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# Analysis of *BRCA1*/2 Mutations and Performance of Manchester Scoring System in High Risk Iranian Breast Cancer Patients: A Pilot Study

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## Abstract

**Background:** Breast cancer is the most frequent malignancy in Iranian women. Pathogenic mutations in *BRCA1* and *BRCA2* genes account for 20% to 25% of familial breast cancer. *BRCA1* and *BRCA2* mutation frequencies differ considerably among various geographic regions and ethnicities. Most studies have primarily used Caucasian populations to delineate the population and family risks associated with germline *BRCA1* and *BRCA2* mutations, leaving patients of other ancestries understudied. As genetic testing for *BRCA1* and *BRCA2* mutations is underused in Iran, it is of great importance to be able to describe the mutation spectrum of these genes and subsequently the genetic risks and testing benefits particular to Iranian population.
**Methods:** We designed a pilot study to identify the full spectrum of *BRCA1* and *BRCA2* sequence variations and large single or multi-exonic deletions in 20 Iranian breast cancer patients with a high likelihood of hereditary predisposition to breast cancer. Manchester score, as a validated scoring system for the probability of carrying a *BRCA1*/2 mutation, was calculated for all patients to determine the cut-off value for genetic testing in Iranian families.
**Results:** Two pathogenic [c.4566C > G (p.Tyr1522Ter), c.1961delA (p.Lys654Serfs*47)] and one likely pathogenic (c.5153-26A > G) variants in *BRCA1* and 2 pathogenic variants [c.8165C > G (p.Thr2722Arg), c.92G > A (p.Trp31Ter)] in *BRCA2* gene were identified. Assuming a Manchester score of 20 points as cut-off value to perform BRCA genetic testing, this scoring system has a sensitivity of 80%, specificity of 60%, positive predictive value (PPV) of 40%, and negative predictive value (NPV) of 90%.
**Conclusions:** Considering the high cost of testing in Iran, it seems that that the cut-off value of 20 points is more appropriate.

*Keywords:* Familial Breast Cancer, Manchester Score, Iran, *BRCA1*, *BRCA2*

## 1. Background

Breast cancer is the most frequent malignancy in women worldwide (1). In Iran, breast cancer is the most common malignancy among women with an estimated age-standardized incidence ratio (ASR) of 28.1 (1). In the United States, around 65% of all women newly diagnosed with breast cancer are > 55 years old, but in most low- and middle-income countries, including Iran, almost half of women with newly diagnosed breast cancer are < 50 years (2). The incidence of breast cancer among Iranian women is increasing. The results of a recent study showed that the rate of mortality from breast cancer in Iran has an increasing trend (3). In order to reduce breast cancer mortality and burden in Iran, preventive and screening programs for breast cancer are needed.

Gene mutations contribute to cancer in 2 ways. Oncogenic mutations that happen in a specific cell after birth are named “somatic cancer mutations” and considered a hallmark of cancer. Genes, in which germline mutations increase the risk of developing cancer, are called cancer predisposition genes. Germline mutations in cancer predisposition genes confer the high or moderate risks of cancer (> 2 fold relative risk) (4). It is estimated that about 3% to 5% of breast cancers are the result of germline mutations in cancer predisposition genes (5). *BRCA1* and *BRCA2* genes are 2 major high penetrance genes associated with early onset and familial breast and ovarian cancer (6, 7). Women with a germline mutation in *BRCA1* or *BRCA2* gene

have a lifetime risk of breast cancer of up to 70%, and once they are diagnosed with breast cancer, they are at high risk of developing second primary breast and ovarian cancers. Mutation carriers are also at increased risk of prostate, pancreas, and male breast cancer compared to the general population (8-10).

More than 3,000 different genetic variants have been reported in *BRCA1* and *BRCA2* universal mutation database (11). Despite the large number of carriers detected, only 9 *BRCA1* and 6 *BRCA2* de novo mutations have been identified (12). It is known that a strong negative fitness effect can result in a high de novo mutation rate (13). Numerous case-control studies between *BRCA1* and *BRCA2* carriers and non-carriers have shown no effect of these mutations on female fertility (14-16). With respect to the relatively high prevalence of *BRCA1* and *BRCA2* mutation carriers in the general population (about 1 in 420) and the absence of a negative fitness effect, it seems that most *BRCA1* and *BRCA2* mutations are inherited and outnumber de novo cases (17). Hereditary breast and ovarian cancer is mainly caused by heterozygous mutation in *BRCA1* or *BRCA2* genes (18). Biallelic mutations in *BRCA2* gene lead to Fanconi Anemia, which is characterized by bone marrow failure and predisposition to cancer (19). In contrast, there is only 1 report of biallelic mutation in *BRCA1* gene, identified in a developmentally delayed patient with early-onset ovarian cancer (20).

Several models and scoring systems have been developed to estimate the probability of carrying a *BRCA1* or *BRCA2* mutation on the basis of personal and family history of breast and/or ovarian cancer (21, 22). The accurate estimation of *BRCA1*/2 pathogenic mutation likelihood in index cases from families suspected of hereditary breast and ovarian cancer prior to genetic testing is crucial in determining which families should perform costly genetic tests. Manchester scoring system is easier and less time-consuming compared to computer-based models, which makes it more appropriate to use in clinical practice. It consists of 12 components with specific sub-scores for *BRCA1* and *BRCA2* genes, which are summed up to give a total score. Each component includes the number of breast, ovarian, prostate, and pancreatic cancers diagnosed at different ages in family members of index cases. Several studies have demonstrated the good predictive performance of Manchester scoring system (23-26). A combined score of 16 points is used for the 10% threshold and 20 points for the 20% threshold of *BRCA1*/2 mutation probability (24).

*BRCA1* and *BRCA2* mutation frequencies differ considerably among various geographic regions and ethnicities (27-29). Most studies have primarily used Caucasian populations to delineate the population and family risks associated with germline *BRCA1* and *BRCA2* mutations, leaving patients of other ancestries understudied. As genetic testing for *BRCA1* and *BRCA2*, mutations is underused in Iran, it is of great importance to be able to describe the mutation spectrum of these genes and subsequently the genetic risks and testing benefits particular to Iranian population. Assessment of the literature reporting *BRCA1* and *BRCA2* mutation frequencies in Iranian population raises concerns about the methodologies and various mutation ascertainment methods used. The identification of mutation carriers leads to the early detection and implementation of strategies to reduce the risk of breast cancer. Furthermore, the management of breast cancer patients who are mutation carriers is different from non-carriers (including bilateral mastectomy and oophorectomy) (30-32). In addition, the results of the TNT trial showed that patients with *BRCA1*/2 mutations have a greater response and a longer progression-free survival with carboplatin compared with docetaxel (33). Therefore, knowledge of mutation status may have an impact on adjuvant and later treatments, contralateral risk-reduction options, and eligibility for clinical trials. We designed a pilot study to identify the full spectrum of *BRCA1* and *BRCA2* sequence variations and large single or multi-exonic deletions in a cohort of Iranian breast cancer patients with a high likelihood of hereditary predisposition to breast cancer. Manchester score was calculated for all patients to determine the cut-off value for genetic testing in Iranian families.

## 2. Methods

Breast and/or ovarian cancer families were identified at Comprehensive Cancer Control Center, Tajrish Shohada Hospital in Tehran, Iran, from September 2014 to April 2017. Twenty index cases diagnosed with invasive breast cancer were selected from 135 unrelated breast and/or ovarian cancer families visited at cancer genetics clinic, with the following inclusion criteria: invasive breast cancer diagnosed $\leq$ 30 years, bilateral invasive breast cancer and both cancers diagnosed $\leq$ 40 years, metachronous, bilateral, or ipsilateral invasive breast cancer with $\geq$ 5 years interval and a first- or second-degree relative with breast cancer diagnosed $\leq$ 50 years, triple negative breast cancer diagnosed $\leq$ 40 years, personal history of epithelial ovarian cancer, a first- or second-degree relative with breast cancer and both cancers diagnosed $\leq$ 40 years, a first- or second-degree relative with epithelial ovarian cancer, male breast cancer with a first- or second-degree relative with breast or ovarian cancer, and a family history with a Manchester score greater than or equal to 16, as previously reported (24).

All study participants provided written informed consent for genetic testing. The study was approved by the

ethics committee of Shahid Beheshti University of Medical Sciences. Pre-test genetic counseling was offered to all patients. Genomic DNA was extracted from peripheral whole blood with QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. DNA quantitation was performed with BioPhotometer (Eppendorf, Hamburg, Germany). DNA purity was determined by A260/280 ratio. The entire coding sequence including exon-intron boundaries of *BRCA1* and *BRCA2* genes was amplified with published primers (34). The primers were synthesized by TAG Copenhagen (Copenhagen, Denmark). The PCR reactions were performed with HotStar Taq Master Mix Kit (QIAGEN, Hilden, Germany) on a Mastercycler (Eppendorf, Hamburg, Germany). The PCR products were sequenced bidirectionally with BigDye Terminator v3.1 Cycle Sequencing Kit on an ABI 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Sequencing traces were analyzed using Chromas 2.6 (Technelysium, Brisbane, Australia). Sequence traces were aligned to *BRCA1* RefSeqGene (LRG_292) and *BRCA2* RefSeqGene (LRG_293) sequences. Sequence variants were described in accordance with the recommendations of the human genome variation society (HGVS). The DNA sequence numbering is based on cDNA sequences for *BRCA1* (NM_007294.3) and *BRCA2* (NM_000059.3). Identified sequence variants were interpreted as pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign, and benign according to American college of medical genetics and genomics (ACMG) guideline (35).

Large single or multi-exonic deletion testing of *BRCA1* and *BRCA2* genes with Multiplex Ligation-dependent Probe Amplification (MLPA) method was performed for patients with no identified pathogenic or likely pathogenic sequence variant by Sanger sequencing. MLPA analysis was performed, using probemix P002 *BRCA1* and P090 *BRCA2* (MRC Holland, Amsterdam, The Netherlands). MLPA results were confirmed by probemix P087 *BRCA1* and P077 *BRCA2*. All MLPA experiments were performed in duplicate, using a Mastercycler (Eppendorf, Hamburg, Germany). Three reference samples (no personal or family history of cancer) were included in each MLPA experiment. MLPA PCR products were separated, using an ABI 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The results were interpreted, using Coffalyser.Net (MRC Holland, Amsterdam, The Netherlands). Post-test genetic counseling was offered to those patients with an identified pathogenic or likely pathogenic sequence variant or large deletion in *BRCA1* or *BRCA2* genes.

## 3. Results

The characteristics of 20 unrelated index patients are shown in Table 1. All of the index cases were female, except for patient 13. The mean age at cancer diagnosis of female index patients was 42 years (range 28 - 57 years). Breast cancer was diagnosed in the male index patient at 66 years. Eleven patients were presented with invasive ductal carcinoma, and 2 patients had triple negative breast cancer. Patient 3 had ER/PR negative breast tumor; HER2 immunohistochemistry was 2+, but HER2 in situ hybridization had not been performed. The pathology report of patient 16 was not available. Patients 2 and 17 were not able to present ovarian cancer pathology reports of their first- and second-degree relatives, respectively. Patients 6 and 20 were diagnosed with metachronous bilateral breast cancer with 6 year interval.

The sequence variants identified in index patients are shown in Tables 2 and 3. Pathogenic or likely pathogenic variants were identified in 5 patients. Patient 1 was carrier of a missense pathogenic mutation in exon 18 of *BRCA2* gene: c.8165C > G (p.Thr2722Arg). Patient 3 carried a likely pathogenic mutation in intron 18 of *BRCA1* gene: c.5153-26A > G. Patient 9 was carrier of a nonsense pathogenic mutation in exon 15 of *BRCA1* gene: c.4566C > G (p.Tyr1522Ter). The MLPA analysis of patient 12 using P002 *BRCA1* probemix revealed exon 2 deletion (Figure 1). This patient carried a variant of uncertain significance in exon 2 of *BRCA1* gene: c.16C > T (p.Leu6Phe). Further analysis using MLPA probemix P087 did not confirm the deletion of *BRCA1* exon 2. Patient 16 was carrier of a frameshift pathogenic mutation in exon 11 of *BRCA1* gene: c.1961delA (p.Lys654Serfs*47) (Figure 2). Patient 19 carried a nonsense pathogenic mutation in exon 3 of *BRCA2* gene: c.92G > A (p.Trp31Ter). Five variants of uncertain significance (VUS) in *BRCA1* gene and 3 variants of uncertain significance in *BRCA2* gene were identified.

## 4. Discussion

Breast cancer imposes a significant health problem in Iran; therefore, dedicated national programs are needed for cancer prevention and early diagnosis. Pathogenic mutations in *BRCA1* and *BRCA2* genes account for 20% to 25% of familial forms of breast cancer. Professional organizations in Europe and North America have published clinical practice guidelines for BRCA genetic testing and management (36, 37). The utilization of BRCA testing in clinical practice has resulted in a large increase of variant classification burden among laboratories. Variability between genetic code of different individuals is common within the general population and between individuals of different ethnic origin,

**Table 1.** Clinical Characteristics of Index Patients and Their Families

| Index Patient (IARC Score) | Age at Breast Ca Diagnosis (Pathology) | Epithelial Ovarian Ca in Index Patient (Pathology) | No. of Female Breast Ca in Family | No. of Epithelial Ovarian Ca in Family (Pathology) | No. of Male Breast Ca in Family | Triple Negative Breast Ca | Bilateral BC | Manchester Score (Pathology-Adjusted) |
|---|---|---|---|---|---|---|---|---|
| **1 (*BRCA2*+) (5)** | 43 (Mixed IDC& ILC) | - | 3 | 0 | 0 | - | - | 20 (21) |
| **2** | 37 (DCIS & Invasive papillary Ca) | - | 1 | 1 (NA) | 0 | - | - | 21 (19) |
| **3 (*BRCA1*+) (4)** | 30 (Invasive medullary Ca) | - | 2 | 1 (Serous cystadenocarcinoma) | 0 | NA | - | 25 (26) |
| **4** | 36 (IDC) | - | 2 | 0 (Dysgerminoma) | 0 | - | - | 10 (6) |
| **5** | 40 (IDC) | - | 2 | 0 | 0 | + (Grade 3) | - | 10 (14) |
| **6** | 43 (Undifferentiated Ca) | - | 1 | 0 | 0 | - | + (6-y interval) | 14 (10) |
| **7** | 41 (IDC) | - | 2 | 0 | 0 | - | - | 14 (10) |
| **8** | 51 (Medullary Ca) | - | 2 | 0 | 0 | + | - | 8 (9) |
| **9 (*BRCA1*+) (5)** | 42 (IDC) | + (Papillary serous cystadenocarcinoma of both ovaries) | 3 | 1 | 0 | + (Grade 3) | - | 31 (35) |
| **10** | 29 (IDC) | - | 3 | 0 | 0 | - | - | 33 (29) |
| **11** | 45 (IDC) | - | 3 | 0 | 0 | - | - | 18 (17) |
| **12** | 57 (IDC) | + (Endometrioid Ca) | 2 | 1 | 0 | - | - | 23 (24) |
| **13** | 66 (IDC) | - | 1 | 0 | 1 | - | - | 18 (17) |
| **14** | 44 (IDC) | - | 2 | 0 | 1 | - | - | 23 (19) |
| **15** | 39 (IDC) | - | 3 | 0 | 0 | - | - | 30 (26) |
| **16 (*BRCA1*+) (5)** | 28 (NA) | - | 3 | 0 | 0 | NA | - | 13 (NA) |
| **17** | 40 (Invasive Tubulolobular Ca) | - | 2 | 1 (NA) | 0 | - | - | 25 (24) |
| **18** | 57 (IDC) | - | 3 | 0 | 0 | - | - | 16 (15) |
| **19 (*BRCA2*+) (5)** | 39 (mixed mucinous carcinoma and IDC) | - | 2 | 0 | 2 | - | - | 31 (30) |
| **20** | 53 (Comedocarcinoma) | - | 2 | 0 | 0 | NA | + (6-y interval) | 16 (NA) |

Abbreviations: DCIS, ductal carcinoma in situ; IARC, International Agency for Research on Cancer; IDC, Invasive ductal carcinoma; ILC, invasive lobular carcinoma; NA, Not available, 6-y: 6-year.

and this intrinsic variability can lead to difficulties in interpreting some types of sequence change. Variants of uncertain significance (VUS) represent a particular challenge since it is not possible to infer the clinical significance from sequence information alone. Most VUS are not associated with a high risk of cancer, but misinterpretation of VUS can

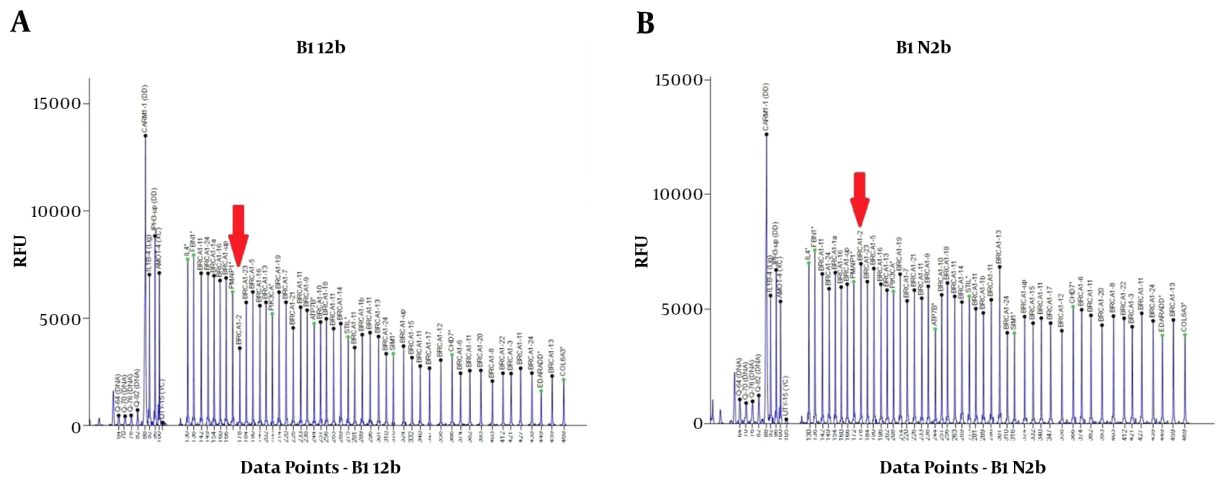


**Figure 1.** MLPA Analysis of Patient 12 Using Probemix P002 *BRCA1* Identified an Exon 2 deletion. Further analysis with probemix P087 *BRCA1* did not confirm this finding. A is fragment analysis of patient 12 using probemix P002 *BRCA1*, which shows deletion of *BRCA1* exon 2 compared to normal (B).

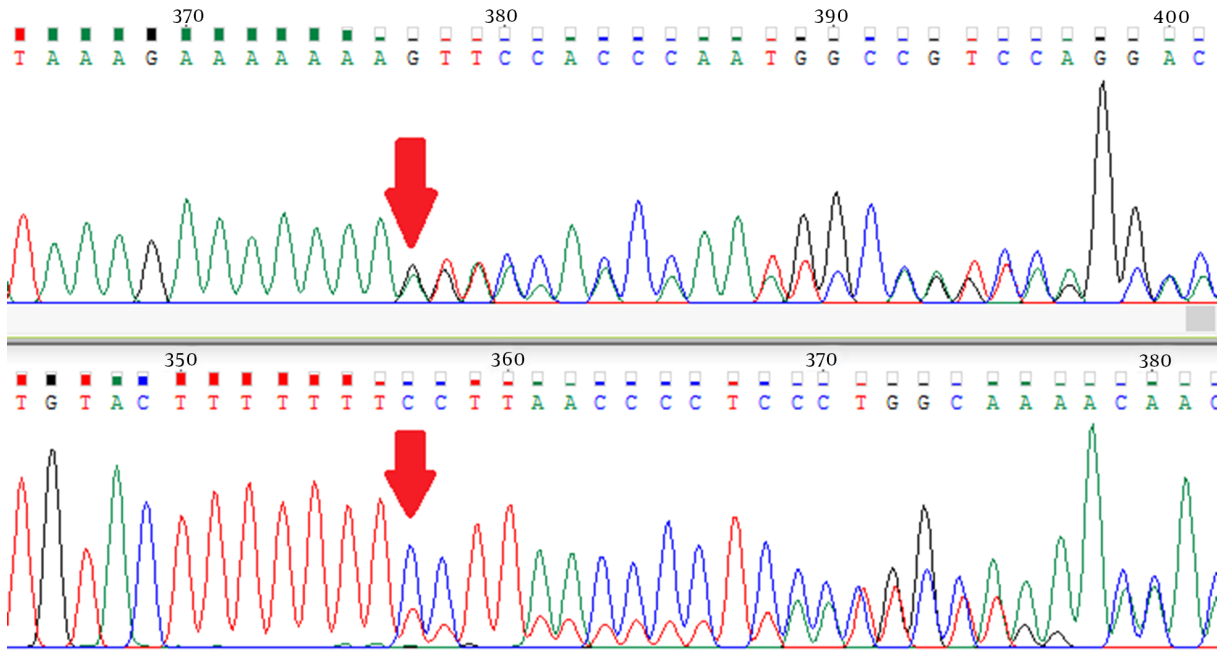


**Figure 2.** Sequencing Analysis of Exon 11 of *BRCA1* Gene in Patient 16 Identified a Pathogenic Variant (*BRCA1* c.1961delA; p.Lys654Serfs*47). Upper and lower figures are forward and reverse sequencing traces, respectively.

lead to mismanagement of both the patients and their relatives.

In the present study, we performed full sequencing and large deletion analysis of *BRCA1* and *BRCA2* genes in index patients of 20 high risk Iranian breast cancer families. Two pathogenic and one likely pathogenic variants in *BRCA1* gene and 2 pathogenic variants in *BRCA2* gene were identified. The MLPA analysis of patient 12 using P002 *BRCA1* probemix showed an exon 2 deletion, but subsequent analysis with P087 *BRCA1* probemix did not confirm this finding. This patient carried a VUS in exon 2 of *BRCA1* gene: c.16C > T (p.Leu6Phe). It is possible that this sequence variant prevented the attachment of P002 exon 2 probe, but not P087 exon 2 probe. Therefore, it is necessary to always con-

**Table 2.** *BRCA1* Gene Variations Detected and Their Clinical Classification

| Location | Variant | Classification | Zygosity (No. of Patients) |
|---|---|---|---|
| **5´ UTR** | c.-134T > C | Benign | Heterozygous (11) |
| **Exon 2** | c.16C > T (p.Leu6Phe) | Uncertain Significance | Heterozygous (1) |
| **Intron 3** | c.134 + 112G > A | Uncertain Significance (VUS) | Heterozygous (2) |
| **Intron 7** | c.442-34C > T | Benign | Heterozygous (10) |
| | | | Homozygous (1) |
| **Intron 8** | c.548-58delT | Benign | Heterozygous (8) |
| | | | Homozygous (1) |
| **Exon 11** | c.1067A > G (p.Gln356Arg) | Benign | Heterozygous (3) |
| **Exon 11** | c.1961delA (p.Lys654Serfs*47) | Pathogenic | Heterozygous (1) |
| **Exon 11** | c.2077G > A (p.Asp693Asn) | Benign | Heterozygous (2) |
| **Exon 11** | c.2082C > T (p.Ser694 =) | Benign | Heterozygous (10) |
| | | | Homozygous (1) |
| **Exon 11** | c.2311T > C (p.Leu771 =) | Benign | Heterozygous (10) |
| | | | Homozygous (1) |
| **Exon 11** | c.2612C > T (p.Pro871Leu) | Benign | Heterozygous (10) |
| | | | Homozygous (2) |
| **Exon 11** | c.3113A > G (p.Glu1038Gly) | Benign | Heterozygous (10) |
| | | | Homozygous (1) |
| **Exon 11** | c.3119G > A (p.Ser1040Asn) | Benign | Heterozygous (1) |
| **Exon 11** | c.3548A > G (p.Lys1183Arg) | Benign | Heterozygous (10) |
| | | | Homozygous (1) |
| **Intron 12** | c.4185 + 112C > A | Benign | Heterozygous (1) |
| **Exon 13** | c.4308T > C (p.Ser1436 =) | Benign | Heterozygous (10) |
| | | | Homozygous (1) |
| **Intron 13** | c.4357 + 116G > A | Uncertain Significance (VUS) | Heterozygous (1) |
| **Intron 13** | c.4358-77A > C | Uncertain Significance (VUS) | Heterozygous (1) |
| **Intron 14** | c.4485-63C > G | Benign | Heterozygous (10) |
| | | | Homozygous (1) |
| **Exon 15** | c.4566C > G (p.Tyr1522Ter) | Pathogenic | Heterozygous (1) |
| **Exon 16** | c.4837A > G (p.Ser1613Gly) | Benign | Heterozygous (10) |
| | | | Homozygous (1) |
| **Intron 16** | c.4986 + 70G > C | Uncertain Significance (VUS) | Heterozygous (1) |
| **Intron 18** | c.5152 + 66G > A | Benign | Heterozygous (10) |
| | | | Homozygous (1) |
| **Intron 18** | c.5153-26A > G | Likely Pathogenic | Heterozygous (1) |
| **Intron 20** | c.5278-191A > T | Benign | Heterozygous (9) |
| | | | Homozygous (1) |
| **Intron 22** | c.5407-54T > G | Benign | Homozygous (1) |

Abbreviations: UTR, untranslated region; VUS, variant of uncertain significance.

**Table 3.** *BRCA2* Variations Detected and Their Clinical Classification

| Location | Variant | Classification | Zygosity (No. of Patients) |
|---|---|---|---|
| **5´ UTR** | c.-26G > A | Benign | Heterozygous (9) |
| | | | Homozygous (3) |
| **Exon 2** | c.125A > G (p.Tyr42Cys) | Benign | Heterozygous (1) |
| **Intron 2** | c.67 + 82C > G | Likely Benign | Heterozygous (1) |
| | | | Homozygous (1) |
| **Exon 3** | c.92G > A (p.Trp31Ter) | Pathogenic | Heterozygous (1) |
| **Intron 3** | c.316 + 18G > A | Uncertain Significance (VUS) | Heterozygous (1) |
| **Intron 8** | c.681 + 56C > T | Benign | Heterozygous (3) |
| **Exon 10** | c.1114A > C (p.Asn372His) | Benign | Heterozygous (9) |
| | | | Homozygous (1) |
| **Exon 11** | c.3396A > G (p.Lys1132 =) | Benign | Heterozygous (12) |
| | | | Homozygous (3) |
| **Exon 11** | c.3516G > A (p.Ser1172 =) | Benign | Heterozygous (1) |
| **Exon 11** | c.3807T > C (p.Val1269 =) | Benign | Heterozygous (9) |
| **Exon 11** | c.4563A > G (p.Leu1521 =) | Benign | Homozygous (18) |
| **Exon 11** | c.5660C > T (p.Thr1887Met) | Uncertain Significance (VUS) | Heterozygous (1) |
| **Exon 11** | c.6131G > C (p.Gly2044Ala) | Uncertain Significance (VUS) | Heterozygous (1) |
| **Exon 11** | c.6513G > C (p.Val2171 =) | Benign | Homozygous (20) |
| **Intron 11** | c.6841 + 24G > A | Likely Benign | Heterozygous (1) |
| **Exon 12** | c.6935A > T (p.Asp2312Val) | Benign | Heterozygous (1) |
| **Intron 12** | c.6938-120T > C | Benign | Homozygous (20) |
| **Exon 14** | c.7242A > G (p.Ser2414 =) | Benign | Heterozygous (10) |
| | | | Homozygous (2) |
| **Intron 16** | c.7806-14T > C | Benign | Heterozygous (9) |
| | | | Homozygous (3) |
| **Exon 18** | c.8165C > G (p.Thr2722Arg) | Pathogenic | Heterozygous (1) |
| **Intron 19** | c.8487 + 82G > A | Benign | Heterozygous (1) |
| **Intron 21** | c.8754 + 102T > C | Likely Benign | Heterozygous (1) |
| **Intron 21** | c.8755-66T > C | Benign | Heterozygous (11) |
| | | | Homozygous (3) |
| **Intron 24** | c.9257-16T > C | Benign | Heterozygous (2) |
| **Exon 27** | c.9976A > T (p.Lys3326Ter) | Benign | Heterozygous (1) |
| **Exon 27** | c.10110G > A (p.Arg3370 =) | Likely Benign | Heterozygous (2) |
| **3´ UTR** | c.*105A > C | Benign | Heterozygous (9) |

Abbreviations: UTR, untranslated region, VUS, variant of uncertain significance.

firm MLPA findings with another probemix. A frameshift mutation in exon 11 of *BRCA1* gene was identified in patient 16: c.1961delA (p.Lys654Serfs*47). She had a personal history of breast cancer at age 28, a paternal aunt with breast cancer at age 70, another paternal aunt with uterine cancer at age 42, and a paternal cousin with uterine cancer at age 39 and breast cancer at age 44. This patient had a deletion of nucleotide adenine at position 1961, which led to a frameshift change in the coded amino acid from Lysine to Serine at position 654 and a subsequent premature termi-

nation of *BRCA1* translation 47 codons later (38-40).

A nonsense mutation in exon 15 of *BRCA1* gene was identified in patient 9: c.4566C > G (p.Tyr1522Ter). She had a personal history of breast cancer at age 42 and epithelial ovarian cancer at age 43, a sister with breast cancer at age 40, and a paternal aunt with breast cancer at age 42. This patient carried a C > G substitution at position 4566, which led to the premature termination of *BRCA1* translation (38, 39, 41). A splicing mutation in intron 18 of *BRCA1* gene was identified in patient 3: c.5153-26A > G. She had a personal history of breast cancer at age 30, and her mother was diagnosed with breast cancer at age 58 and epithelial ovarian cancer at age 59. This patient carried an A > G substitution in intron 18 of *BRCA1* gene. The analysis of this variant with Human Splicing Finder version 3.0 identified a cryptic new acceptor site at position -37 (42). MutationTaster software identified this variant as disease causing (43). Allele G has neither been found in ExAC (Exome Aggregation Consortium) nor in 1000 Genome projects. COVAR project (COsegregation of VARiants in the *BRCA1*/2 Gene) by Institute Curie has classified this variant as likely pathogenic on the basis of its family co-segregation (41).

A nonsense mutation in exon 3 of *BRCA2* gene was identified in patient 19: c.92G > A (p.Trp31Ter). She had a personal history of breast cancer at age 39, a paternal uncle with breast cancer at age 46, and another paternal uncle with breast cancer at age 80. One of her paternal cousins was diagnosed with breast cancer at age 35. This patient carried a G > A substitution at position 92, which resulted in premature termination of *BRCA2* translation (39, 44). A missense mutation in exon 18 of *BRCA2* gene was identified in patient 1: c.8165C > G (p.Thr2722Arg). She had a personal history of breast cancer at age 39, a sister with breast cancer at age 41, and her mother was diagnosed with breast cancer at age 45. She also had a maternal aunt with uterine cancer at age 53. This patient carried a C > G substitution at position 8165, leading to substitution of amino acid threonine at position 2722 by arginine. This amino acid is located in the DNA binding domain (DBD) of *BRCA2* protein and is considered a pathogenic variant (44, 45).

Assuming a Manchester score of 16 points as cut-off value (10% cut-off) to perform BRCA genetic testing, this scoring system has a sensitivity of 80%, specificity of 33%, positive predictive value (PPV) of 29%, and negative predictive value (NPV) of 83%. On the other hand, when the cut-off value of 20 points (20% cut-off) is chosen, this scoring system has a sensitivity of 80%, specificity of 60%, PPV of 40%, and NPV of 90%. This is similar to the findings of other published studies. Antoniou et al., by analysis of 2140 families in the UK, showed that at 10% cut-off, Manchester score had a sensitivity of 92.3%, specificity of 33.4%, PPV of 24.4%, and NPV of 94.9%. But, when 20% cut-off was chosen, Manchester score had a sensitivity of 87.1%, specificity of 43.4%, PPV of 26.4%, and NPV of 93.6% (26). Similarly, Kast et al. by analysis of 9,390 families in Germany, showed that sensitivity of Manchester score at 10% mutation probability cut-off was 92.2% and specificity was 25.4% (23).

In conclusion, in the present study, we identified 5 different *BRCA1* and *BRCA2* pathogenic or likely pathogenic mutations in 20 index patients from high risk Iranian breast cancer families. Considering the high cost of BRCA testing in Iran, it seems that Manchester score cut-off value of 20 points is more appropriate in Iranian population. As this scoring system is not capable of identifying all BRCA mutation carriers, a complementary set of criteria similar to the current study and recommendations of other organizations is needed.

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## Footnotes

**Authors' Contribution:** Sanaz Tabarstani and Mohammad Esmaeil Akbari designed the study. Mohammad Esmaeil Akbari, Mona Malekzadeh Moghani, and Leyla Shojaee sent patients for genetic counseling. Sanaz Tabarestani performed genetic counseling. Sanaz Tabarestani and Marzieh Motallebi performed laboratory tests. Sanaz Tabrestani analyzed the genetic data and wrote the manuscript.

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