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## Analysis of 6174delT Mutation in BRCA2 Gene by Mutagenically Separated PCR Among Libyan Patients with Breast Cancer

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## ABSTRACT

**Background:** Breast cancer is the most common malignancy among women. It is estimated that 1 in 10 women worldwide is affected by breast cancer during their lifetime. In 5 to 10% of breast cancer patients, the disease results from a hereditary predisposition, which can be attributable to mutations in either of two tumor suppressor genes, BRCA1 and BRCA2 to a large extent. BRCA2 6174delT mutation constitutes the common mutant alleles which predispose to hereditary breast cancer in the Ashkenazi population with a reported carrier frequency of 1.52%. In this study, we investigated the presence of the 6174delT mutation of the BRCA2 gene in Libyan woman affected with breast cancer and compared the results with those of other population groups.

**Methods:** Eighty- five Libyan women with breast cancer in additions to 5 relatives of the patients (healthy individuals) were recruited to this study. We obtained clinical information, family history, and peripheral blood for DNA extraction and analyzed the data using multiplex mutagenic polymerase chain reaction (MS-PCR) for detection of 6174delT mutation in the BRCA2 gene .

**Results:** The 6174delT of the BRCA2 gene was not detected either in the 85 patients with breast cancer (18 with familial breast cancer and 67 with sporadic breast cancer) nor in the 5 healthy individuals.

**Conclusions:** The present study showed that the 6174delT of the BRCA2 gene was not detectable using mutagenic PCR in the Libyan patients with breast cancer and can be considered to be exceedingly rare.

**Introduction**

Cancer is a major public health problem throughout the world. It is considered to be the second most common cause of death in developed countries and the fourth most common in developing nations. In

Arab countries, within the years 1982–1987, cancer deaths comprised more than 10% of all deaths in Bahrain, Iraq and Kuwait.<sup>1,2</sup> Cancer was responsible of 8.7 percent of all deaths in Benghazi Municipality within the 6-year period 1991–96. This can be compared to 2.0% in 1970–73 and 3.9% in 1980–83. Among women, the proportional cancer mortality ratio per 1000 deaths was 83.3 for females.<sup>3</sup> Cancer registries in North Africa (Morocco, Algeria, Tunisia, Libya, Egypt) increased in number within the past few years from 1 to 9%, and now cover 13 percent of the region population.<sup>3</sup> The incidence rates of breast cancers per 100,000 population in

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North African countries ranged between 23.3 in Benghazi, Libya (the year 2004) to 60.5 in Algiers (the year 2006).<sup>3</sup>

Hereditary breast cancer is considered to account for a small proportion of all breast cancer cases.<sup>4</sup> However, a positive family history for breast cancer is a very important risk factor for the development of this malignancy. About 4–5 percent of breast cancer cases are considered to be related to inheritance of a dominant cancer-predisposing gene.<sup>5</sup> In the other 5 to 10% of breast cancer patients, the disease results from a hereditary predisposition, which can be attributable to mutations in either of two tumor suppressor genes, BRCA1 and BRCA2 to a large extent.<sup>6–8</sup> Currently, hundreds of BRCA1 and BRCA2 mutations are known. Most of these mutations are nonsense or frameshift mutations, which can be detected throughout the entire gene sequence and produce truncated proteins. The prevalence of BRCA1 and BRCA2 mutations varies in different populations due to founder effects and other geographical and environmental factors.<sup>9–15</sup> Founder mutations are known to be mutations detected frequently in a particular population due to geographic, cultural, or ethnic isolation. Individuals of Ashkenazi Jewish community have an especially high carrier rate for three mutations, which predispose them to hereditary breast and ovarian cancer syndrome: the 185delAG and the 5382insC in BRCA1 and the 6174delT in BRCA2 with a prevalence of about 2.5 percent in that population.<sup>15–19</sup>

The BRCA2 6174delT mutation constitutes the most common mutation alleles predisposing to hereditary breast cancer in the Ashkenazi population with a carrier frequency of 1.52%.<sup>15</sup> Yet, the calculated contribution of the BRCA2 6174delT mutation to breast cancer diagnosed in Ashkenazi women before the age of 50 is about 8 percent.<sup>16–18</sup>

Few mutations have been described in BRCA1 and BRCA2 in high-risk non-Ashkenazi Jews population. In a family from Libya, the 1100delAT BRCA1 mutation was found and the 8765delAG BRCA2 mutation was previously described in two Jewish Yemenite-families.<sup>20</sup> Moreover, 185delAG BRCA1 mutation has been detected in Moroccan Jewish women.<sup>21</sup>

The history of the Libyan population reflects a heterogeneous genetic pool of Arabs, Amazighs (Berbers), Romans, Tuaregs, Tebus, Africans, Turks, Greeks, and Jews.<sup>22, 23</sup> Here, we investigated the 6174delT mutation in BRCA2 by mutagenically separated PCR for Libyan women diagnosed with

breast cancer and compared the results with those of other population groups.

## Methods

### Study Samples

The patients enrolled in this study were selected from the patients in Breast Cancer Follow-Up Clinic in Tripoli Central Hospital and African Oncology Institute "Sabratha". Informed consent was obtained from all participants and the study was approved by the Board of the Libyan Academy for Higher Studies, School of Biological Sciences. A total of 90 samples were included in this project, of whom 85 were diagnosed as patients, complaining of breast cancer, while 5 were diagnosed as normal (control group). The study patients were 26–70 years old. Three to five milliliters blood samples were collected in vacutainer tubes with EDTA as the anticoagulant and transported to the Laboratory of Genetic Engineering Department (Biotechnology Research Center) and frozen at -20 °C until needed for DNA extraction and subsequently, PCR analysis.

### DNA extraction

A total number of 90 DNA samples were extracted according to Sambrook *et al.*<sup>24</sup> The frozen blood samples were thawed, treated with 1X SSC buffer, Na Acetate, 10% SDS and 5 µl proteinase K, and vortexed briefly and incubated for 1 hour at 55 °C. The DNA was isolated using the phenol/chloroform method and precipitated with Ethanol as described by Sambrook *et al.*<sup>24</sup> The DNA quantity and quality were determined according to Sambrook *et al.*<sup>24</sup> using a spectrophotometer and agarose gel electrophoresis. All chemicals used in this study were of the molecular grade.

### Multiplex Mutagenically Separated PCR method (MS-PCR)

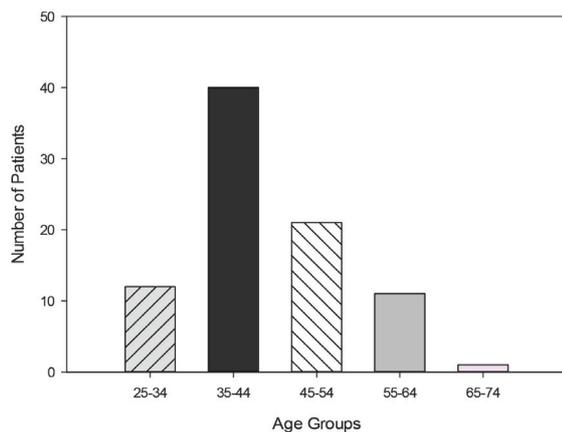
A simple and rapid method for detection of commonly analyzed mutation (6174delT) in BRCA2 were used as described in a previously published article.<sup>25</sup> In general, three primers designed for the mutation (one common, one specific for the mutant, and one specific for the wild-type allele). The competing mutant and wild-type primers were designed to differ by ~20 bp in size, in order to allow easy detection of the PCR products by routine electrophoresis and ultraviolet illumination after ethidium bromide staining. The sequences of the primers were designed as described elsewhere.<sup>25</sup> The primers sequences, annealing temperatures and the sizes of PCR products are demonstrated in Table 1.

**Table 1.** Nucleotide sequences of the primer set

Primer	Primer sequence	Size of amplicon
Common reverse (P1)	5'-agctggtctgaatgttcgtact	151 bp
Wild-type forward (P2)	5'-gtgggatttttagcacagctagt	171 bp
Mutant forward (P3)	5'-cagtctcatctgcaatacttcagggatttttagcacagcatgg	



In general, the genotype of the 6174Tdel was determined using a mutagenically separated PCR method as described.<sup>25</sup> The final volume used for the PCR assay was 25  $\mu$ l, in which 25ng genomic DNA was amplified with 0.9  $\mu$ l 20mM of the three primers included in the reaction, 0.125 $\mu$ l 5 Go Taq® Flexi DNA Polymerase, 5 $\mu$ l 5X Green Go Taq® Flexi Buffer (Promega), 4 $\mu$ l 25Mm MgCl Solution and 0.5  $\mu$ l 10mM dNTP were employed using an Applied Biosystem Thermocycler. PCR amplification comprised of an initial denaturation step at 95 °C for 5 minutes followed by 30 cycles of denaturation at 94 °C for 1 minute, annealing at 59 °C for 30 seconds, extension at 72 °C for 1 minute, and a final extension step at 72 °C for 7 minutes. The PCR products for the mutagenically separated PCR were analyzed by electrophoresis on a 3% agarose gel, stained with ethidium bromide and visualized using a UV transilluminator for the presence of wild or mutant allele.

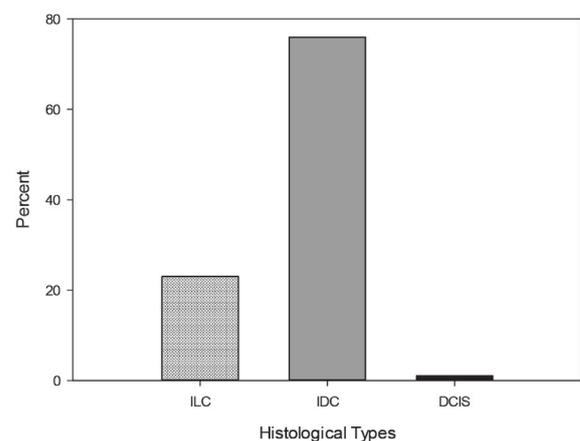


**Figure 1.** The correlation between age and the incidence of breast cancer among Libyan women

and ductal carcinoma in-situ in 1% (n=1) of the patients in the left breast and infiltrating ductal carcinoma in 39% (n=33), and infiltrating lobular carcinoma in 12% (n=10) of the patients in the right breast (Table 2). A family history can always play an important role in developing any medical disease, including breast cancer. In this study, we focused on first and second degree family relationships, including parents, children, siblings, grandparents, and aunts. Regarding positive family history of breast cancer, 21% (18 patients) had a past history of breast cancer in the family. Fourteen percent (12 patients) of the patients had a positive history of breast cancer in the first degree relatives and 7% (6 patients) in the second degree family members (Table 3). Moreover, the data showed that 40% of the patients (n=36) had a family history of cancer and 27% (14 patients) had a family history of other cancers except breast cancer. Nine percent of the patients reported a family history of other cancers

## Results

The mean age of the patients included in this study was  $44 \pm 9$  years (range: 26 – 70 years). Breast cancer was detected in 14% of the patients (12 patients) in the age group 25-34 years and 25% of the patients (21 patients) in the age group 45-54 years old, reach a maximum of 47% (40 patients) in the age group 35- 44 years, and declined to 13% (11 patients) in the age group 55-64 years, and was detected in 1% of the patients (one patient) in the age group 65-74 years (Figure 1). This study showed that 51% of the patients (43 patients) had breast cancer in the right breast and 49% (42 patients) had breast cancer in the left side. The pathology reports revealed that types of breast cancer in our study were ductal carcinoma in-situ (1%, 1 patient), infiltrating lobular carcinoma (24%, 20 patients), and infiltrating ductal carcinoma (75%, 64 patients) (Figure 2). The types of breast cancer were infiltrating ductal carcinoma in 36% (n = 31), infiltrating lobular carcinoma in 12% (n = 10)



**Figure 2.** The histological types of breast cancer among Libyan women (ILC; invasive lobular carcinoma, IDC; invasive ductal carcinoma, DCIS; ductal carcinoma in-situ)

except breast cancer (n=8) in first degree relatives and 18% (16 patients) in the second degree relatives (Table 3)

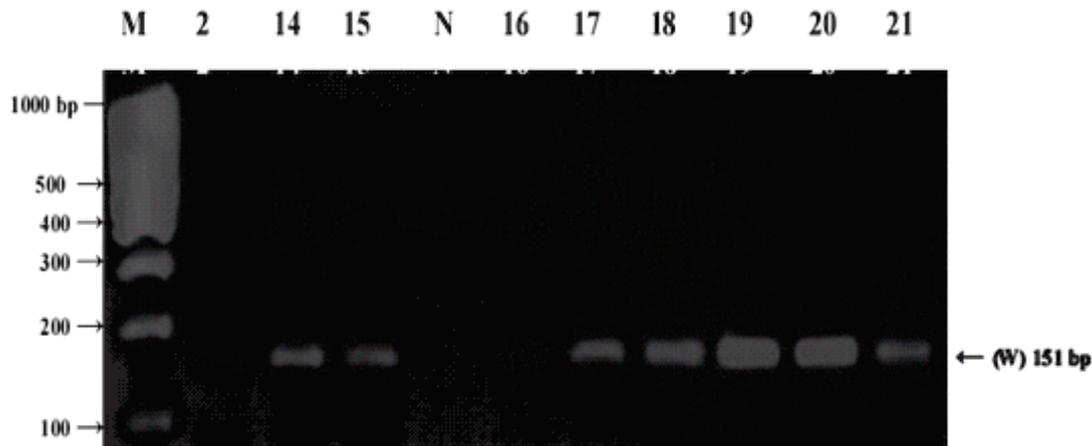
Mutagenically separated PCR (MS-PCR) was done for all DNA from 85 cases for the 6174delT mutation in the BRCA2 gene and 5 controls (All 85 cases were negative for 6174delT) with the

**Table 2.** The laterality and the histological type of breast cancer

Histological type	N (%)
Left breast	
Infiltrating ductal carcinoma	31 (16%)
Infiltrating lobular carcinoma	10 (12%)
Ductal carcinoma in-situ	1 (1%)
Right breast	
Infiltrating ductal carcinoma	33 (39%)
Infiltrating lobular carcinoma	10 (12%)

**Table 3.** Family history of breast cancer and other cancers

Family history	Breast cancer	Other cancers
Negative	67 (79%)	66 (73%)
Positive (first degree)	12 (14%)	8 (9%)
Positive (second degree)	6 (7%)	16 (18%)

**Figure 3.** Electrophoretogram of multiplex mutagenic PCR (MS-PCR) products on the 3% agarose gel. Lane M: molecular size of the DNA marker. Lane N: negative control. Lane 2: PCR products of a normal individual. Lanes 14 to 21: PCR products of breast cancer patients. W: Wild – type product.

appearance of one band (151bp) on agarose gel electrophoresis indicating no mutation in any allele in exon11 (Figure 3).

### Discussion

The risk of breast cancer in Libya is highest among women younger than 50 years of age, which is 10 years younger than reported in other countries.<sup>3</sup> Almost all women diagnosed with breast cancer had history of breastfeeding to one or more children. The present results indicated that the mean age of our patients was  $44 \pm 9$  years (range: 26-70 years), which is in agreement with the results of previous study that reported that the median age of 46 years (range: 21–76 years) for breast cancer patients in National Cancer Institute in Egypt between 1994 and 1998.<sup>26</sup> Another study from Victorian Cancer Registry reported the mean age of  $43.5 \pm 8.2$  years (range: 23-60 years).<sup>27</sup> One study reported that the median age at diagnosis was 52.5 years in Lebanese breast cancer females.<sup>28</sup> The present study revealed that the rate of breast cancer was 14% in the age group 25-34 years and 25% in the age group 45-54 years, reached a maximum of 47% in the age group 35-44 years, then declined to 13% in the age group 55-64 years, and was 1% in the age group 65-74 years. This finding is in agreement with another study which showed that the frequency of breast cancer was 14% in the age group 26-34 years, 30% in the age group 45-54 years, reached a maximum of 45% in the age group 35-44 years, and then declined to 11% in the age group 55-60 years.<sup>27</sup> There was an increase in the incidence of breast cancer in young women under 40 years of age in France.<sup>29-31</sup> On the other hand, frequency of breast

cancer was 8% in the age group 25-34 years, 27% in the age group 35-44 years, 35% in the age group 45-54 years, 22% in the age group 55-64 years, and 9% in the age group 65-70 years.<sup>32</sup> The present study showed that 51% of the patients had cancer in the right breast and 49% of them had cancer in the left breast; this finding is compatible with the results of another study.<sup>33</sup>

A large number of distinct mutations in the BRCA1 and BRCA2 genes have been reported through the world, and many methods have been reported for the study of BRCA mutations, including allele-specific oligonucleotide hybridization, allele-specific PCR, PCR-mediated site-directed mutagenesis, heteroduplex analysis (HDA), single-strand conformation polymorphism, and the protein truncation test.<sup>14</sup> Identification of BRCA1 and BRCA2 mutations carriers is an important focus in prevention and early detection of the breast cancer risk. In this study, we used the Mutagenically Separated PCR method to detect the 6174delT mutation in BRCA2 gene among Libyan patients with breast cancer; this method is considered to be a simple and reliable, and can be considered for routine use, but it needs high-resolution electrophoresis to detect this mutation. We observed in our study that neither the patients (67 cases with sporadic breast cancer and 18 familial breast cancer patients) nor the 5 healthy individuals had the 6174delT mutation in the BRCA2 gene by this simple method which requires high-resolution electrophoresis; however, the 6174delT mutation of the BRCA2 gene was not present in these samples which might be due to the small number of samples or the fact that the samples



were not taken from families carrying the BRCA2 susceptibility gene mutations. In addition, this mutation is present mostly in certain ethnic groups such as Ashkenazi Jews. However, our results are consistent with several studies that did not detect a 6174delT mutation in the BRCA2 gene in other populations, although they used different methods for detecting mutations in BRCA genes (data not shown).

In conclusion, the study indicated the absence of the 6174delT mutation of the BRCA2 gene in Libyan breast cancer patients and in controls. A complete BRCA2 gene sequence analysis might be necessary for identification of specific mutations in Libya, a country with an ethnically diverse population.

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#### Conflicts of interest

The authors state that no conflicts of interest exist.

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