

Study of *BRCA2* Gene Mutations in Egyptian Females with Breast Cancer

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ABSTRACT: In this study we assessed the frequency of two founder mutations in *BRCA2* gene which are 999del5 and 6174delT. This study was carried out on fifty Egyptian females, including twenty healthy females as controls and thirty patients with breast cancer. DNA was extracted from the blood. PCR was done for amplification of the founder mutations 999del5 and 6174delT in *BRCA2* gene (exons 9 and 11 respectively), gel electrophoresis was done for separation of amplified DNA bands. Our results showed that the frequency of 999del5 was 14%, and 6174delT founder mutation was 4%, both are from the patient group with family history. We concluded from the founder effect of 999del5 on Egyptian population, and the low frequency of 6174delT founder mutation. It was apparent from the study findings that women with a strong family history were still at higher risk for developing the disease.

KEYWORDS: Breast cancer, *BRCA2* gene, founder mutation, familial cancer.

I. INTRODUCTION

Worldwide, breast cancer is the most common cancer affecting women and one of the most common human cancers. Between 2008 and 2012 breast cancer incidence increased by 20%, while mortality has increased by 14% [1]. While most tumors are sporadic, about 5 to 10% are caused by germ line mutations in certain genes. Breast cancer susceptibility gene1 (*BRCA1*) and breast cancer susceptibility gene2 (*BRCA2*) are responsible for approximately 20-40% of inherited breast cancer [2].

A wide variation in the *BRCA2* mutation spectrum and frequency has been reported for different populations [3]. Some of the *BRCA2* mutations represent founder mutations; the knowledge of founder mutations can shorten the search for an inherited disease-associated mutation. So, in geographic areas where breast cancer population genetics has not yet been widely studied, founder mutations can provide a starting place for understanding of the public health impact of inherited predisposing genes [4].

II. MATERIALS AND METHODS

This prospective study was performed at biochemistry department, Benha University hospital. The study was carried out on fifty Egyptian females divided into two groups as following, control group (group I) including twenty healthy females, subdivided into two subgroups, group Ia: ten healthy females without a family history of breast &/or ovarian cancers, group Ib: ten healthy females with a family history of breast &/or ovarian cancer. Breast cancer group (group II) including thirty female patients attending general surgery department, Benha university hospital, subdivided into two subgroups: group IIa: fifteen female patients without a family history of breast &/or ovarian cancers, group IIb: fifteen female patients with a family history of breast &/or ovarian cancers. Consents were taken from the subjects after being informed the aim of the study, full history was taken (Age, menstrual history, marital status, parity, lactation, contraception and family history). All patients included were diagnosed to have invasive breast carcinoma. 5ml of venous blood from each subject were collected on tube containing EDTA, the sample mixed well and stored at -80 for

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further processing. DNA was extracted from the blood using G-spin™ Total DNA Extraction Mini Kit – (iNtRON Biotechnology, INC. - Korean Biotech Database). PCR was done for amplification of the founder mutations 999del5 and 6174delT in *BRCA2* gene [5]. Detection of 6174delT founder mutation of exon 11 was carried out by digestion of amplified products of exon 11 with BstXI restriction Endonuclease. Agarose gel electrophoresis was done using separated on 2% agarose gel and 100 bp DNA ladder; the bands were stained with ethidium bromide [6]. Detection of the other founder mutation 999del5 was done using 7.5% polyacrylamide gels and 50 bp DNA ladder, stained with ethidium bromide; the mutation was identified by the presence of an extra band as compared with the pattern in carriers of only the wild-type allele [7]. Statistical package for social science (SPSS) and Microsoft Office Excel were used for data processing and data analysis. For all the tests, a p value of 0.05 or less was considered for statistical significance.

III. RESULTS

Our results showed that there was no statistically significant difference among the studied groups regarding to age, age of menarche, lactation, parity and use of oral contraceptives. The percentage of family history of breast cancer is much more than the ovarian cancer; also this study showed that the percentage of the first degree relative with breast and/or ovarian cancer is extremely higher than that of the second degree relative in the control and breast cancer groups with family history (table1).

Table 1: Percentage of 1st degree and 2nd degree relatives & the history of breast and ovarian cancers among studied groups.

Group` Item		Control Group With +ve Family History	Breast Cancer Group With +ve Family History
		Number (%)	Number (%)
Degree of family relative	1 st degree	10/10 (100)	14/15 (93.3)
	2 nd degree	0 (0)	1/15 (6.7)
Type of cancer	Breast cancer	9 (90)	14 (93.3)
	Ovarian cancer	1 (10)	1 (6.7)

In this study, the frequency of 999del5 was 14%, confirming the founder effect of this mutation in Egyptian population. The other mutation screened in this study was the founder mutation 6174delT, we found two cases showing this founder mutation, both are from the patient group with family history (table2).

Table 2: Founder mutations frequencies in *BRCA2* gene (exon9 & exon 11 respectively), among studied groups.

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Groups Exons Mutation Frequency	Control Group		Breast Cancer Group		X ²	P
	Without family history N =10 (Group Ia)	With family history N=10 (Group Ib)	Without family history N =15 (GroupIIa)	With family history N=15 (Group IIb)		
	Number (%)	Number (%)	Number (%)	Number (%)		
Exon 9 (999del5)	0/10 (0)	2/10 (20)	0/15 (0)	5/15 (33.3)	24.2	< 0.05
Exon 11 (6174delT)	0/10 (0)	0/10 (0)	0/15 (0)	2/15 (13.3)	46.5	>0.05

The mutations found in this study were of heterozygous genotype (figure1 and figure2).

Figure 1: Polyacrylamide gel amplified DNA products in normal subjects and subjects having 999del5 mutation exon 9 of BRCA2 gene.

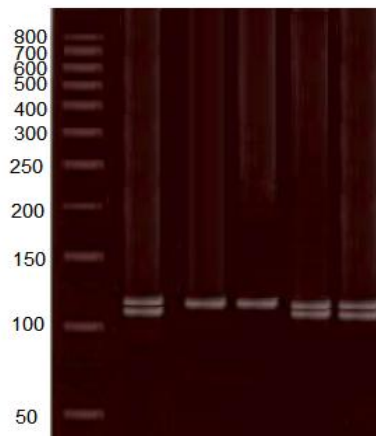
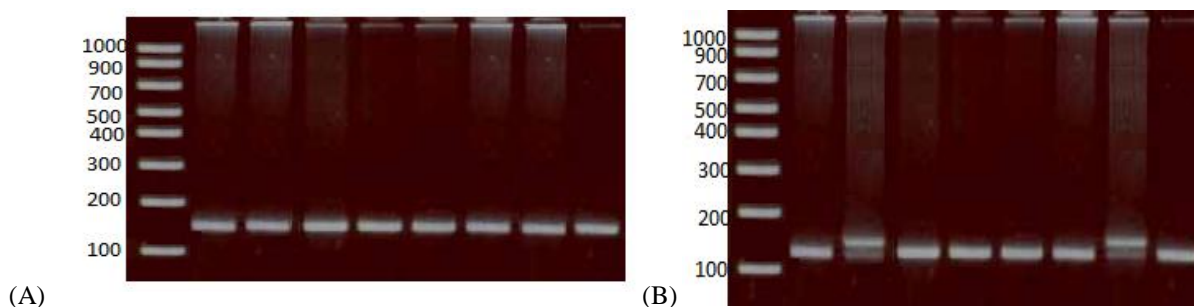


Figure 1 shows amplified DNA products in normal and mutant subjects, lane 1 indicate 50bp DNA marker, lanes 3 and 4 show amplified DNA bands at 114bp denoting normal wild type alleles, lanes 2, 5 and 6 show two amplified DNA bands one at 114bp denoting wild type allele and the other at 109bp indicating mutant allele.

Figure2: Agarose gel amplified DNA products in normal and mutant subjects before and after digestion by BstXI for detection of 6174delT mutation in exon 11 of BRCA2 gene.



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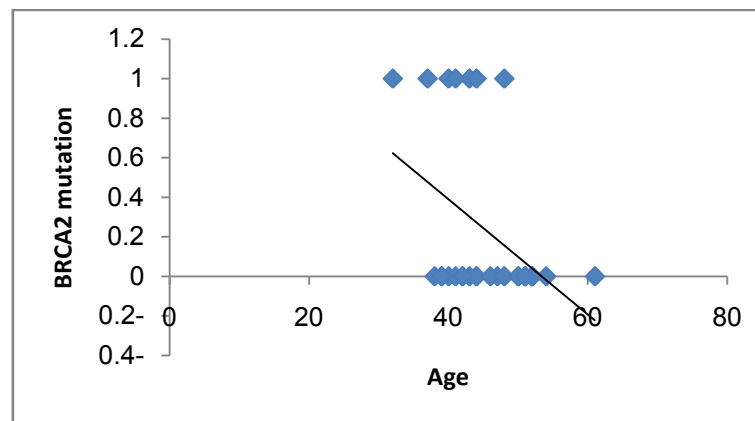
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Figure2 shows amplified DNA products in normal and mutant subjects before and after digestion by BstXI for detection of 6174delT mutation in exon 11 of BRCA2 gene. (A): Amplified DNA products before digestion, lane 1 indicates 100bp DNA marker, wild type alleles (at 159bp) not differentiated from mutant alleles (158bp), as mutant alleles is only one base pair deficient. (B): After digestion, lanes 2,4,5,6,7 and 9 show amplified DNA bands at 133bp denoting wild type allele (normally restricted at the cutting site of the enzyme BstXI, removing 26bp DNA fragment from the normal 159bp amplified DNA product), lane3 and 8 show amplified DNA bands, one at 133bp denoting one wild allele, and the other at 158bp denoting mutant allele, the mutant allele is one bp (T) at the nucleotide 6174 missed and not restricted as it lost the recognition site for restriction by BstXI.

According to this study, there was statistically significant negative correlation between frequency *BRCA2* gene mutations and female age in the studied groups, $r = -0.42$, $p < 0.05$ (figure3).

Figure 3: Correlation coefficient (r) between age and *BRCA2* gene mutations in the breast cancer groups.



IV. DISCUSSION

Breast cancer is the most common cancer in women and its impact on mortality and morbidity is significant and well documented. It is also the most common cause of death from cancer among women worldwide. About 10% of breast cancer is due to strong inherited risk conferred by a dominant gene mutation. Significant proportion of hereditary breast cancer is due to germline mutations in either of the two predisposing genes, *BRCA1* and *BRCA2*.

A wide variation in the *BRCA2* mutation spectrum and frequency has been reported for different populations. Some of the *BRCA2* mutations represent founder mutations; founder mutations can provide a starting place for understanding of the public health impact of inherited predisposing genes. This study was carried out on fifty Egyptian females with and without family history of breast and/or ovarian cancer to allow identification of individuals at high risk. The mean age of breast cancer patients with family history was 44.9 and those without family history was 45.9 years. There was no statistically significant difference between age of breast cancer patient with family history and those without family history. The total mean age of our patients was less than 46 years, as the likelihood of finding a mutation is highly age dependent [8]. However, this age is lower as compared with the onset age (60 years) of Caucasian patients with breast cancer as reported by Markus and co-workers [9]. As regards to the mean age at onset of menarche, we found that it was 12.6 for breast cancer patients with family history and for those without family history was 12 years, this was consistent with previous studies which found that the age at onset of menarche for breast cancer patients ranged from 11.3 to 12.8 [10], and not consistent with other studies demonstrated that women who had their first menstrual period before age 12 are at an increased risk of breast cancer [11].

Several studies demonstrated some important risk factors which may enhance the appearance and/or progress of breast cancer. These factors include lactation and parity [12]. However, this study demonstrated no significant difference in number of lactating females and the number of nullipara females between breast cancer and control groups. This also was demonstrated by other previous studies [13]. Previous studies found that using oral contraceptives have a greater

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risk of breast cancer than women who have never used them, but this risk seems to decline once their use is stopped. Women who stopped using oral contraceptives more than 10 years ago do not appear to have any increased breast cancer risk [14]. This study revealed no significant difference in number of females utilizing oral contraceptives between the studied groups and this goes with the study of Hall et al (2005), who found that use of oral contraceptives had a low priority as a risk factor for breast cancer as in his results there was limited, if any, evidence of an important association with breast cancer [15].

This study showed that the percentage of family history of breast cancer in the control and breast cancer groups with positive family history was 90% and 93.3% respectively. On the other hand, the percentage of family history of ovarian cancer in the control and breast cancer groups with positive family history was 10% and 6.7% respectively in each. This means that the percentage of family history of breast cancer is much more than the ovarian cancer; also this study showed that the percentage of the first degree relative with breast and/or ovarian cancer is extremely higher than that of the second degree relative in the control and breast cancer groups with family history. Women with first- and second-degree relatives with breast cancer have a higher risk of the disease themselves. This risk is higher when the affected relative was a mother or a sister (first degree) compared to aunts (second degree) or grandmothers (first degree), as found in a meta-analysis of 74 studies [16].

A large number of distinct mutations in the *BRCA2* gene have been reported worldwide, but little is known regarding the role of this susceptibility gene on breast cancer in Egypt. In this study we searched for two founder mutations of *BRCA2* gene 999del5 in exon 9 and 6174delT in exon 11. The founder mutation of *BRCA2* gene 999del5 is a frame shift (five bases) deletion mutation in exon 9. The deletion starts at nucleotide 999, leading to a stop codon at nucleotide 1047 and to premature truncation of protein translation [17]. This mutation is recurrent and proposed as an ancient founder mutation. It has been identified as the single, strong *BRCA2* founder mutation in Iceland and the most common *BRCA1/2* founder mutation in Finland [18].

In this study, the frequency of 999del5 was 14%, (5 breast cancer patient and 2 of healthy first degree relatives), this result was lower than that reported by Ibrahim et al (2010), who examined Egyptian female patients with breast cancer and found that 26.7 % carried the mutation [5], while it was higher than that reported by Bensam et al (2014), who found that the frequency of this mutation was 6% of the examined fifty Egyptian study subjects [19]. Our finding confirmed the founder effect of this mutation in Egyptian population.

The other mutation screened in this study was the founder mutation 6174delT. In this mutation there is one nucleotide (a thymine – denoted as “T”) missing at nucleotide 6174 (6174delT) in exon 11, it is frame shift deletion. This founder mutation is mainly described in Ashkenazi Jewish patients ranging from 7% to 30% in the different studies of this population [20]. There are several studies reporting this founder mutation in other populations. Here, we investigated the potential role of this founder mutation in Egyptians, we found two cases showing this founder mutation, both are from the patient group with family history, representing 4% of the study subjects, this result is consistent with several studies describe the presence of this founder mutation in low frequencies (3% or less) as non Ashkenazi Jewish [21], Russian [22], Slovak [23], Costa Rica [24], Caucasian [25] and Poland [26]. However, some other studies did not find this mutation in the studied population such as Iranian [27], Colombian [28], Chilean [29], South African [30], Turkish [31] and Indian [32].

The mutations found in this study were of heterozygous genotype and this goes with previous researches which conclude that human *BRCA1/2* homozygotes are likely to be biologically non-viable and are unknown to exist. Among the consanguineous couples, there are excess deaths (abortions, stillbirths, perinatal and early-childhood deaths) especially in younger females. It has been suggested that, in part, the excess deaths are due to *BRCA1/2* and other still undiscovered tumor gene homozygotes [33]. According to this study, there was statistically significant negative correlation between frequency *BRCA2* gene mutations and female age in the studied groups. This also was found by Eerola et al (2005) [34]. However, studies demonstrate that *BRCA2* mutations were mostly found in breast cancer patients with disease diagnosis before the age of 50 years. Moreover, in cases with familial clustering of site-specific breast cancer, *BRCA2* mostly accounted for tumors diagnosed before age 40 years [35].

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V. CONCLUSION

We concluded from this study that there is high frequency of exon 9 founder mutation (999del5) of *BRCA2* gene, confirming its founder effect on Egyptian population, and low frequency of exon 11 founder mutation (6174delT). It was apparent from the study's finding that women with a strong family history were still at higher risk for developing the disease.

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