Association of the nibrin gene (*NBN*) variants with breast cancer

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Abstract. Nibrin, encoded by the *NBN* gene, participates in DNA repair. Mutations in the *NBN* gene lead to Nijemen breakage syndrome, which may result in several types of diseases, particularly susceptibility to cancer, including breast cancer. Polymorphic variants and defective mutations occurring in the *NBN* gene increase the risk of breast cancer through the double-stranded break repair mechanism. The aim of the present study was to investigate a possible association between breast cancer and *NBN* genetic variants, *NBN* 924 T>C, 8360 G>C and 30537 G>C, in women with breast cancer. Locus-specific primers were designed to study 3 genetic variants in DNA samples isolated from peripheral blood samples of 101 women with breast cancer and 115 healthy controls. Subsequently, 3 polymerase chain reaction-restriction fragment length polymorphism methods were performed and the obtained results were statistically analysed. The *NBN* gene 924 T>C variant was found to be significantly associated with breast cancer (χ^2=5.722, P=0.017). There were no statistically significant differences between cases and controls in the *NBN* gene 8360 G>C variant (χ^2=1,125, P=0.570) or the *NBN* gene 30537 G>C variant (χ^2=4.301, P=0.116). In conclusion, the *NBN* gene 924 T>C variant may be a genetic risk factor for breast cancer development in women with breast cancer.

Introduction

Breast cancer is the most frequent type of cancer and the second most common cause of mortality from cancer in women. In addition, it is the first cause of fatality in women between the ages of 40-59 years. In the USA, the estimated number of new cases for breast cancer per year is ~230,000, and additionally, it is predicted that <40,000 of these will succumb from this disease. During a lifetime, the breast cancer risk is 1/8 (1-3).

Epidemiological studies have revealed that genetic, epigenetic and environmental factors may have a role in the aetiology of breast cancer. Although genetic factors are particularly critical in the familial breast cancer, genetic and epigenetic, and environmental factors may be responsible for sporadic breast cancer. Thus, it is beneficial to focus on the identification of genetic factors causing breast cancer to illustrate the primary basis behind the disease.

In addition to the familial breast cancer development with genes such as *BRCA1/2*, *TP53* and *ATM*, importance of the genetic factors, particularly affecting the DNA repair system, have been shown in certain studies in which a significant association was identified between insufficient DNA repair and breast cancer susceptibility (1,4). Ionizing radiation and free radicals may damage DNA causing DNA double-strand breaks (DSBs), leading to genomic instability, thus increasing the risk of developing breast cancer (5).

In humans, DSBs are repaired by homologous recombination and non-homologous end-joining (NHEJ). During the initial step of NHEJ, the MRN protein complex, including meiotic recombination 11 homolog 1, RAD50 and nibrin (NBN) recognize DSBs. The main regulator of this complex is NBN, which supports enzymatic activity of other proteins in the complex and recruits them to the DNA damage site in the early stages (4,5). NBN also forms the nuclear focal point and induces cell-cycle checkpoint to provide genomic stability (4).

NBN is encoded by the *NBN* gene, located on chromosome 8q21. Mutations in the *NBN* gene lead to autosomal recessive disorder such as Nijemen breakage syndrome (NBS), which is a radiation-sensitivity disorder that may result in microcephaly, growth retardation, immunodeficiency, and in particular, susceptibility to cancer (4,6,7). The majority of fatalities originating from the *NBN* gene are due to cancer development (4). In addition, studies on the *NBN* gene suggest that polymorphic variants and defective mutations occurring in this gene increase the risk of breast cancer through the DSB repair mechanism (8-10).

The frequency of the *NBN* gene polymorphisms varies among populations. According to the HapMap database, for the *NBN* gene 924 T>C variant, small differences in terms of allele frequencies are observed between European populations and Japanese and Chinese populations (C allele frequency: 0.100 and 0.150, respectively) (11).

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By contrast, the difference of the allele frequencies between European and Asian populations for the *NBN* gene 8360 G>C variant decreases from 0.699 to 0.550 for the C allele, respectively. Additionally, the C allele frequency as 0.813 tends to be greater in Sub-Saharan Africans (12). Furthermore, for the *NBN* gene 30537 G>C variant, the C allele appears to be more frequent for all populations in the database with nearly a 1.000 frequency, except for European populations with a 0.642 frequency (13) (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=1805787).

Apart from these genetic variants, other polymorphisms were identified to differ in the allele frequencies among populations. For instance, the *NBN* gene 553 G>C variant, which is essential for relocalization of the MRN complex, may lead to cancer. The *NBN* gene 553 C allele was frequent in Slovenian populations and Caucasian populations. Conversely, the *NBN* gene 553 G allele was identified more frequently in Polish, Hungarian, Romanian, Slovakian and Czech populations (14). Furthermore, Lu *et al* (15) reported that the C allele of the *NBN* gene 8360 G>C variant in breast cancer patients has a greater frequency in Chinese compared to Caucasians residing in North Carolina. Even the type of disease-causing variations change between populations. Smith *et al* (16) reported that although the *NBN* gene 8360 G>C variant was significant in leading to the breast cancer risk in Caucasians, it was not identified as significant in African-Americans.

The location of genetic variants, *NBN* gene 924 T>C, 8360 G>C and 30537 G>C, is important in the *NBN* gene function. Bioinformatically, it has been shown that the *NBN* gene 924 T>C variant is found at the 5' untranslated region of the *NBN* gene where the transcription factor GATA-1 binds. The activation domains of GATA-1 are responsible for transcription activation in mammalian cells through the GATA motifs. The *NBN* gene 924 C variant results in the loss of the GATA-1 binding site, thus affecting *NBN* gene expression. The *NBN* gene 8360 G>C variant is found at the coding region of NBN gene C-terminal domain that eases the interaction between NBN and breast cancer 1 (BRCA1), early onset. This interaction provides the formation of the BRCA1-associated genome surveillance complex, which has a role in the recognition and repair of damaged DNA. Thus, the *NBN* gene 8360 G>C variant, which results in amino acid change, possibly disturbs the interaction between proteins, thus leading to an increased risk of breast cancer (4). A study by Mavaddat *et al* (17) in which 4,500 breast cancer cases and controls were used, suggested that the *NBN* gene 30537 G>C variant was significantly associated with breast cancer (P<0.0065).

In the present study, whether there were a possible association between the *NBN* gene 924 T>C, 8360 G>C and 30537 G>C variants and breast cancer was investigated in a case-control study of 101 breast cancer patients and 115 well-matched healthy controls from a Turkish population.

Materials and methods

Subjects. Between October 2010 and October 2014, histopathologically confirmed, unrelated primary female breast cancer patients who registered at the University of Kocaeli Hospital General Surgery Clinic (Kocaeli, Turkey) were recruited. The subjects were limited to women of ~52.71±10.5 years of age and were of Caucasian origin (Table I). A total of 101 breast cancer cases were recruited, consisting of 89 invasive ductal, 9 invasive lobular, 1 mixed and 2 Paget's carcinoma *in situ* carcinomas (Table II). For the staging classifications, there were 59 cases of early stage and 42 locally advanced stage (Table II). The study population consisted of 101 unrelated consecutive breast cancer patients and 115 age-matched controls (52.03±9.9 years). The study was approved by the Institutional Review Board, and written informed consent was obtained from all the subjects. Additional information on the menopausal status, age at first full-term pregnancy, number of live births, weight, height and education level, and use of hormone replacement or oral contraceptives were available for the subjects. Each subject was requested to donate 10 ml of blood following obtaining the informed concent.

Table I. Demographic data of the cases and controls.

Demographics	Cases	Controls
Mean age ± SD, year	52.71±10.5	52.03±9.9
Gender, n (women/men)	101/0	115/0

SD, standard deviation.

Genotyping. Genomic DNA was isolated with a conventional salting-out method from the blood (18). Genotypes of the subjects were determined using the polymerase chain reaction (PCR)-restriction fragment length polymorphism method, as described by Lu *et al* (19). The PCR cycling conditions for the *NBN* 924 T>C (rs13312840) variant were as follows: Briefly, genomic DNA was denatured at 95˚C for 5 min followed by 35 cycles at 95˚C for 30 sec, 61˚C for 45 sec, 72˚C for 50 sec and with a final extension step of 72˚C for 10 min. The digestion of the amplified 141-base pair (bp) fragment with the *Bcl*I restriction endonuclease was performed at 55˚C overnight. The *NBN* 924 T>C polymorphism had 121- and 20-bp fragments for the TT genotype, 141-bp for the CC genotype, and 141-, 121- and 20-bp for the CT genotype. The PCR cycling conditions for the 8360 G>C (rs1805794) variant were as follows: Briefly, genomic DNA was denatured at 94˚C for 5 min followed by 35 cycles at 95˚C for 45 sec, 54˚C for 45 sec, 72˚C for 1 min and with a final extension step of 72˚C for 10 min. The digestion of the amplified 174-bp fragment with the *Hin*fI restriction endonuclease was carried out at 37˚C overnight. The *NBN* 8360 G>C polymorphism had 2 fragments of 125- and 49-bp for the GG genotype, one fragment of 174-bp for the CC genotype and three fragments of 174-, 125- and 49-bp for the GC genotype. The PCR cycling conditions for the 30537 G>C (rs1805787) variant were as follows: Briefly, genomic DNA was denatured at 95˚C for 5 min followed by 35 cycles at 95˚C for 30 sec, 61˚C for 45 sec, 72˚C for 50 sec and with a final extension step of 72˚C for 10 min. The digestion of the amplified 197-bp fragment with the *Ear*I restriction endonuclease was carried out at 37˚C overnight. The *NBN* 30537 G>C polymorphism had one fragment of 197-bp for the GG genotype, and 2 fragments of 172- and 25-bp for the CC genotype, and 3 fragments

Table II. Clinical features of the breast cancer patients.

Clinical features	Patients, n
Breast cancer types	
Invasive ductal	89
Invasive lobular	9
Mixed type	1
Paget's carcinoma in situ	2
Surgery technique	
Modified radical mastectomy	72
Breast conserving surgery	29
Family history	
Present	16
Absent	85
Stages	
Early stage	59
Locally advanced	42
Metastatic	0

of 197-, 172- and 25-bp for the GC genotype. Subsequently the digested fragments were run on a 10% PAGE for 30 min at 20 watts, followed by silver staining and scanning (20). All the genotyping was performed using the procedure presented before without any failure.

Statistical analysis. χ^2 tests were used to compare the distribution of demographic variables, genotypes and alleles. Unconditional logistical regression analysis was used to calculate the odds ratios (ORs) and 95% confidence intervals (CIs) without adjustment for genotypes or demographics. The logistic regression analysis was also used for the trend test. Crude ORs are presented. All the statistical tests were two-sided, and $P<0.05$ was considered to indicate a statistically significant difference, assessed using SPSS software (version 21; IBM Corp., Armonk, NY, USA).

Results

Characteristics. In total, 216 subjects, 101 women with breast cancer and 115 healthy women as the controls, participated in the study. The mean age was 52 years. There was no statistically significant difference for the age between the cases and controls. Histopathologically, invasive lobular carcinoma was diagnosed in 9 of the patients, Paget's carcinoma in 2, mixed invasive carcinoma in 1 and invasive ductal carcinoma in 89. Modified radical mastectomy (72 patients) and breast conserving surgery (29 patients) were applied to patients as the surgery techniques. All the patients were in the early and locally advanced stages, and patients with metastatic breast carcinoma were not included in the study. There was a family history of breast cancer in 16 patients. Family history of breast cancer was present in 1 first-degree relative of 5 patients; 1 second-degree relative of 9 patients and both first and second-degree relative of 2 patients (Table II). To obtain a statistical power of 0.80, 318 samples should have been collected; however, only 101 patients were recruited.

NBN genetic variants for genotype distribution and breast cancer risk. Genotypes and alleles of *NBN* 924 T>C, 8360 G>C and 30537 G>C polymorphisms were compared between the cases and controls. The genotype frequencies of these 3 regions were in Hardy-Weinberg equilibrium (P=1.00, 0.145 and 0.139, respectively) (Table III).

NBN gene 924 T>C variant genotype frequencies in the cases and controls. The *NBN* gene 924 TT genotype was compared with the homozygote 924 CC carriers and heterozygote 924 TC carriers. The *NBN* 924 T>C variant was associated with breast cancer (χ^2=5.722, P=0.017). The *NBN* 924 TT genotype was present in 92 of the cases (91.1%) and 113 of the control group (98.3%). The *NBN* gene 924 TC genotype was present in 9 of the cases (8.9%) and 2 of the control groups (1.7%). The *NBN* 924 CC genotype was not observed in the cases or the controls (0.0%). The *NBN* gene 924 C allele was 0.045% in the cases, and 0.009% in the controls. The *NBN* gene 924 T>C variant was associated with the breast cancer risk (χ^2=5.722, P=0.017). The individuals with the *NBN* 924 TT genotype showed protection against breast cancer (χ^2=5.722; P=0.017; OR=0.181, 95% CI: 0.038-0.858). By contrast, the individuals with the *NBN* 924 TC genotype revealed a 5.5-fold increased risk for breast cancer (χ^2=5.522; P=0.017; OR=5.527, 95% CI: 1.165-26.216). The individuals with the *NBN* 924 C allele conferred a 5.5-fold increased risk for breast cancer (Table III).

NBN gene 8360 G>C variant genotype frequencies in the cases and controls. The genotype frequencies of the *NBN* gene 8360 G>C polymorphism were 42.6% GG, 38.6% GC and 18.8% CC in the cases and 47.8% GG, 38.3% GC and 13.9% CC in the controls. There was no statistically significant difference between the cases and controls (χ^2=1,125, P=0.570). The *NBN* C allele was 0.38% in the cases, and 0.33% in the controls, and it was not statistically significant (P=0.439) (Table III).

NBN gene 30537 G>C variant genotype frequencies in cases and controls. The genotype frequencies of the *NBN* gene 30537 G>C variant were 59.4% GG, 32.7% GC and 7.9% CC in the cases, and 52.2% GG, 44.3% GC and 3.5% CC in the controls. The *NBN* gene 30537 C allele was 0.24% in the cases, and 0.26% in the controls, which was not statistically significant (P=0.286). Consequently the *NBN* gene 30537 G>C variant was not associated with breast cancer (χ^2=4.301, P=0.116) (Table III).

Discussion

Understanding the breast cancer risk factors will allow the identification of women at a high risk and to intervene to change the risk in time. The *BRCA1* and *BRCA2* genes are associated with familial breast cancer. However, *BRCA1/2* mutations do not indicate a 100% risk for developing breast cancer. Penetrance of these mutations changes from 40 to 90%. Additionally, all the genetic information responsible for breast cancer has not been disclosed yet. In particular, the susceptible genes have not been defined in detail to determine the environmental and personal factors that interact with them. Increased knowledge on these issues will require the creation of information and consulting services on individuals who are first at risk.

Table III. *NBN* genotypes and allele frequencies and logistic regression analysis for the association with breast cancer risk.

Genotypes	Cases, n (%)	Controls, n (%)	χ^2	P-value	Crude OR (95% CI)
Subjects, no.	101 (100.0)	115 (100.0)			
Alleles, no.	202	230			
NBN 924 T>C	101 (100.0)	115 (100.0)	5.722	0.017	
TT	92 (91.1)	113 (98.3)	5.722	0.017	0.181 (0.038-0.858)
TC	9 (8.9)	2 (1.7)	5.722	0.017	5.527 (1.165-26.216)
CC	0 (0.0)	0 (0.0)	-	-	-
C allele	9 (0.045)	2 (0.009)	5.722	0.017	
HWE (exact)	1.00	1.00			
SP	0.35	0.39			
NBN 8360 G>C	101 (100.0)	115 (100.0)	1.125	0.570	
GG	43 (42.6)	55 (47.8)	0.598	0.439	0.809 (0.472-1.385)
GC	39 (38.6)	44 (38.3)	0.003	0.958	1.015 (0.586-1.758)
CC	19 (18.8)	16 (13.9)	0.950	0.330	1.434 (0.722-2.117)
C allele	77 (0.4)	76 (0.3)	0.598	0.439	
HWE (exact)	0.090	0.145			
NBN 30537 G>C	101 (100.0)	115 (100.0)	4.301	0.116	
GG	60 (59.4)	60 (52.2)	1.139	0.286	1.341 (0.782-2.302)
GC	33 (32.7)	51 (44.3)	3.084	0.079	0.609 (0.350-1.061)
CC	8 (7.9)	4 (3.5)	2.023	0.155	2.387 (0.697-8.179)
C allele	49 (0.2)	59 (0.3)	1.139	0.286	
HWE (exact)	0.280	0.139			

NBN, nibrin; HWE, Hardy-Weinberg equilibrium; SP, statistical power; OR, odds ratio; CI, confidence interval.

The *NBN* gene generating an intermediate risk in the breast cancer etiology was studied and identified as statistically significant for certain populations. When DNA DSBs cannot be repaired, it can cause genomic instability and cancer. The NBN protein is one of the key proteins involved in repair of DSBs (6).

Effective variants of the *NBN* gene on the breast cancer risk remain to be elucidated. At the same time, studies have revealed an increased spontaneous chromosomal instability in the cells of *NBN* heterozygote carriers (21).

The hypothesis that *NBN* was associated with breast cancer, particularly in young women (≤55 years), was confirmed by previous case-control studies (4,22,23). Additionally, certain studies have indicated that genetic variants and haplotypes of the *NBN* gene may be associated with breast cancer in young non-Hispanic Caucasian women (24).

Based on these observations, the 924 T>C, 8360 G>C and 30537 G>C *NBN* genetic variants were compared for the first time in a Turkish population between breast cancer patients and healthy controls with the hypothesis that NBN genetic variants were associated with breast cancer.

In this case-control study, the *NBN* gene 924 T>C variant was associated with breast cancer (χ^2=5.722, P=0.017). This region is located in the binding side of the GATA-1 transcription factor that may be important for cancer development. Results supporting breast cancer susceptibility were obtained in variant studies of promoter region. *NBN* variations were present in high-risk breast cancer cases, even though they were identified at a low frequency (4). In the present study, 6 of 8 breast cancer patients, who were heterozygote in the *NBN* gene 924 T>C variant, were in the locally advanced stage and 2 were in the early stage. Patients who were in the advanced stage with the *NBN* gene 924 TC genotype suggested that this variant was associated with the more advanced stage and poor prognosis in breast cancer. However, a more ample patient population is required to make a definite conclusion. For precise and clear results, large patient populations are required for further studies.

The 8360 G>C variant is one of the most studied genetic variants of the *NBN* gene and the results obtained with this variant in different populations are contradictory (15). In the present study, this region was not associated with breast cancer (χ^2=1.125, P=0.570). However, in previous study, a meta-analysis of 10 case-control studies on the genetic variants of this region shows that it was associated with breast cancer (15). Furthermore, other meta-analysis studies suggested that the *NBN* gene 8360 G>C variant genotype (8360 GC/CC) exhibited an increase in breast cancer, particularly in Caucasian patients (11). In the present study, the *NBN* gene 8360 CC genotype was identified in 18.8% of the cases and 13.9% of the controls. However, it was not identified to be significant, and the frequency of this genotype was more frequent in the cases. Therefore, it should be studied in larger populations. In total, 9 of the *NBN* gene 8360 CC genotypes were identified in the cases with the locally advanced stage and 10 of them were in the early stages of breast cancer. The similar number of patients with the *NBN* gene 8360 CC genotype in the advanced stage and in the early stage suggested that this genetic variant was not associated with staging.

The third studied region in the *NBN* gene, the 30537 G>C variant, was not associated with breast cancer (χ^2=4.301, P=0.116). The *NBN* 30537 CC genotype was identified in 7.9% of the cases and 3.5% of the controls. In total, 2 of these genotypes were in the locally advanced stage and 6 were in the early stage of breast cancer. The *NBN* gene 30537 CC genotype may be associated with the early stage as it was identified more frequently in patients with the early stage of breast cancer. However, as with the other studied regions, it is difficult to deduce a definite conclusion due to the small population size.

Cancer develops in ~40% of *NBN* patients with NBS who are homozygous for pathogenic *NBN* mutations before the age of 21 years (11). In addition, *NBN* variants identified under the age of 55 years suggest that this gene may be important in terms of breast cancer risk for younger patient groups. The average age in the present patient group was 52 years. The *NBN* genetic variants were observed in 2 patients with Paget's carcinoma diagnosed *in situ* and the age of these 2 patients was 36 years. Consequently, cases with breast cancer at the early age should be checked for *NBN* genetic variants.

The limitation of the present study was the insufficient sample size. Although there were tendencies detected in the *NBN* gene 8360 CC and 30537 CC genotypes towards an association, the results were statistically insignificant. Therefore, they should be studied in larger populations. More homogenous groups, such as Caucasian, Hispanics or African-Americans, have been investigated in previous studies. The present study investigated cases that were hospital-based and may not represent the general population as a whole.

To the best of our knowledge, the present study investigated these genetic variants for the first time in a Turkish population. The *NBN* gene 924 T>C or 30537 G>C variants and their association with cancer risk have been examined in only one study previously (4). Therefore, this study has contributed to the literature regarding breast cancer.

Family history was not observed in any patients with the *NBN* gene 924 TC and 30537 CC genotypes; however, 2 of 19 patients with the *NBN* gene 8360 CC genotype had a family history. Therefore, these results clearly indicate that these genetic variants were effective on sporadic cases rather than familial cases.

In conclusion, the present retrospective case-control study identified, for the first time, that the *NBN* gene 924 T>C variant was a genetic risk factor for breast cancer in the Turkish population studied. Therefore, this *NBN* variant may contribute to breast cancer particularly in young women. Further studies should be carried out in larger populations.

References

1. Ergul E and Sazci A: Molecular genetics of breast cancer. Turk J Med Sci 31: 1-14, 2001.
2. Ergul E, Sazci A, Utkan Z and Canturk NZ: Polymorphisms in the MTHFR gene are associated with breast cancer. Tumour Biol 24: 286-290, 2003.
3. Siegel R, Naishadham D and Jemal A: Cancer statistics, 2012. CA Cancer J Clin 62: 10-29, 2012.
4. Lu J, Wei Q, Bondy ML, Yu TK, Li D, Brewster A, Shete S, Sahin A, Meric-Bernstam F and Wang LE: Promoter polymorphism (-786t>C) in the endothelial nitric oxide synthase gene is associated with risk of sporadic breast cancer in non-Hispanic white women age younger than 55 years. Cancer 107: 2245-2253, 2006.
5. Wang Z, Cui D and Lu W: NBS1 8360G > C polymorphism is associated with breast cancer risk: A meta-analysis. Breast Cancer Res Treat 123: 557-561, 2010.
6. Bogdanova N, Feshchenko S, Schürmann P, Waltes R, Wieland B, Hillemanns P, Rogov YI, Dammann O, Bremer M, Karstens JH, *et al*: Nijmegen Breakage Syndrome mutations and risk of breast cancer. Int J Cancer 122: 802-806, 2008.
7. di Masi A and Antoccia A: NBS1 heterozygosity and cancer risk. Curr Genomics 9: 275-281, 2008.
8. Tessitore A, Biordi L, Flati V, Toniato E, Marchetti P, Ricevuto E, Ficorella C, Scotto L, Giannini G, Frati L, *et al*: New mutations and protein variants of NBS1 are identified in cancer cell lines. Genes Chromosomes Cancer 36: 198-204, 2003.
9. Hsu HMWH, Wang HC, Chen ST, Hsu GC, Shen CY and Yu JC: Breast cancer risk is associated with the genes encoding the DNA double-strand break repair Mre11/Rad50/Nbs1 complex. Cancer Epidemiol Biomarkers Prev 16: 2024-2032, 2007.
10. Damiola F, Pertesi M, Oliver J, Le Calvez-Kelm F, Voegele C, Young EL, Robinot N, Forey N, Durand G, Vallée MP, *et al*: Rare key functional domain missense substitutions in MRE11A, RAD50, and NBN contribute to breast cancer susceptibility: Results from a breast cancer family registry case-control mutation-screening study. Breast Cancer Res 16: R58, 2014.
11. The International Nijmegen Breakage Syndrome Study Group: Nijmegen breakage syndrome. Arch Dis Child 82: 400-406, 2000.
12. International HapMap Consortium: The International HapMap Project. Nature 426: 789-796, 2003.
13. Reference SNP (refSNP) Cluster Report: rs1805787. http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=1805787. Accessed July 6, 2015.
14. Goricar K, Erculj N, Zadel M and Dolzan V: Genetic polymorphisms in homologous recombination repair genes in healthy Slovenian population and their influence on DNA damage. Radiol Oncol 46: 46-53, 2012.
15. Lu M, Lu J, Yang X, Yang M, Tan H, Yun B and Shi L: Association between the NBS1 E185Q polymorphism and cancer risk: A meta-analysis. BMC Cancer 9: 124, 2009.
16. Smith TR, Levine EA, Freimanis RI, Akman SA, Allen GO, Hoang KN, Liu-Mares W and Hu JJ: Polygenic model of DNA repair genetic polymorphisms in human breast cancer risk. Carcinogenesis 29: 2132-2138, 2008.
17. Mavaddat N, Dunning AM, Ponder BA, Easton DF and Pharoah PD: Common genetic variation in candidate genes and susceptibility to subtypes of breast cancer. Cancer Epidemiol Biomarkers Prev 18: 255-259, 2009.
18. Miller SA, Dykes DD and Polesky HF: A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16: 1215, 1988.
19. Lu J, Wei Q, Bondy ML, Li D, Brewster A, Shete S, Yu TK, Sahin A, Meric-Bernstam F, Hunt KK, Singletary SE, Ross MI and Wang LE: Polymorphisms and haplotypes of the NBS1 gene are associated with risk of sporadic breast cancer in non-Hispanic white women <or=55 years. Carcinogenesis 27: 2209-2216, 2006.
20. Ergul E, Ozel MD, Sazci A and Idrisoglu HA: TP53 Arg72Pro polymorphism in Turkish patients with sporadic amyotrophic lateral sclerosis. Neurobiol Aging 32: 2107.e2101-2102, 2011.
21. Heikkinen K, Rapakko K, Karppinen SM, Erkko H, Knuutila S, Lundán T, Mannermaa A, Børresen-Dale AL, Borg A, Barkardottir RB, *et al*: RAD50 and NBS1 are breast cancer susceptibility genes associated with genomic instability. Carcinogenesis 27: 1593-1599, 2006.
22. Xiong P, Bondy ML, Li D, Shen H, Wang LE, Singletary SE, Spitz MR and Wei Q: Sensitivity to benzo(a)pyrene diol-epoxide associated with risk of breast cancer in young women and modulation by glutathione S-transferase polymorphisms: A case-control study. Cancer Res 61: 8465-8469, 2001.
23. Shi Q, Wang LE, Bondy ML, Brewster A, Singletary SE and Wei Q: Reduced DNA repair of benzo[a]pyrene diol epoxide-induced adducts and common XPD polymorphisms in breast cancer patients. Carcinogenesis 25: 1695-1700, 2004.
24. Desjardins S, Beauparlant JC, Labrie Y, Ouellette G and Durocher F; INHERIT BRCAs: Variations in the NBN/NBS1 gene and the risk of breast cancer in non-BRCA1/2 French Canadian families with high risk of breast cancer. BMC Cancer 9: 181, 2009.