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# Molecular study of *BRCA*-1,2 and *P53* gene polymorphisms among post-operative breast cancer patients

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ABSTRACT

Breast cancer is a malignant tumor i group of cancer cells that may develop into (invade) or spread to distant body regions around tissues. In both advanced and developing nations and in many parts of the globe, the burden of breast cancer is rising. It's the most prevalent malicious person illness in females, with 18% of all female cancers and the third most prevalent cause of cancer death globally. This case-control study was organized to explore the potential role of chosen genetic parameters in the Al-Diwanyia province in random samples of breast cancer patients the research, 5 ml of blood samples from 50 women with post-operative breast cancer attending the outpatient oncology department at Al Diwaniyia Teaching Hospital were employed compared to 50 women without cancer, patient ages and control ranged from 18 to 80 years. Among the three susceptibility genes studied, BRCA In BRCA-1 GG genotype evidently proposed a risk factor for tumor as had an (OR 5.3191) and risk factor (EF 0.065); AG & AA genotypes, on the other hand, played a rather preventive part as they had no risk factor (PF) of 0.0476 & 0.1667 respectively and low OR (0.7619 & 0.7917 respectively) and patients had 16%, and 84% of patients had G and A alleles respectively. The genotype of BRCA-2 AG As had the risk factor (OR 13.4146) and the risk factor (EF 0.1851), the AA genotype, on the other hand, did not have a risk factor role since it had a protective fraction (PF) of 0.9103 and a low OR (0.0731). The GC genotype, on the other hand, did not have a risk factor as it had (PF) of 0.087 and low OR (0.4565) and patients had 56 percent of G allele and 44 percent of C allele compared to 52 percent of G and 48 percent of C control.

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## INTRODUCTION

Breast cancer is a malignant tumor that begins with breast cells. A malignant tumor is a group of cancer cells that may develop into (invade) surrounding tissues or spread (metastasize) to remote body regions. The disease happens almost completely in females, but it is also possible for males to get it (Bray *et al.*, 2013). In both advanced and developing nations, and in many areas, the burden of breast cancer is rising Malignant illness commonly occurring in females; comprising 18% of all female cancers and the fifth most prevalent cause of cancer death globally. Around 1.4 million females glob-

ally were diagnosed with breast cancer in 2008, with an associated death rate of 460,000 (Ferlay, 2010). These risk factors, though, Different breast cancer relationships have been shown in different world ethnic communities (Abdulrahman *et al.*, 2012). Breast cancer is, therefore, clinically considered a heterogeneous and complicated illness that encompasses a broad range of pathological entities and a range of clinical behaviors (Cavallaro, 2012). The extent of breast genetic anomalies Cancer has been affected by various genetic methods, one of which is the assessment of genomic instability (Alwan, 2010; Saaed, 2011). Genomic instability in cancer can be regarded as chromosomal instability (CIN), where most tumors have abnormal karyotypes involving either chromosomal rearrangement and/or aneuploidy. Classified as tumors with CIN. Various studies stated a substantial rise in chromosomal aberrations (CAs) in crop peripheral blood lymphocytes (PBLs) in strong tumor cancer patients (Bonassi *et al.*, 2004; Doak, 2008). Breast cancer is often caused by genetic and epigenetic modifications in genes that control the function of the breast cancer mammary epithelial cells, and to prevent the development of breast cancer, diverse intrinsic tumor suppressor mechanisms induce senescence or apoptosis of neoplastic cells (Harsimran *et al.*, 2009; Nicholls, 2012).

### Aim of Study

Study of some predisposing genes and tumor markers to reach to more frequent and dangerous factor among breast cancer patients through the following objective,

Study of genetic variation in *BRCA-1 &-2*, and *P53* as a predisposing genes and response to a tumor by using RFLP-PCR.

## MATERIALS AND METHODS

### Subject

This research was carried out on 100 women (50 groups of nurses and 50 groups of controls) The patients were women with breast cancer (post-operative phase). Both groups include 18-80-year-old women. The patients were referred to Al-Diwanya Teaching hospital, department of oncology, during the period March-November 2016. The diagnosis was created by the pathologist specialist, all patients in the post-operative phase. Demographic and risk factor data were gathered using a brief structured questionnaire including information on age, weight, height, marital status, number of pregnancies and kids, age at first birth, average lactation period, breast cancer family history or other cancers (first-degree relatives), age at menarche and age at marriage. Another group include healthy females without any family history of breast cancer also included in this study as a control group.

### Genomic DNA Extraction

Genomic DNA from blood samples were extracted by using a Genomic DNA mini kit extraction kit (Frozen Blood) Geneaid. The USA, and done according to company instructions.

### Genotyping

RFLP-PCR for BRCA1-185delAG mix was prepared using DdeI restriction enzyme (New England Biolabs. the UK), and this master mix was made independently as instructed by the company. After that, this master mix was placed in Exispin vortex centrifuge at 3000rpm for 3 minutes, then incubated at 37°C for overnight. After that, RFLP-PCR product was analysis by 3% agarose gel electrophoresis methods. The genotyping of BRCA1 gene including AA (homozygous) by two bands at (150, 26bp), GG (homozygous) as a non-digested band at 176bp, A/G (heterozygous) of three bands at bp, 150bp, and 26bp.

### RFLP-PCR

Mix for (BRCA2-A / G) RFLP-PCR mix was prepared using BspHI restriction enzyme (New England Biolabs. the UK), and this master mix was made independently as per company instructions. Afterwards, this master mix was placed in an Exispin vortex centrifuge at 3000rpm for 3 minutes, then incubated at 37 ° C for overnight. The RFLP-PCR product was subsequently analyzed using 3% agarose gel electrophoresis techniques The genotyping of BRCA2 gene, including AA (homozygous) by two bands at 296bp and 50bp, GG (homozygous) three-band at 235bp, 61bp, and 50bp, A/G (heterozygous) of four bands at 296bp, 235bp, 61bp, and 50bp.

### RFLP-PCR mix for (p53 intron 6G13964C)

RFLP-PCR mix was prepared by using a high restriction enzyme (New England Biolabs. the UK) and this master mix done independent, according to company instructions, After that, this master mix placed in an Exispin vortex centrifuge at 3000rpm for 3 minutes, then incubation at 37°C for overnight. After that, RFLP-PCR product was analysis by 3% agarose gel electrophoresis The genotyping of p53 gene, including GG (homozygous) by two bands at 33bp and 98bp, CC (homozygous) as a non-digested band at 131bp, G/C (heterozygous) of four bands at 33bp, 98bp, and 131bp.

### Statistical analysis

Statistical analysis was performed by Social Sci-

**Table 1: The case-control difference in mean age**

| Demographic features | Case (breast cancer) | Healthy controls |
|---|---|---|
| Age Groups (years) | N (%) | N (%) |
| 19-29 | 5(10) | 6 (12) |
| 30-39 | 10 (20) | 9 (18) |
| 40-50 | 20 (40) | 23(46) |
| 51-60 | 6 (12) | 4(8) |
| 61-80 | 9 (18) | 8(16) |
| Total Number | 50 | 50 |
| Range | 19-80 | 19-80 |
| Mean | 46.38 | 45.6 |
| SD | 14.31 | 14.34 |
| SE | 2.023 | 2.028 |
| P-value | 0.9369 (NS) | |

NS= NotSignificant ($p > 0.05$), SD= Standard Deviation, SE= Standard Error, N= Number

**Table 2: distribution of genotyp and alleles of BRCA1 gene in case & control**

| BRCA1 gene | Patier N (%) | Control N (%) | OR | 95% CI OR | X2 | P (X2) | EF | PF |
|---|---|---|---|---|---|---|---|---|
| BRCA1 genotypes | | | | | | | | |
| AA | 38 (76) | 40 (80) | 0.7917 | 0.306 -2.046 | 0.233 | 0.629 | *** | 0.1667 |
| GG | 4 (8) | 0 (0) | 5.3191 | 0.599 -47.229 | 5.233 | 0.022 | 0.065 | *** |
| AG | 8 (16) | 10 (20) | 0.7619 | 0.273 -2.125 | 0.271 | 0.603 | *** | 0.0476 |
| Total number | 50 | 50 | | | | | | |
| BRCA1 Allele | | | | | | | | |
| A | 84 (84) | 90 (90) | 0.5833 | 0.251 -1.357 | 1.591 | 0.208 | *** | 0.3750 |
| G | 16 (16) | 10 (10) | 1.7143 | 0.737 -3.988 | 1.59 | 0.207 | 0.0667 | *** |
| Total number | 100 | 100 | | | | | | |

OR=Odd ratio, EF= Etiology fraction, PF=Preventive fraction, $X^2$ = chi square

ence Statistics and the Statistical Package For Social Sciences version 19 for Windows Software and Microsoft Excel 2010. Continuous random variables of age and serum concentration of immunological makers that normally distributed are described by mean, SD (standard deviation), SE (standard error), and the parametric statistical tests of significance. ANOVA test are used to analysis the statistical significance of the difference in mean between more than 2 groups and when ANOVA model shows statistically significant differences, additional exploration of the statistical significance of the difference in mean between each 2 groups was assessed by Bonferonni t-test. The statistical significance, direction and strength of linear correlation between 2 quantitative variables was measured by Spearman's rank and Pearson linear correlations coefficient as in a state of serum markers. Moreover measure the strength of association between 2 categorical variables, such as the presence of certain genotype and disease status, the odds ratio (OR) and Chi-square (c2) test were used. P-value calculated from different tests depend on variables, and that less than the 0.05 level of significance was considered statistically significant (Walters, 2004).

**Table 3: Distribution of genotypes and alleles of BRCA2 gene in case & control**

| BRCA2 gene | Patient N (%) | Control N (%) | OR | 95% CI OR | X2 | P (x2) | EF | PF |
|---|---|---|---|---|---|---|---|---|
| BRCA2 genotype | | | | | | | | |
| AA | 40 (80) | 50 (100) | 0.0731 | 0.009 - 0.5897 | 11.11 | 0.001 | *** | 0.9103 |
| GG | 0 (0) | 0 (0) | *** | *** | *** | *** | *** | *** |
| AG | 10 (20) | 0 (0) | 13.4146 | 1.662-108.282 | 11.10 | 0.0009 | 0.1851 | *** |
| Total number | 50 | 50 | | | | | | |
| BRCA2 Allele | | | | | | | | |
| A | 90 (90) | 100(100) | 0.0819 | 0.010 - 0.647 | 10.50 | 0.0012 | *** | 0.9098 |
| G | 10 (10) | 0 (0) | 12.2088 | 1.546 - 96.430 | 10.53 | 0.0010 | 0.0918 | *** |
| Total number | 100 | 100 | | | | | | |

OR=Odd ratio, EF= Etiology fraction, PF=Preventive fraction, $X^2$ =chi-square

## RESULTS AND DISCUSSION

### Demographic Features Of The Study

The present case-control study were based on the analysis of a random sample of 50 females with precise diagnosis of breast cancer, their ages ranged from 19 to 80 years with a mean of 46.38 (SD 14.31) and 50 (cancer-free health) controls females their ages ranged 19 to 80 years with a mean of 45.6 (SD14.34) as in Table 1, that also show not significant ($p > 0.05$) association between mean age of cases and controls.

### Detection of *BRCA-1* Polymorphism

The distribution of *BRCA-1* polymorphism was detected by PCR-RFLP technique, at this locus there're three genotype; homozygote lane (AA) homozygous as non-digested band , lane (GG) homozygous at 150 and 26bp and lane (G/A) heterozygous at bp, 150bp, and 26bp shown in Figure 1.

In *BRCA-1* GG genotype has obviously suggested an etiology for tumor, as had an (OR 5.3191) and Etiologic Fraction (EF 0. 065) as in Table 2, In contrast, the AG & AA genotypes had a rather preventive role as it had Protective Fraction (PF) of 0.0476 & 0.1667 respectively and low OR (0.7619 & 0.7917 respectively). Figure 2 show patient have 76% of AA, 8% of GG and 16% of AG compared with control showed 20% of AG, 80% of AA and 0% of GG. Figure 3 show patient have 16%, and 84% of patient have G and A respectively compared with the control they have 10% and 90% of G and A respectively.

### Detection of *BRCA-2* Polymorphism

The distribution of *BRCA-2* polymorphism was detected by PCR-RFLP technique, at this locus there're three genotypes; lane (GG) homozygous at 296bp and 50bp, lane (AA) homozygous at 235bp, 61bp, and 50bp, and lane (G/A) heterozygous at 296bp, 235bp, 61bp, and 50bp, Figure 2.

In *BRCA-2* AG genotype has obviously suggested an etiology for tumor, as had an (OR 13.4146) and Etiologic Fraction (EF 0.1851)as in Table 3, In contrast, the AA genotype had a rather preventive role as it had Protective Fraction (PF) of 0.9103 and low OR (0.0731. Figure 2 show patient have 80% of AA and 20% of AG compared with control show 100% of AA and 0% of AG Figure 3 show patient have 10% of G and 90% of A compared with the control they have 100% of A only.

### 4-Detection of *p53 intron 6G13964C* Polymorphism

The distribution of *P53* polymorphism was detected by PCR-RFLP technique, at this locus there're three genotypes; lane (GG) homozygous at 33bp and 98bp, lane (CC) homozygous as a non-digested band at 131bp, and lane (G/C) heterozygous at 33bp, 98bp, and 131bp. Figure 3.

In *P53* CC genotype has obviously suggested an etiology for tumor, as had an (OR 1.2941) and Etiologic Fraction (EF 0.091), In contrast, the GC genotype had a rather preventive role as it had Protective Fraction (PF) of 0.087 and low OR (0.4565). Figure 3 show patient have 52% of GG, 40% of CC and 8% of GC compared with control showed 50% of GG, 34 % of CC and 16% of GC. Present study show patient

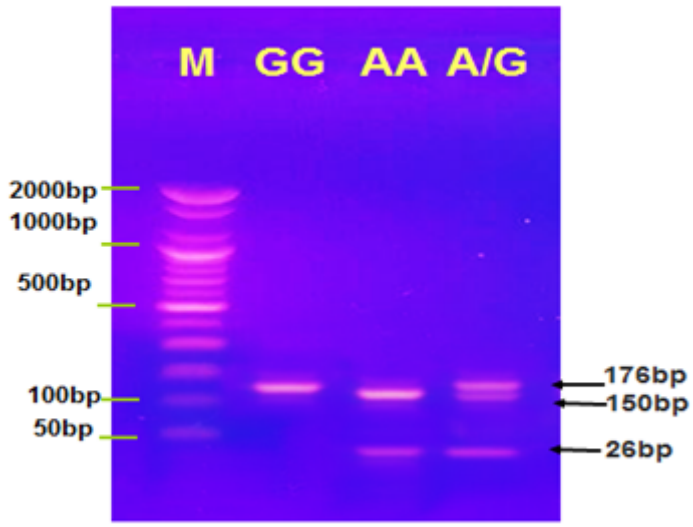


**Figure 1: Agarose gel electrophoresis image that shows the RFLP-PCR product analysis of BRCA1185delAG gene polymorphism by using DdeI restriction enzyme. Where M: marker (2000-50bp), lane (GG) homozygous at 150 and 26bp, lane(AA) homozygous as non-digested band 176bp, and lane (G/A) heterozygous at bp,150bp, and 26bp**

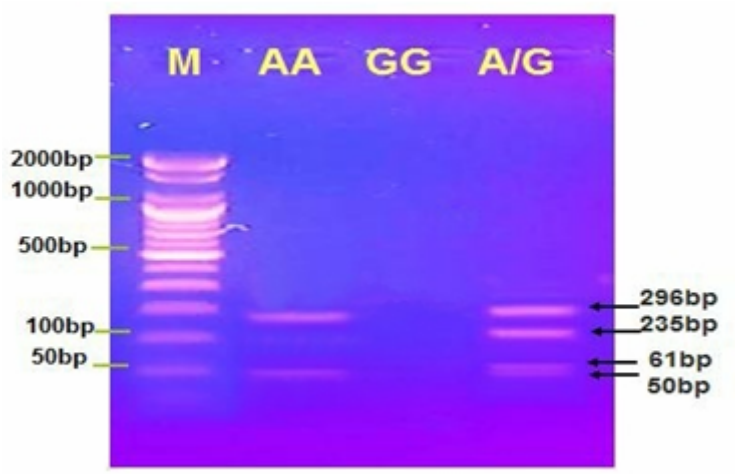


**Figure 2: Agarose gel electrophoresis image that shows the RFLP-PCR product analysis of BRCA2185delAG gene polymorphism by using BspHI restriction enzyme. Where M:marker (2000-50bp), lane (GG) homozygous at 296bp and 50bp, lane (AA)homozygous at 235bp, 61bp, and 50bp, and lane (G/A) heterozygous at 296bp,235bp, 61bp, and 50bp**

have 56% of G and 44% of C compared with the control they have 52% of G and 48% of C.

**1-Demographic characteristics**

The age characteristic of patients who have breast cancer in the present study, revealed that the highest frequency of breast cancer patients among (40-50) years old (40%), followed by the age group of ( 30-39) years old (20%) , and the less frequency in the age (19-29) years (10%) , which has no significant differences as compared with control group ($p > 0.05$) mean 46.38 years (SD14.34) , so breast cancer is a disease of all ages, considering the entire lifespan (Walters, 2004). The results of our present study are agreed with (Walters, 2004) since the results of their study which included 200 Bulgarian females with breast cancer (postoperative and the age ranged from 25 to74 years) selected by the established genetic testing criteria, the mean age of the patients at diagnosis was 49.5 years , and no significant association between patients group and controls group ($p > 0.05$) (Dodova and Ivanova, 2015). So our findings are comparable with a study conducted an aver-

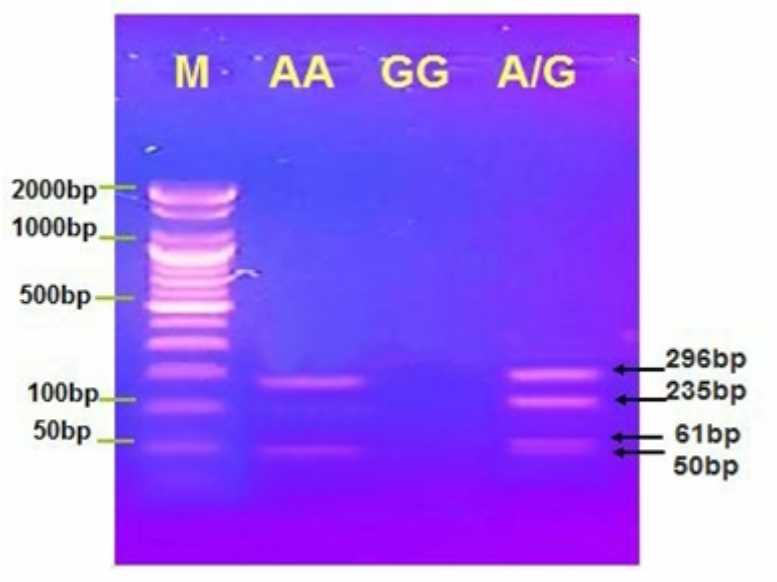


**Figure 3: Agarose gel electrophoresis image that shows the RFLP-PCR product analysis of p53 intron 6G13964C gene polymorphism by using HhaI restriction enzyme. Where M:marker (2000-50bp), lane (GG) homozygous at 33bp and 98bp, lane (CC) homozygousas a non-digested band at 131bp, and lane (G/C) heterozygous at 33bp, 98bp, and131bp**

age 12% of women worldwide related breast cancer, their ages ranged between <40 - >70 years and showed 48.5 years mean of patients ages (Mcguire, 2015). Other studies documented an age mean of 50.3 years (Joyce, 2015). So this results that is consistence with (Barthelemy, 2011) who found the mean age of breast cancer patients 45.1 years , and no significant differences with control group (P = 0.903) , another study performed by (Han and Kang, 2010), stated in their study a mean age 44.7 years of patients with breast cancer which was not different from control group (p=0.19) , and a similar findings was reported by (Partridge, 2013) who found 42.95 years as a mean age of breast cancer patients. The *BRCA-1*, *BRCA-2* and *P53* genotypes were assessed for their roles in predicting the risk of having breast cancer. Each compared of a control group, (general population without history family for breast cancer in any degree). The results of the present study showed the *BRCA-1* genotypes, had significant predictive power. The G allele had the strongest association and significantly increases the risk of having breast cancer 16% (OR= 1.7143, 95% CI OR=0.737 -3.988, EF=0.0667) compared to general population control. In a lesser degree the A allele had a statistically significant protective effect 84% (OR= 0.5833, 95% CI OR= (0.25 - 1.357), PF= 0.3750). the homozygous GG genotype increases the risk of the disease 8% (OR=5.3191, 95% CI OR= 0.599 -47.229, EF=0.065. While the wild AA genotype showed a statistically significant protective effect 76%(OR= 0.7917, 95% CI OR= (0.306 -2.046), PF=0.1667 (Parvin *et al.*, 2015). So the heterozygous AG genotype showed a statistically significant protective effect 16%(OR= 0.7619, 95% CI OR= (0.273 -2.125), PF=0.047), compared with control group they have (0% GG, 80% AA and 20% AG) . This result have similarity with results of (Haytural, 2013), she tested (310) patients with breast cancers were recruited from different public and private hospitals of Bangladesh and as controls (250) Bangladeshi women, and found GG genotype increase the risk of malignant tumor in breast (OR=4.9, 95% CI=0.59 to 41.09, p=0.14). So our result that is consistence with study (Hansa *et al.*, 2012), who study on 106 consecutive breast cancer patients who were admitted to Istanbul Training and Research Hospital, Department of General Surgery and, they found GG responsible for risk to breast cancer (OR=8.54, 95% CI; 1.07- 68.27). So our present study have similarity with the findings from most other previous studies in breast cancer patients with mutations in BRCA1 and BRCA2 such as studies of (Campeau *et al.*, 2008; Chakraborty, 2013) they referred to G allele had the strongest association and significantly increases the risk of having breast cancer in GG genotype (OR= 1.812, 95% CI OR=0.691 -3.312) and (OR= 1.911, 95% CI OR=0.599 -3.018 ). The results in this study showed the *BRCA-2* genotypes, so had significant predic-

tive power. The G allele had the strongest association and significantly increases the risk of having breast cancer (OR= 12.2088, 95% CI OR=1.546 - 96.430, EF=0.0918) compared with the control group. In a lesser degree the A allele had significant protective role (OR= 0.0819, 95% CI OR= (0.010 - 0.647), PF= 0.9098). The heterozygous AG genotype increases the risk of the disease by (OR=13.4146, 95% CI OR= 1.662- 108.282, EF=0.1851. While the wild AA genotype showed a statistically significant protective effect (OR= 0.0731, 95% CI OR= (0.009 - 0.5897), PF=0.9103 (This results agreed with most studies such as (Gholipoorfeshkecheh and Arjunan, 2014), who study on 106 Turkish patients with breast cancer and they reached to AG genotype increase the risk for breast malignancies (OR=12.6, 95% CI, 43.91-3.67, EF=0.203) , (Gholipoorfeshkecheh and Arjunan, 2014) they , their result showed to (OR= 11.412, 95% CI, 1.20-24.65, EF= 0.154) , So (Evans *et al.*, 2005), they found AG increase risk of malignant tumor of breast(OR= 14.211, 95% CI, 2.03-28.55, EF= 0.106). The results in this study showed the *p53* genotypes, so had significant predictive power. The C allele had the strongest association and significantly increases the risk of having breast cancer 44%(OR= 1.0850, 95% CI OR=0.6198 - 1.8996, EF=0.0345) compared with the control group. To a lesser degree the G allele had significant protective role56% (OR= 0.9216, 95% CI OR= (0.526 - 1.614), PF= 0.0455). The homozygous CC genotype increase the risk of the disease by40 % (OR=1.2941, 95% CI OR= 0.573 - 2.921, EF=0.091) and the heterozygous GC genotype showed a statistically significant protective effect 8% (OR= 0.4565, 95% CI OR= (0.128 - 1.627), PF=0.087. while wild type GG genotype doesn't have any role in increasing risk or protective effect 52% (OR= 1, 95%CIOR=0.495 - 2.374). This present study agreed with (Zhang, 2010), and their result referred CC genotype increased risk for breast cancer (OR = 0.87, 95% CI: 0.78–0.97) while GC have protective effect (OR = 0.91, 95% CI: 0.83–1.00), So there are similarity between our results and (Zhang, 2010) who study on Tunisian women, and who found increasing risk of disease by CC and presence of protection belong to GC genotype (OR=0.81 and OR=0.79 respectively). *P53*, which is a tumor suppressor gene, creating a protein that repairs DNA and prevents carcinogenesis. Every cell in mutation carriers has been demonstrated to lack one functional allele (i.e. the tumor-suppressor function of that gene is lost); a situation that favors cancer development, so *P53* is a tumor suppressor gene that is mutated or changed in more than 50 percent of tumors (Evans *et al.*, 2005).

## CONCLUSION

Patients how to have a history family considered a risk for breast cancer disease because of the presence of mutations in *BRCA*-1 and *BRCA-2* genes, breast cancer considered a disease for all ages.

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