RESEARCH ARTICLE

BRCA1 and BRCA2 Common Mutations in Iranian Breast Cancer Patients: a Meta Analysis

Mohammad Forat-Yazdi¹, Hossein Neamatzadeh²*, Mohammad Hasan Sheikhha³, Masoud Zare-Shehneh², Mortaza Fattahi³

Abstract

Background: To date several common mutations in BRCA1 and BRCA2 associated with breast cancer have been reported in different populations. However, the common BRCA1 and BRCA2 mutations among breast cancer patients in Iran have not been described in detail. Materials and Methods: To comprehensively assess the frequency and distribution of the most common BRCA1 and BRCA2 mutations in Iranian breast cancer patients, we conducted this meta-analysis on 13 relevant published studies identified in a literature search on PubMed and SID. Results: A total of 11 BRCA1 and BRCA2 distinct common mutations were identified, reported twice or more in the articles, of which 10 (c.2311T>C, c.3113A>G, c.4308T>C, c.4837A>G, c.2612C>T, c.3119G>A, c.3548A>G, c.5213G>A c.IVS16-92A/G, and c.IVS16-68A/G) mutations were in BRCA1, and 1 (c.4770A>G) was in BRCA2. The mutations were in exon 11, exon 13, intron 16, and exon 20 of BRCA1 and exon 11 of BRCA2. All have been previously reported in different populations. Conclusions: These meta analysis results should be helpful in understanding the possibility of any first true founder mutation of BRCA1/BRCA2 in the Iranian population. In addition, they will be of significance for diagnostic testing, genetic counseling and for epidemiological studies.

Keywords: Breast cancer - BRCA1 - BRCA2 - common mutations - Iran

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Introduction

Breast cancer is the most common cause of mortality among women accounting for 23% all cancers (Perez et al., 2009). In recent years, the mortality rate from breast cancer has increased rapidly in all countries. Breast cancer is the most prevalent cancer in Iranian women with an increase in incidence rates in recent years. In Iran, even though the prevalence of breast cancer is lower compared to western countries, it is the most common malignancy among women (Rezaianzadeh et al., 2011). There is an increasing trend for breast cancer mortality in Iran during 1995 to 2004 from 1.40 to 3.52 per 100,000 (Taghavi et al., 2012). In the next decades, Iran will face an upsurge in the incidence of the disease (Dey and Soliman, 2010).

Breast-ovarian cancer (BOC)-causing mutations and other genetic variants are distributed along the entire coding and non-coding regions of BRCA1 and BRCA2, and more than 3400 gene variants have been described in the Breast Cancer Information Core (Solano et al., 2012). New variants continue to be detected worldwide, mostly in BRCA1. Individuals with an inherited inactivating mutation in BRCA1 (MIM# 113705) or BRCA2 (MIM# 600185) have an increased risk of developing early-onset breast and ovarian cancers (Welch and King et al., 2001). Inherited mutations in BRCA1 account for 40-45% of all hereditary BC cases, but approximately 80% of cases in families with multiple cases of breast and ovarian cancers (Easton et al., 1999).

The aim of this study was to survey the spectrum of most common mutations among Iranian breast cancer patients.

Materials and Methods

Search strategy

The electronic databases PubMed, EMBASE, and Scientific Information Database (SID) were searched up to October 30, 2014. The final search strategy used for each database was based on keywords “BRCA1,” “BRCA2,” “Iran,” “Breast cancer,” and “polymorphism.” Synonyms and different styles of the search terms were also used in the search in order to obtain every relevant paper. Case-control studies containing available BRCA1 and BRCA2

¹Department of Internal Medicine, ²Department of Medical Genetics, Shahid Sadoughi Hospital, Hematology, Oncology and Genetics Research Center, Shahid Sadoughi University of Medical Sciences, ³Department of Cellular and Molecular Biology, Islamic Azad University Ashkzar Branch, Yazd, Iran *For correspondence: hn_1364@yahoo.com
genes mutations frequencies were chosen. Only research articles were included and the language was not limited. Reference lists of the included studies on related topics were also screened for additional studies.

Data extraction
Data were carefully and independently extracted from the relevant papers by two of the authors (HN and MZS) using the same standardized form. The following data were collected from each study: first author, year of publication, source of controls, and genotype distribution. In case of disagreement, a third reviewer assessed the articles until an agreement was reached. The following items were collected from each article: first author, publication year, country or region of study, objective of study, study design, number of patients, mutation carriers information, and the enrollment criteria describing study population, mutation scanning method, and the results of screening. Table 1 summarizes a synopsis of the included studies, briefly indicating the main characteristics. A flow diagram of the study selection process is shown in Figure 1.

Statistical analysis
The strength of association between common mutations in BRCA1 and BRCA2 and breast cancer risk was assessed for each study by using the pooled ORs with 95% CIs. For all studies, only the frequency of mutation which repeatedly reported twice or more in different articles was evaluated. Frequency of each BRCA mutation carriers was more than 1% in meta-analyses by using the number of patients with event mutation and the total number of patients. The statistical heterogeneity among studies was assessed with the I² test was used to quantify inconsistency. An I² value≥50% was considered to represent significant statistical heterogeneity. The fixed-effects model (the Mantel-Haenszel method) was used to calculate the pooled OR with 95% CI; Otherwise we applied the random effects model (the DerSimonian and Laird method) (Begg et al., 1994; Egger et al., 1997). These two models provided similar results when between-studies heterogeneity was absent. Funnel plots and the linear regression asymmetry test by Egger et al., were applied to evaluate potential publication bias (Egger et al., 1997). In these analyses, the ORs with 95% CIs were calculated with StatsDirect (v.3.0; StatsDirect, Ltd, Cheshire, UK) and Review Manage (v.4.2; Oxford, England), using two-sided P values.

Results
Study characteristics
Our systematic literature search identified 22 studies that met the inclusion criteria. After deduplication and exclusion of the clearly irrelevant studies, we eventually included 13 studies (Bar-Sade et al., 1998; Ghaderi et al., 2001; Yassaee et al., 2002; Moslehi et al., 2003; Pietschmann et al., 2005; Quintana-Murci et al., 2005; Mehdipour et al., 2006; Rassi et al., 2008; Fattahi et al., 2009; Saleh gohari et al., 2012, Keshavarzi et al., 2012; Keshavarzi et al., 2013) involving 1183 breast cancer patients. Figure 1 shows the study selection process.

Baseline characteristics of the 13 studies are summarized in the included studies, briefly describing study population, mutation scanning method, mutation carriers information, and the enrollment criteria of families and subjects (Tables 1, 2). The 13 studies included report on 7 different screening methods for the detection of BRCA1 and BRCA2 mutations: In these studies, the diagnosis of breast cancer was based on the presence of a palpable mass, mammographic density, mammographic abnormality, or clinical examination.

Table 1. Characteristics of All 13 Studies Retrieved with the Searching Strategy for BRCA1

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>NO.</th>
<th>Age(yr)</th>
<th>Study population</th>
<th>Technique</th>
<th>Exo/Int</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ghaderi et al</td>
<td>2001</td>
<td>22</td>
<td>37</td>
<td>(BC) 18 (control)</td>
<td>Direct sequencing</td>
<td>2, 11, 20</td>
</tr>
<tr>
<td>Yassaee et al</td>
<td>2002</td>
<td>83</td>
<td>&lt;45</td>
<td>Family with 4 cases (HOBS)</td>
<td>SSCP-PCR</td>
<td>2</td>
</tr>
<tr>
<td>Moslehi et al</td>
<td>2003</td>
<td>442</td>
<td>45-67</td>
<td>(only males)</td>
<td>SSCP-PCR</td>
<td>2</td>
</tr>
<tr>
<td>Pietschmann et al</td>
<td>2005</td>
<td>10</td>
<td>56</td>
<td>high risk breast cancer families</td>
<td>RFLP-PCR</td>
<td>2</td>
</tr>
<tr>
<td>Mehdipour et al</td>
<td>2006</td>
<td>396</td>
<td>48.8±11.3</td>
<td>Female, 4(Male)</td>
<td>Direct sequencing</td>
<td>16, 17, 18, 24</td>
</tr>
<tr>
<td>Rassi et al</td>
<td>2008</td>
<td>16</td>
<td>15-95, 25-80</td>
<td>(FBC), 18(NFBC)</td>
<td>PCR</td>
<td>2</td>
</tr>
<tr>
<td>Fattahi et al</td>
<td>2009</td>
<td>250</td>
<td>45.1±9.2</td>
<td>(SBC), 55(FBC), 200(HF)</td>
<td>Multiplex-PCR</td>
<td>2</td>
</tr>
<tr>
<td>Keshavarzi et al</td>
<td>2011</td>
<td>27</td>
<td>≥35</td>
<td>(BC), 50(HF)</td>
<td>Direct sequencing</td>
<td>7, 9, 11, 13, 16, 20</td>
</tr>
<tr>
<td>Saleh-gohari et al</td>
<td>2012</td>
<td>22</td>
<td>51</td>
<td>(female), 8 (male)</td>
<td>Direct sequencing</td>
<td>2, 11, 11</td>
</tr>
<tr>
<td>Keshavarzi et al</td>
<td>2012</td>
<td>36</td>
<td>≥35</td>
<td>(FBC), 49(NFBC), 61(Control)</td>
<td>Direct sequencing</td>
<td>16, 20</td>
</tr>
<tr>
<td>Kooshyar et al</td>
<td>2013</td>
<td>39</td>
<td>49.3</td>
<td>(BC) 29(HR)</td>
<td>SSCP-PCR</td>
<td>2</td>
</tr>
</tbody>
</table>

Figure 1. Flow Diagram of the Study Selection Process

BRCA1 and BRCA2 Common Mutations in Iranian Breast Cancer Patients: A Meta Analysis

Methods, Direct sequencing was more than one evaluation study identified and the distribution of these screening methods was as follows: 9 DHPLC, 7 SSCP, 6 PTT, 4 HA, 2 DGGE, 2 CSGE and 4 studies conducted sequencing to confirm each germline BRCA1/2 variant. As shown in Figure 2, 11 common mutations were identified from all 13 studies, at exon 11, exon 13, intron 16, and exon 20 of BRCA1 and exon 11 of BRCA2.

To evaluate the frequency of 11 common mutations which repeatedly reported twice or more in different articles, we conducted meta-analysis and the results were shown in Table 1. For those 11 mutations, 4 studies had repeatedly reported the c.2311T>C, c.3113A>G, c.4308T>C, c.4837A>G, and c.2311T>C, 3 studies had repeatedly reported the c.2612C>T, and c.3119G>A, and 2 studies had repeatedly reported the c.3548A>G, c.IVS16-92A/G, c.IVS16-68A/G, and c.5213G>A. For those mutations which were not repeatedly reported in all 13 studies, we only selectively listed the common mutations (frequency more than 5%) in our study. For the BRCA1 (Figure 3), after conducting meta-analysis, we found that the overall frequency of c.3113A>G was 0.3 (95% CI 0.24-0.44, p=0.00 for heterogeneity test), the frequency of c.2311T>C was 0.13 (95% CI 0.08-0.20, p=0.00 for heterogeneity test), the frequency of c.2612C>T was 0.27 (95% CI 0.17-0.57, p=0.00 for heterogeneity test), the frequency of c.3548A>G was 0.24 (95% CI 0.01-0.13, p=0.00 for heterogeneity test), the frequency of c.4837A>G was 0.20 (95% CI 0.14-0.28, p=0.00 for heterogeneity test), and the

Table 2. Characteristics of All 13 Studies Retrieved with the Searching Strategy for BRCA2

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>NO.</th>
<th>Age(yr)</th>
<th>Technique</th>
<th>Exo/Int</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yassaee et al</td>
<td>2002</td>
<td>83</td>
<td>&lt;45</td>
<td>PTT, DS, SSCP/HA</td>
<td>11, 17, 18, 23</td>
</tr>
<tr>
<td>Moslehi et al</td>
<td>2003</td>
<td>1</td>
<td>Family with 4 cases (HOBS)</td>
<td>56</td>
<td>PTT</td>
</tr>
<tr>
<td>Pietschmann et al</td>
<td>2005</td>
<td>20</td>
<td>50</td>
<td>Direct sequencing</td>
<td>2, 8, 10, 11, 14, 16, 21</td>
</tr>
<tr>
<td>Fattahi et al</td>
<td>2009</td>
<td>55</td>
<td>45.1±9.2</td>
<td>Multiplex-PCR</td>
<td>2, 5</td>
</tr>
</tbody>
</table>

Figure 2. Position of Common Mutations with in BRCA1 in Iranian BC Patients

Methods, Direct sequencing was more than one evaluation study identified and the distribution of these screening methods was as follows: 9 DHPLC, 7 SSCP, 6 PTT, 4 HA, 2 DGGE, 2 CSGE and 4 studies conducted sequencing to confirm each germline BRCA1/2 variant.

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Table 3. BRCA1 and BRCA2 Common Mutations Identified in 13 Studies

<table>
<thead>
<tr>
<th>BRCA1 mutation</th>
<th>Exon/intron</th>
<th>Mutation effect</th>
<th>n/Na</th>
<th>Frequency</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.2311T&gt;C</td>
<td>11</td>
<td>Synonymous</td>
<td>17/154</td>
<td>0.131</td>
<td>Ghaderi et al., 2001; Pietschmann et al., 2005; Keshavarzi et al., 2011; Keshavarzi et al., 2012</td>
</tr>
<tr>
<td>c.2612C&gt;T</td>
<td>11</td>
<td>Missense</td>
<td>18/69</td>
<td>0.269</td>
<td>Ghaderi et al., 2001; Pietschmann et al., 2005; Keshavarzi et al., 2011</td>
</tr>
<tr>
<td>c.3113A&gt;G</td>
<td>11</td>
<td>Missense</td>
<td>35/105</td>
<td>0.334</td>
<td>Ghaderi et al., 2001; Pietschmann et al., 2005; Keshavarzi et al., 2011; Keshavarzi et al., 2012</td>
</tr>
<tr>
<td>c.3119G&gt;A</td>
<td>11</td>
<td>Missense</td>
<td>26/132</td>
<td>0.202</td>
<td>Ghaderi et al., 2001; Pietschmann et al., 2005; Keshavarzi et al., 2011</td>
</tr>
<tr>
<td>c.3548A&gt;G</td>
<td>11</td>
<td>Missense</td>
<td>Oct-42</td>
<td>0.243</td>
<td>Ghaderi et al., 2001; Pietschmann et al., 2005</td>
</tr>
<tr>
<td>c.4308T&gt;C</td>
<td>13</td>
<td>Synonymous</td>
<td>26/154</td>
<td>0.175</td>
<td>Ghaderi et al., 2001; Pietschmann et al., 2005; Keshavarzi et al., 2011; Keshavarzi et al., 2012</td>
</tr>
<tr>
<td>c.4837A&gt;G</td>
<td>16</td>
<td>Missense</td>
<td>30/154</td>
<td>0.201</td>
<td>Ghaderi et al., 2001; Pietschmann et al., 2005; Keshavarzi et al., 2011; Keshavarzi et al., 2012</td>
</tr>
<tr>
<td>IVS16-92A/G</td>
<td>16</td>
<td>Unknown</td>
<td>6/42</td>
<td>0.147</td>
<td>Ghaderi et al., 2001; Pietschmann et al., 2005; Keshavarzi et al., 2011</td>
</tr>
<tr>
<td>IVS16-68A/G</td>
<td>16</td>
<td>Unknown</td>
<td>9/44</td>
<td>0.219</td>
<td>Ghaderi et al., 2001; Pietschmann et al., 2005</td>
</tr>
<tr>
<td>c.5213G&gt;A</td>
<td>20</td>
<td>Missense</td>
<td>9/112</td>
<td>0.096</td>
<td>Keshavarzi et al., 2011; Keshavarzi et al., 2012</td>
</tr>
<tr>
<td>BRCA2</td>
<td>4075delGT</td>
<td>Synonymous</td>
<td>5/122</td>
<td>0.045</td>
<td>Keshavarzi et al., 2011; Keshavarzi et al., 2012</td>
</tr>
</tbody>
</table>

*a n referred to the number of patients with event mutation, N referred to the total number of patients in total families; b These data were calculated through cumulative outcomes from all related articles; c These frequencies were obtained from meta-analysis results from all related articles
The majority of these 11 mutations described here are frequent in other populations. Recently, Medimegh et al., found that c.2082C>T, c.3113A>G, c.3119G>A, c.3548A>G and c.4837A>G SNPs were not associated with breast cancer disease (FBC or SBC) with P value>0.05.

The most commonly observed mutation in the present study was the c.3113A>G, which accounted for 29.8% of all common mutations. This mutation has previously been reported in various ethnic groups. However, Medimegh et al., have found c.3113A>G was not associated with breast cancer disease (Medimegh et al., 2014). In addition, Dombernowsky et al., in a large study, evaluated risk associated of breast and/or ovarian cancer by 9 missense polymorphisms in BRCA1 c.1067A>G, c.2612C>T, c.3113A>G, c.4837A>G, c.4956G>A and BRCA2 c.865A>C, c.1114A>C, c.4258G>T, and c.5744C>T. They found no association between heterozygosity or homozygosity for any of the nine polymorphisms and risk of breast and/or ovarian cancer in either study (Dombernowsky et al., 2009).

Medimegh et al., Wild-type alleles and genotypes of c.442-58 delT, c.2311T>C, c.2612 C>T and c.4308T>C are clearly associated with familial breast cancer with an odds ratio ranging from 2.49 to 4.66. They found that among the four associated SNPs to familial breast cancer, the c.2612 C>T variant could have an effect on the protein sequence with an amino acid change (Proline to Leucine) at position 871, suggesting an alteration on the protein function and an ambivalent role of wild allele to familial breast cancer susceptibility. However, Dombernowsky et al., found no association between c.2612 C>T and breast cancer (Dombernowsky et al., 2009).

The BRCA1 c.2311T>C missense mutation in exon 11 accounted for 4.5% of families with mutations. This mutation has previously been reported in various ethnic groups. Combination between TT genotype of c.3548A>G (p.Lys1183Arg) were more frequently present in breast cancer relatives belonging to families tested negative for BRCA1 and BRCA2 mutations (Pilato et al., 2011). Also, Cherbala et al., have reported that the c.3548A>G missense has high frequency in patients who were tested negative for BRCA1 and BRCA2 mutations in Algerian breast/ovarian cancer families (Cherbala et al., 2012).

We have found two unclassified intronic variants (IVS16-68 G>A, IVS16-92 G>A) that were reported twice or more in the studies. These two intronic mutations previously reported in Greek, Netherlands, Belgium, Argentina breast/ovarian cancer families and Singapore Malay women with early onset breast/ovarian cancer (Konstantopoulou et al., 2000; Sng et al., 2003). BRCA1 IVS16-68 G>A and IVS16-92 G>A have been reported previously in the BIC database, and they are among the top 20 mutation frequencies that have been described by...
Nevertheless, this meta-analysis also had some limitations. Firstly, the sample size for the association about BRCA1/2 and breast cancer common mutations and articles about Iranian populations are too small to provide strong information and more original studies are needed to further confirm our findings. Secondly, previous study only screened particular region of the BRCA1/2 genes might naturally exclude many potential common polymorphism in other region, so our results may not have sufficient statistical power to identify and analysis common polymorphism in the Iranian breast cancer patients.

Although there are some limitations in this meta-analysis, values of this study should also be highlighted. Firstly, as far as we know, this is the first meta-analysis to examine the common mutation of the BRCA genes in the Iranian breast cancer patients. Finally, any obvious evidence of publication bias could be detected in all genetic models.

In conclusion, this Meta-analysis results will be helpful to understand the possibility of the first true founder mutation of BRCA1/BRCA2 identified in the Iranian population, establish a genetic screening strategy, to provide individual risk assessment, and to design better therapeutic strategies in the Iranian population. However, the relatively high frequency of these mutations in the Iranian breast cancer patients cannot be explained by founder effects and It is possible that we have not yet identified all recurrent mutations that occur in Iran. Therefore, further population surveys of BRCA mutations will be necessary.

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References


