to allow a complete extension of PCR fragment. The PCR products were separated on 2% agarose gel and visualized by ethidium bromide staining. Genotype results were regularly confirmed by randomly selecting 20% of the samples that were regenotyped by another laboratory member to improve the quality of genotyping and its validity and no discrepancy in genotyping was found.

Statistical analysis

Data management and analysis was performed using Medcalc software (version 12.1, Mariakerke, Belgium). Genotype frequencies between cases and controls were compared by the χ^2 test. To estimate the association between the *ApE1* -656T>G and 1349T>G variants and the risk of breast cancer, Odds ratios and 95% confidence intervals (95%CI) were calculated by logistic regression and differences in demographic variables (including smoking history, body mass index (BMI), and family history of cancer) between patients and control subjects were compared by chi-squared ($\chi 2$) test. The association between the *ApE1* -656T>G and 1349T>G variants and risk of breast cancer was investigated by treating the three genotypes (major allele homozygous, heterozygous and variant allele homozygous) as ordinal variables in the analysis. The homozygosity with the more frequent allele among controls was set as the references group. A value of $P < 0.05$ was considered statistically significant.

Results

In the present study 300 subjects, including 150 breast cancer patients and 150 cancer-free control subjects were evaluated. The mean age of cases (51.5 ± 4.6) at diagnosis was slightly older than the reference age for controls (50.1 ± 2.3) and was no significantly different between patients and controls, (P>0.05). The main characteristics of patients are summarized in Table 2. Analysis indicated that age, smoking status and family history of breast cancer were not significantly different between cases and controls. Genotyping of -656T>G and 1349T>G was done by T–ARMS-PCR method. The size of -656T>G digestion products was 384 bp for wild allele and 446 bp for polymorphic one that are shown in Figure 1. For the 1349T>G polymorphism, both alleles were represented by PCR products with sizes of 152 and 450 bp, which are provided in Figure 2 as amplified for three patients. All information about allele and genotype frequencies and associated ORs (95% CI) for cases and controls are presented in Table 3. The frequencies of the TT, TG and GG genotypes of *ApE1* -656T>G were 27.3%, 68.0% and 4.7% in controls and 38.6%, 53.4% and 8.0% in patients with breast cancer, respectively. Significant association were observed in genotypes distributions of *ApE1* -656T>G between breast cancer patients and controls (P= 0.03). In the subgroup with TG genotypes, the results suggested that the protective

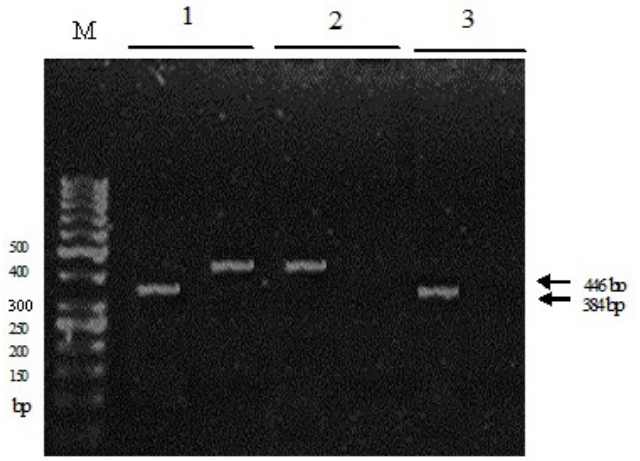

Figure 1. Agarose gel electrophoresis stained by ethidium bromide after tetra – primer amplification refractory mutation system PCR. Lanes: (1), fragments presenting T and G alleles for heterozygous patients; (2), fragments indicating the G allele for the mutant homozygous patients; (3), fragments showing the T allele for the wild type homozygous patients, M: 50 bp DNA marker

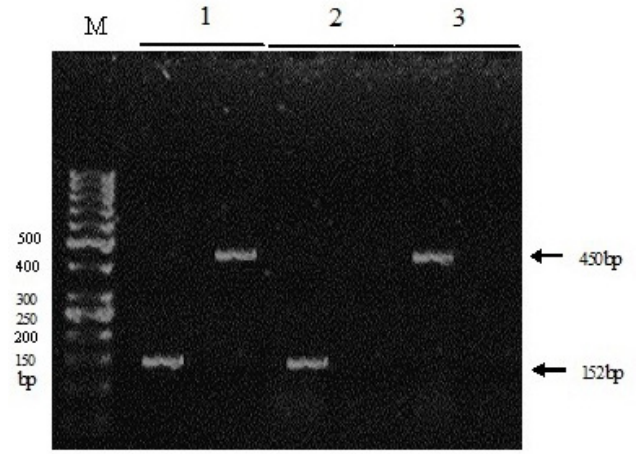

Figure 2. Detection of 1349T>G polymorphism at codon 148 by T-ARMS- PCR. The T allele is represented by fragments of 152 bp while the G allele is of 450 bp. The molecular weight marker is shown in the left part of the gel. Lanes 1, 2 and 3 show the heterozygous TG, the homozygous TT, and the homozygous GG, respectively

effect of this genotype was more apparent among others (OR= 0.55, 95% CI= 0.33 – 0.91, *P*= 0.019). Moreover, no significant differences were found in allele frequencies (*P*= 0.351). Furthermore, the frequencies of the TT, TG and GG genotypes of *ApE1* 1349T>G were 30.0%, 56.6%, 15.4% in controls, and 31.3%, 52.0%, 16.7% in patients, respectively. No significant differences were found in allele and genotype distributions of *ApE1* 1349T>G between cases and controls (*P*= 0.93 and *P*= 0.89). Moreover, the variant (GG and TG/GG) and wild type (TT and TG/TT) genotypes were evaluated that the results did not suggest any statistical evidence of an association between those and breast cancer.

Discussion

In this case-control study the role of -656T>G (rs1760944) and 1349T>G (rs1130409) variants in 150 patients and 150 cancer-free controls was investigated. The results of this research indicated that -656T>G polymorphism has a possible protective effect on breast cancer risk. Significant association were came across in genotype distributions between breast cancer cases and controls (*P*=0.03). The individuals with TG genotypes were associated with decreased breast cancer risk and the protective effect of this genotype was more predominant among other subgroups (OR= 0.55, 95% CI= 0.33 – 0.91, *P*= 0.019).

Breast cancer is the most common cancer and one of the main causes of cancer-related death all over the world that has become a major public health concern (17). The relations between breast cancer and inter-individual variations in DNA damage and repair process are explained. DNA base excision repair is the main pathway to repair DNA base damages caused by oxidation, radiation, and the loss of bases. There are several enzymes that take part in this pathway, such as DNA polymerase β, DNA ligase III and apurinic/apyrimidinic endonuclease 1 (APE1) to protect the genome entirety. The DNA repair activity of APE1 resides in the C-terminal region, that its endonuclease function makes a nick directly adjoining to 5′ of an apurinic/apyrimidinic (AP) site and produces a hydroxyl group at the 3’ terminus upstream of the nick (18). Also, *ApE1* has a role in the reductive activation of many transcription factors and this function is related with other crucial cellular processes including responses to oxidative stress, regulation of transcription factors, cell cycle control and apoptosis. The cytoplasmic expression of *APE1* is involved in redox/transcriptional regulation and *APE1*/Ref-1 is constitutively activated in cancer cells (10). These findings led us to hypothesize that functional polymorphisms within *ApE1* gene might be linked to cancer and repair process after DNA damage in breast cells.

Polymorphic variants of BER genes may alter DNA repair capacity and influence both cancer progression and clinical responses to chemotherapy and radiotherapy. It is known that 1349T>G polymorphism is the only renowned non-synonymous *ApE1* coding region variant (19). It has been shown that genetic variations can disorder *ApE1* function and play an important role in susceptibility to many kind of cancers. Luo et al demonstrated that the combined effects of polymorphisms of BER genes may contribute to tumorigenesis in a chinese population (20). Gu et al indicated the *ApE1* 1349T>G polymorphism may contribute to genetic susceptibility of cancer (21). Recently, two independent research groups have reported the association between the 1349T>G polymorphism and breast cancer risk (22,23) Zhao and colleagues found that TG genotype of *ApE1* 1349T>G was a risk factor for breast cancer susceptibility among Asian population. Also, Smith et al. detected 1349T>G was significantly associated with breast cancer risk (OR= 1.49, 95% CI= 1.03-2.00). While, in two other studies (24,25) Wu et al. and Peng et al. revealed that 1349T>G polymorphism is not associated with increased breast cancer risk (OR= 1.00, 95% CI= 0.873-1.155). Previous research findings have been inconsistent and contradictory, that the conflicting results of these reports may be due to the differences in sample sizes, and the impact of other genetic and environmental factors.

The other important SNP of *ApE1* that is located in the promoter region is -656T>G (rs1760944), which only -141bp upstream from the transcription initiation site. The promoter and 3′UTR region of a gene have

Table 2. Demographic characteristics of study population by age and breast cancer stage.

Variable	Categories	Age		P Value	Tumor stage		P Value
		Age ≥ 50 (N = 105)	Age < 50 (N = 45)		Early (N = 90)	Advanced† (N = 60)	
Age	Mean ± SD	61.3 ± 5.9	41.8 ± 3.3		58.4 ± 12.9	55.1 ± 13.2	0.13
	< 40		11 (25 %)		9 (10%)	6 (10%)	
	40-49		34 (75 %)		18 (20%)	15 (25%)	
	50-59	36 (34%)			21 (24%)	15 (25%)	
	60-69	37 (35%)			24 (26%)	10 (17%)	
	≥ 70	32 (31%)			18 (20%)	14 (23%)	0.69
Family history	None	84 (80%)	37 (82%)		71 (78.8)	48 (80%)	
	Mother and/ or sister	21 (20%)	8 (18%)	0.92	19 (21%)	12 (20%)	0.96
Smoking history	Never	60 (58%)	25 (58%)		52 (58%)	35 (58%)	
	Former	30 (29%)	12 (27%)		27 (30%)	15 (25%)	
	Current	13 (13%)	7 (15%)	0.86	11 (12%)	9 (15%)	0.76
	Missing	2	1		0	1	
Age at menarche	≤ 12	48 (48%)	19 (43%)		38 (43%)	28 (47%)	
	13-14	40 (39%)	20 (44%)		38 (43%)	21 (35%)	
	≥ 15	14 (13%)	6 (13%)	0.82	12 (14%)	10 (18%)	0.63
	Missing	3	0		2	1	
BMI	Mean ± SD	28.36 ± 5.94	27.15 ± 7.23	0.28	27.56 ± 6.18	28.81 ± 6.68	0.24
	< 25 Kg/m^2	36 (35%)	21 (48%)		37 (42%)	19 (32%)	
	25-29.9 Kg/ m^2	36 (35%)	14 (32%)		29 (33%)	20 (34%)	
	≥ 30 Kg/m^2	31 (30%)	9 (20%)	0.29	22 (25%)	20 (34%)	0.38
	Missing	2	1		2	1	
Age at first live birth	≤ 24	66 (63%)	18 (42%)		52 (58%)	34 (58%)	
	25-29	22 (21%)	8 (19%)		18 (20%)	11 (19%)	
	≥ 30	6 (6%)	7 (17%)		8 (9%)	4 (8%)	
	Nulliparous	11 (10%)	10 (22%)	0.02	11 (13%)	9 (15%)	0.92
	Missing	0	2		1	2	

† Early = Stages 0-I, Advanced = Stages II-IV.

Table 3. Allele and genotype frequencies of *ApE1* -656T>G and 1349T>G polymorphisms among cases and controls and the associations with risk of breast cancer.

		Controls (n =150)	Patients (n =150)			
		n (%)	n (%)	OR (95% CI)	P[a]	P[b]
Alleles (-656T>G)	T	184 (61.3)	196 (65.3)	1.00 (reference)	0.351	-
	G	116 (38.6)	104 (34.6)	0.84 (0.60- 1.17)	-	0.309
Genotypes (-656T>G)	TT	41 (27.3)	58 (38.6)	1.00 (reference)	0.031	-
	TG	102 (68.0)	80 (53.4)	0.55 (0.33 – 0.91)	-	0.019
	GG	7 (4.7)	12 (8.0)	1.21 (0.43 – 3.34)	-	0.710
	TT vs GG + TG	109 (72.6)	92 (61.3)	0.59 (0.36 – 0.97)	-	0.037
Alleles (1349T>G)	T	172 (58.0)	172 (57.0)	1.00 (reference)	0.934	-
	G	128 (35.0)	128 (34.3)	1.00 (0.72- 1.38)	-	1.000
Genotypes (1349T>G)	TT	45 (30.0)	47 (31.3)	1.00 (reference)	0.892	-
	TG	82 (56.6)	78 (52.0)	0.91 (0.54 - 1.52)	-	0.720
	GG	23 (15.4)	25 (16.7)	1.04 (0.51 - 2.09)	-	0.910
	TT vs GG + TG	105 (70.0)	103 (68.6)	0.93 (0.57 – 1.53)	-	0.802

[a] allele and genotype frequencies in cases and controls were compared using χ^2 test

[b] significance level for allele and genotype frequencies in cases and controls

several functions including mRNA stability, regulating transcription and gene production. Variants in the promoter region can function by modulating transcription and leading to unusual protein expression (26,27). Functional studies on -656T>G polymorphism have shown that G allele may have altered endonuclease and DNA-binding activity, reduced ability to communicate with other BER proteins (14) and increased cell cycle G_2 delay (12).

The findings of the current study are consistent with those of Huafeng Kang who found that the variant genotype of *ApE1* -656T>G was significantly associa-

ted with decreased breast cancer risk (OR= 0.71, 95% CI= 0.56-0.91), but no significant association has been shown between the 1349T>G polymorphism and breast cancer (28). Finally, some important limitations need to be considered. We only evaluated two SNP_s in the *ApE1* gene, which was inadequate to assess breast cancer risk for the gene studies. In addition, these data must be interpreted with caution because our population that was studied was not large enough.

In conclusion, our results suggests that the *ApE1* -656T>G polymorphism has a possible protective effect on breast cancer risk. We also found that 1349T>G appeared to be unrelated to the risk of breast cancer in our population. Moreover, future studies with larger numbers of patients and controls are required to confirm our results.

Acknowledgments

This study was supported financially by the University of Guilan.

References

1. Jemal, A., Bray, F., Center, M., Ferlay, J., Ward, E., Forman, D., Global cancer statistics. *CA cancer J. Clin.* 2011, **61(2):** 69-90. doi: 10.3322/caac.20107.

2. Lichtenstein, P., Holm, N.V., Verkasalo, P.K., Iliadou, A., Kaprio, J., Koskenvuo, M., Pukkala, E., Skytthe, A., Hemminki, K., Environmental and heritable factors in the causation of cancer-analyses of cohorts of twins from Sweden, Denmark, and Finland. *N. Engl. J. Med.* 2000, **343(2):** 78-85. doi: 10.1056/NEJM200007133430201.

3. Johnson-Thompson, M.C., Guthrie, J., Ongoing research to identify environmental risk factors in breast carcinoma. *Cancer.* 2000, **88:** 1224–1229.

4. Tyrer, J., Duffy, S.W., Cuzick, J., A breast cancer prediction model incorporating familial and personal risk factors. *Stat. Med.* 2004, **23:** 1111–1130. doi: 10.1002/sim.1668.

5. Goode, E.L., Ulrich, C.M., Potter, J.D., Polymorphisms in DNA repair genes and associations with cancer risk. *Cancer Epidemiol Biomarkers Prev.* 2002, **11:** 1513–1530.

6. Benhamou, S., Sarasin, A., ERCC2/XPD gene polymorphisms and lung cancer: a HuGE review. *Am J Epidemiol.* 2005, **161:** 1– 14. doi: 10.1093/aje/kwi018.

7. Wood, R.D., Mitchell, M., Sgouros, J., Lindahl, T., Human DNA repair genes. Science. 2001, **291:** 1284–1289. doi: *10.1126/science.1056154.*

8. Izumi, T., Hazra, T.K., Boldogh, I., Tomkinson, A.E., Park, M.S., Ikeda, S., Mitra, S., Requirement for human AP endonuclease 1 for repair of 3′-blocking damage at DNA single-strand breaks induced by reactive oxygen species. *Carcinogenesis.* 2000, **21:** 1329–1334. doi: 10.1093/carcin/21.7.1329.

9. Kelley, M.R., Cheng, L., Foster, R., Tritt, R., Jiang, J., Broshears, J., Koch, M., Elevated and altered expression of the multifunctional DNA base excision repair and redox enzyme Ape1/ref-1 in prostate cancer. *Clin Cancer Res.* 2001, **7:** 824–830.

10. Evans, A.R., Limp-Foster, M., Kelley, M. R., Going APE over Ref-1. *Mutation Research*. 2000, **461(2):** 83–108.

11. Spitz, M.R., Wu, X., Wang, Y., Wang, L.E., Shete, S., Amos, C.I., Guo, Z., Lei, L., Mohrenweiser, H., Wei, Q., Modulation of nucleotide excision repair capacity by XPD polymorphisms in lung cancer patients. *Cancer Res.* 2001, **61:** 1354–1357.

12. Hu, J.J., Smith, T.R., Miller, M.S., Mohrenweiser, H.W., Golden, A., Case, L.D., Amino acid substitution variants of APE1 and XRCC1 genes associated with ionizing radiation sensitivity. *Carcinogenesis.* 2001, **22:** 917–922. doi: 10.1093/carcin/22.6.917.

13. Xi, T., Jones, I.M., Mohrenweiser, H.W., Many amino acid substitution variants identified in DNA repair genes during human population screenings are predicted to impact protein function. *Genomics.* 2004, **83:** 970–979. doi:10.1016/j.ygeno.2003.12.016.

14. Hadi, M.Z., Coleman, M.A., Fidelis, K., Mohrenweiser, H.W., Wilson, D.M., Functional characterization of Ape1 variants identified in the human population. *Nucleic Acids Res.* 2000, **28:** 3871–3879.

15. Zhou, B., Shan, H., Su, Y., Xia, K., Shao, X., Mao, W., Shao, Q., The association of APE1 −656T > G and 1349 T > G polymorphisms and cancer risk: a meta-analysis based on 37 case-control studies. *BMC Cancer.* 2011, **11:** 521. doi:10.1186/1471-2407-11-521.

16. Hillman, G.G., Singh-Gupta, V., Soy isoflavones sensitize cancer cells to radiotherapy. *Free Radical Biology and Medicine*. 2011, **51(2):** 289–298. doi: 10.1016/j.freeradbiomed.2011.04.039.

17. Parkin, D.M., Bray, F., Ferlay, J., Pisani, P., Global cancer statistics. *CA Cancer J Clin.* 2005, **55:** 74–108.

18. Chou, K.M., Cheng, Y.C., The Exonuclease Activity of Human Apurinic/Apyrimidinic Endonuclease (APE1). *THE JOURNAL OF BIOLOGICAL CHEMISTRY.* 2003, **276:** 18289–18296. doi: 10.1074/jbc.M212143200.

19. Liu, C., Yin, Q., Li, L., Zhuang, Y.Z., Zu, X., Wang, Y., ApE1 Asp148Glu gene polymorphism and bladder cancer risk. *Mol Biol Rep.* 2013, **40:** 171-176. doi: 10.1007/s11033-012-2046-5.

20. Luo, H., Li, Z., Qing, Y., Zhang, S.H., Peng, Y., Li, Q., Wang, D., Single Nucleotide Polymorphisms of DNA Base-excision Repair Genes (APE1, OGG1 and XRCC1) Associated with Breast Cancer Risk in a Chinese Population. *Asian Pac J Cancer Prev.* 2014, **15 (3):** 1133-1140.

21. Gu, D., Wang, M., Wang, S., Zhang, Z., Chen, J., The DNA repair gene APE1 T1349G polymorphism and cancer risk. *Mutagenesis.* 2009, **24:** 507–512. doi: 10.1093/mutage/gep036.

22. Zhao, Z., Liu, C., Zeng, Y., Gu, L., Ying, M., Wang, N., Hao, B., Yao, H., Su, C., Wang, Y., Ma, Y., The association between the APE1 Asp148Glu polymorphism and breast cancer susceptibility: a meta-analysis based on case–control studies. *Tumor Biol.* 2014, doi: 10.1007/s13277-014-1618-5.

23. Smith, T.R., Levine, E.A., Freimanis, R.I., Akman, S.A., Allen, G.O., Hoang, K.N., Liu-Mares, W., Hu, J.J., Polygenic model of DNA repair genetic polymorphisms in human breast cancer risk. *Carcinogenesis.* 2008, **29:** 2132–2138. doi: 10.1093/carcin/bgn193.

24. Wu, B., Liu, H.L., Zhang, S., Dong, X.R., Wu, G., Lack of an Association between Two BER Gene Polymorphisms and Breast Cancer Risk. *Plose One.* 2012, **7(12):** e50857. doi: 10.1371/journal.pone.0050857.

25. Peng, Q., Lu, Y., Lao, X., Chen, Z., Li, R., Sui, J., Qin, X., Li, S., Association between OGG1 Ser326Cys and APEX1 Asp148Glu polymorphisms and breast cancer risk: a meta-analysis. *Diagnostic Pathology.* 2014, **9:** 108. doi: 10.1186/1746-1596-9-108.

26. De Gobbi, M., Viprakasit, V., Hughes, J.R., Fisher, C., Buckle, V.J., Ayyub, H., Gibbons, R.J., Vernimmen, D., Yoshinaga, Y., de Jong, P., Cheng, J.F., Rubin, E.M., Wood, W.G., Bowden, D., Higgs, D.R., A regulatory SNP causes a human genetic disease by creating a new transcriptional promoter. *Science.* 2006, **312:** 1215-1217. doi:10.1126/science.1126431.

27. Buckland, P.R., The importance and identification of regulatory polymorphisms and their mechanisms of action. *Biochim Biophys Acta.* 2006, **1762:** 17-28. doi:10.1016/j.bbadis.2005.10.004.

28. Kang, H., Dai, Z., Ma, X., Ma, L., Jin, Y., Liu, X., Wang, X., A genetic variant in the promoter of APE1 gene (−656 TNG) is associated with breast cancer risk and progression in a Chinese population. *GENE.* 2013, **38953:** 4C. doi: 10.1016/j.gene.2013.08.052.