High Frequency of Germ-Line \textit{BRCA2} Mutations among Hungarian Male Breast Cancer Patients without Family History$^1$

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Abstract

To determine the contribution of \textit{BRCA1} and \textit{BRCA2} mutations to the pathogenesis of male breast cancer in Hungary, the country with the highest male breast cancer mortality rates in continental Europe, a series of 18 male breast cancer patients and three patients with gynecomastia were analyzed for germ-line mutations in both \textit{BRCA1} and \textit{BRCA2}. Although no germ-line \textit{BRCA1} mutation was observed, 6 of the 18 male breast cancer cases (33\%) carried truncating mutations in the \textit{BRCA2} gene. Unexpectedly, none of them reported a family history for breast/ovarian cancer. Four of six truncating mutations were novel, and two mutations were recurrent. Four patients (22\%) had a family history of breast/ovarian cancer in at least one first- or second-degree relative; however, no \textit{BRCA2} mutation was identified among them. No mutation was identified in either of the genes in the gynecomastias. These results provide evidence for a strong genetic component of male breast cancer in Hungary.

Introduction

Male breast cancer is a rare disease, comprising <1\% of all malignancies in men in the western world; however, in some developing areas, it is more frequent, accounting for 6–15\% of all breast cancers.$^3$ In continental Europe, the Hungarians have the highest male breast cancer mortality rates (1). The biggest risk factor for breast cancer in both genders seems to be inherited predisposition. Approximately 5–10\% of female breast cancer cases are due to inheritance of autosomal dominant susceptibility genes (2). In the vast majority of inherited cases, the recently isolated genes \textit{BRCA1} and \textit{BRCA2} are thought to be responsible for the disease (3). \textit{BRCA1} is estimated to account for most female breast/ovarian cancer families, but it can be infrequently detected in families with male breast cancer cases. However, breast-cancer-prone families in whom male breast cancer occurred have been shown to be linked to or have mutation in \textit{BRCA2} (4). Our earlier results did not reveal \textit{BRCA2} carrier male breast cancer patients in Hungarian breast cancer families, but two individuals without family history were found to carry \textit{BRCA2} mutations (5).

Very little is yet known about the genetic background of male breast cancer. Somatic mutations of the $p53$ gene were identified in 41\% of male breast cancer patients studied (6). Defects in only a few genes have been associated with predisposition to the development of this disease. Genetic abnormalities of the androgen receptor have been reported in association with male breast cancer cases (7). There are some reports of \textit{BRCA1} mutation involvement in male breast cancer (8–10), but in all cases the patients with male breast cancer were members of families carrying \textit{BRCA1} mutation. There is much more evidence that inherited \textit{BRCA2} mutations increase the risk for developing male breast cancer. Several families that also contained male breast cancer patients were reported to carry \textit{BRCA2} mutations (4, 11–13). Men who carry a germ-line \textit{BRCA2} mutation have an increased risk of breast cancer (14).

Although men with breast cancer also often have gynecomastia, it is still unknown whether gynecomastia per se predisposes the male breast to malignant disease. Some authors report that as many as 20–36\% of the male breast cancer patients in their studies have a history of gynecomastia (15, 16).

In the present study, we have determined the contribution of germ-line mutations in the \textit{BRCA1} and \textit{BRCA2} genes to the pathogenesis of male breast cancer in Hungary by screening a series of 18 unselected male breast cancer patients and three patients with gynecomastia.

Materials and Methods

Patients. Blood samples were collected from all consenting patients with male breast cancer or gynecomastia at the National Institute of Oncology (Budapest, Hungary) in the period of 1996–1998. The histopathological typing of the cases was completed by board-certified pathologists. This panel represents consecutive cases who were not selected for family history of breast/ovarian cancer; however, information on family history was available for all patients. The patients with gynecomastia did not have a medical history of Klinefelter syndrome.

\textit{BRCA1} and \textit{BRCA2} Mutation Screening. Genomic DNA was extracted by standard methods from peripheral lymphocytes from all patients. PCR amplification of 160–550-bp DNA fragments covering the entire \textit{BRCA2} coding region and splice junctions was carried out by using a set of 44 primer pairs (5, 11). Similarly, all \textit{BRCA1} exons with the exception of exon 11 were amplified with a set of 23 primer pairs; exon 11 was screened in three overlapping protein truncation test fragments as reported previously (5). Mutation screening was performed in germ-line DNA by combined multiple heteroduplex analysis and/or by single-strand conformation analysis. Mutation screening in both genes was performed irrespective of identification of a mutation in one gene, because it is possible that mutations of both \textit{BRCA1} and \textit{BRCA2} may be segregating in a family, as we reported in a Hungarian breast/ovarian cancer patient (17). PCR products from variant conformers were purified using Wizard PCR Prep DNA Purification System (Promega Corp.) and sequenced using the Thermosequenase Cyclo Sequencing kit (USB-Amersham).

Results

The entire coding regions and intronic splice sites of both \textit{BRCA1} and \textit{BRCA2} were screened using multiple heteroduplex analysis/single-strand conformation analysis and protein truncation test, followed by direct sequencing of abnormalities. Six of the 18 male breast cancer cases (33\%) were observed to carry truncating mutations in the \textit{BRCA2} gene (Table 1). Four of the six truncating mutations were novel; the other two alterations (277delAC and 9326insA) were reported earlier (5, 18, 19). A review was conducted of the clinical and family history of the mutation carriers.
None of them reported a family history for breast/ovarian cancer. Two patients (11%) had a family history of breast/ovarian cancer in at least one first-degree relative and another two (11%) in at least one second-degree relative in this unselected study; however, no BRCA2 mutation was observed among them.

No germ-line BRCA1 mutations were detected in the male breast cancer cases, nor was a mutation identified in either BRCA1 or BRCA2 in the three cases of gynecomastia; however, one patient (G2) from this group carried an unclassified BRCA2 variant allele with unclear significance (Table 2). There was no difference between mutation carriers and noncarriers with respect to clinicopathological features nor age at diagnosis (Table 3). The average age at diagnosis was 62 years; there was only an insignificant difference between the mean age of onset of carriers (58 years) and noncarriers (64 years).

**Discussion**

In the first studies of BRCA2 analysis (4, 11), the subjects of scrutiny were mostly large families that sometimes also contained members with male breast cancer. In more recent reports (20–22), sets of population-based male breast cancer cases were studied for the frequency of germ-line BRCA2 carriers. Here we analyzed male breast cancer patients from a hospital-based study to determine the contribution of BRCA1 and BRCA2 mutations to the pathogenesis of male breast cancer in Hungary. Frequencies reported of BRCA2 mutations in male breast cancer vary between 4% (21) and 40% (23), the latter distribution of either mutation spectra in male breast cancers from other studies (Fig. 1) or of all BRCA2 mutations. Thus, there is no evidence to date that mutations in specific domains of BRCA2 may be associated with male breast cancer.

The 18 breast cancer patients screened for germ-line BRCA mutations were selected without any regard for family history. However, information on family history was available for all patients (Table 3). In the present study, the proportion of patients with family history of breast/ovarian cancer in at least one first-degree relative was 11% (2 of 18), and in at least one second-degree relative, also 11% (2/18). This is in agreement with the studies of Rosenblatt et al. (24) and Friedman et al. (21), who found similar proportions in their studies (18–10% and 17–13%, respectively). Surprisingly, no BRCA2 mutation was identified among the cases with family history; neither did the mutation carriers report family histories of breast/ovarian cancer. This is in contrast with the results of Couch et al. (11), who identified germ-line BRCA2 mutations in 7 of 50 (14%) male breast cancer cases, 6 of 7 (85%) of whom had a family history of breast cancer. Friedman et al. (21) found two mutation carriers in their panel, one of whom had a family history. Similarly to our results, Haraldsson et al. (22) reported recently that only one of seven BRCA2 mutation carriers had a positive family history of breast cancer.

No evidence for differences in clinicopathological features was seen between mutation carriers and noncarriers. Most patients had invasive ductal carcinoma (17 of 18; 95%), including all germ-line BRCA2 mutation carriers (Table 3). This is consistent with literature data that invasive ductal carcinomas account for 84–93% of cases. The average age of men at diagnosis of breast cancer is close to 65, about 5 years older than the average age for women.³ In our studies, the average age at diagnosis in carriers was not significantly lower than that of noncarriers. Compared with other BRCA2 mutations, 9326insA appears to be linked with younger age of onset in female breast cancer (26) and possibly also in male breast cancer (22).

No mutation was identified in either of the genes in the three cases of gynecomastia; however, one patient (G2) from this group carried a missense BRCA2 variant allele (7081A→G) with unclear significance. This alteration was reported eight times in the Breast Cancer Information Core as an unclassified variant and lies in a conserved region of the gene in human, rat, and mouse; however, expression analysis would be required to elucidate the nature of this type of alteration.

³ Unpublished data.
4 E. Olah et al., manuscript in preparation.
The data presented here imply that germ-line mutations in the \textit{BRCA2} gene are involved in the development of one-third of male breast cancer cases in Hungary. There may be other genetic factors that are responsible for some of the male breast cancers that cannot be explained by mutations in the \textit{BRCA2} gene. There is no evidence for a male breast cancer-specific cluster region in the \textit{BRCA2} gene thus far, but because the majority of \textit{BRCA2} mutations found in male breast cancer are unique and not identified in female breast cancer cases, it is possible that at least some \textit{BRCA2} mutations may predispose to male rather than female breast cancer. On the other hand, certain mutations (e.g., 9326insA) result in both male and female breast cancers and can be associated with various phenotypes in different families. This could be explained by modifying factors in disease development different in males and females. The first evidence of a modifying genetic factor of \textit{BRCA2} in male breast cancer was provided recently (27), reporting that an interstitial tandem duplication on 9p increases the risk of breast cancer in male patients conferred by \textit{BRCA2}. The lack of a positive family history in all carriers suggests that at least some \textit{BRCA2} mutations may have a lower penetrance than others, and selection of putative \textit{BRCA2} mutation carriers among male breast cancer patients cannot be based on the family background of the disease. Our data provide further evidence that in certain populations there is a high genetic component in the development of this disease, and in these populations all patients with male breast cancer, regardless of their family history, should be screened for \textit{BRCA2} mutations.

![Fig. 1. BRCA2 mutation spectrum in male breast cancer patients.](image)

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**Table 2. BRCA2 polymorphisms and unclassified variants**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Variant</th>
<th>Codon</th>
<th>Exon</th>
<th>Effect</th>
<th>BIC</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBC-10</td>
<td>5972 C/T</td>
<td>Thr1915Met</td>
<td>11</td>
<td>Missense</td>
<td>Reported 1 time (6%)</td>
<td>Frequent polymorphism</td>
</tr>
<tr>
<td>MBC-14, 15</td>
<td>IVS11 + 79del14</td>
<td>Intron 11</td>
<td>None</td>
<td>Reported 1 time (28%)</td>
<td>Frequent polymorphism</td>
<td></td>
</tr>
<tr>
<td>G2</td>
<td>7081 A/G</td>
<td>Ile2285Val</td>
<td>12</td>
<td>Missense</td>
<td>Reported 8 times</td>
<td>Rare polymorphism</td>
</tr>
</tbody>
</table>

* MBC, male breast cancer.
* IVS, intronic variant.
* G, Gynecomastia.

**Table 3. Clinicopathological characteristics of patients tested in this study**

<table>
<thead>
<tr>
<th>Patient</th>
<th>\textit{BRCA2} mutation</th>
<th>Phenotype of individual</th>
<th>Age at diagnosis</th>
<th>Family history of cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBC-1</td>
<td>No</td>
<td>Inv. ductal carcinoma</td>
<td>80</td>
<td>Negative</td>
</tr>
<tr>
<td>MBC-2</td>
<td>4232insA</td>
<td>Inv. ductal carcinoma</td>
<td>60</td>
<td>Negative</td>
</tr>
<tr>
<td>MBC-3</td>
<td>277delAC</td>
<td>Inv. ductal carcinoma</td>
<td>75</td>
<td>Negative</td>
</tr>
<tr>
<td>MBC-4</td>
<td>No</td>
<td>Inv. ductal carcinoma</td>
<td>61</td>
<td>Negative</td>
</tr>
<tr>
<td>MBC-6</td>
<td>No</td>
<td>Inv. ductal carcinoma</td>
<td>79</td>
<td>Negative</td>
</tr>
<tr>
<td>MBC-7</td>
<td>No</td>
<td>Inv. ductal carcinoma</td>
<td>66</td>
<td>Grandfather: stomach, prostate</td>
</tr>
<tr>
<td>MBC-8</td>
<td>No</td>
<td>Inv. ductal carcinoma</td>
<td>63</td>
<td>Negative</td>
</tr>
<tr>
<td>MBC-9</td>
<td>3806insT</td>
<td>Inv. ductal carcinoma</td>
<td>68</td>
<td>Negative</td>
</tr>
<tr>
<td>MBC-10</td>
<td>No</td>
<td>Tubular adenocarcinoma</td>
<td>62</td>
<td>Negative</td>
</tr>
<tr>
<td>MBC-11</td>
<td>No</td>
<td>Inv. ductal carcinoma</td>
<td>46</td>
<td>Negative</td>
</tr>
<tr>
<td>MBC-12</td>
<td>No</td>
<td>Inv. ductal carcinoma</td>
<td>85</td>
<td>Daughter: breast; Sister: ovary</td>
</tr>
<tr>
<td>MBC-13</td>
<td>No</td>
<td>Inv. ductal carcinoma</td>
<td>77</td>
<td>Negative</td>
</tr>
<tr>
<td>MBC-14</td>
<td>No</td>
<td>Inv. ductal carcinoma</td>
<td>46</td>
<td>Grandmother: lung</td>
</tr>
<tr>
<td>MBC-15</td>
<td>No</td>
<td>Inv. ductal carcinoma</td>
<td>49</td>
<td>Aunt: breast; Cousin: brain</td>
</tr>
<tr>
<td>MBC-16</td>
<td>No</td>
<td>Inv. ductal carcinoma</td>
<td>52</td>
<td>Father: stomach; Grandmother: lymphoma</td>
</tr>
<tr>
<td>MBC-17</td>
<td>No</td>
<td>Inv. ductal carcinoma</td>
<td>53</td>
<td>Mother: breast; Aunt: ovary</td>
</tr>
<tr>
<td>MBC-18</td>
<td>No</td>
<td>Inv. ductal carcinoma</td>
<td>48</td>
<td>Cousin: bilateral breast</td>
</tr>
<tr>
<td>MBC-19</td>
<td>841insCTTA</td>
<td>Inv. ductal carcinoma</td>
<td>48</td>
<td>Negative</td>
</tr>
<tr>
<td>G1</td>
<td>No</td>
<td>Gynecomastia</td>
<td>84</td>
<td>Negative</td>
</tr>
<tr>
<td>G2</td>
<td>7081 A → G</td>
<td>Gynecomastia</td>
<td>21</td>
<td>Negative</td>
</tr>
<tr>
<td>G3</td>
<td>No</td>
<td>Gynecomastia</td>
<td>40</td>
<td>Mother: uterus; Sister: colon; Aunt: colon</td>
</tr>
</tbody>
</table>

* MBC, male breast cancer; Inv., invasive.
Acknowledgments

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References

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