Original article

BRCA2 mutation analysis of 87 Spanish breast/ovarian cancer families

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Summary

Background: It is estimated that about 5%-10% of breast cancer (BC) cases is due to inherited predisposition. Early works reported that 45%-50% of site-specific BC families had BRCA1 mutations and 25%-35% BRCA2 mutations. However, these percentages could have been overestimated and likely vary among the populations studied.

Patients and methods: We analysed the BRCA2 gene in 87 Spanish breast/ovarian cancer families in which the BRCA1 mutation screening was negative.

Results: We detected 15 (17.2%) disease-causing mutations and 11 polymorphisms and unclassified variants. Four mutations were recurrent, and five were novel. Seven (47%) mutations were found in site-specific female BC families, five (33%) in families with OC cases, and three (20%) mutations in families with male BC cases. There was incomplete penetrance of the mutations in some families, and considerable phenotypic variations with respect to the age of diagnosis and cancer types.

Conclusions: The percentage of mutations detected reinforces the possibility that some of these families have mutations in genes other than BRCA1 or BRCA2 that confer lower BC risks.

Key words: BRCA2, hereditary breast cancer, mutation

Introduction

Breast cancer is the most common malignancy among women in developed countries. A family history of breast cancer (BC) and/or ovarian cancer (OC) is one of the main risk factors in the development of the disease [1]. It is estimated that about 5%-10% of BC cases is due to inherited predisposition, but the number of predisposing genes is unknown.

Epidemiological studies have provided evidence of at least two susceptibility genes: BRCA1 (17q21) [2] and BRCA2 (13q12-13) [3]. The BRCA2 gene has a large coding sequence, composed of 27 exons that span 10254 bp and encode a protein of 3418 amino acids with no significant homology to the BRCA1 protein [4]. Most disease-causing BRCA2 mutations result in a premature termination of translation or in an absence of transcript. These mutations are distributed throughout the gene. Mutation analysis of BRCA2 in BC/OC families has revealed an increased risk in individuals carrying mutations on this gene [5–6].

Early works reported that 45%-50% of site-specific BC families had BRCA1 mutations [7] and about 25%-35% BRCA2 mutations [8]. These preliminary studies also suggested that most hereditary BC/OC is attributable to BRCA1 mutations, whereas male BC families were over-represented among those with BRCA2 mutations. However, recent data show that these percentages could have been overestimated and that the proportion of hereditary BC and OC attributable to BRCA1 and BRCA2 mutations varies considerably among populations analysed [9, 10].

We present the results obtained in 87 BC/OC Spanish families in which BRCA1 mutations had not been detected. Our objective was to characterise the prevalence and spectrum of mutations in the cancer-predisposing gene BRCA2 in this study group.

Patients and methods

Selection of families

Genetic counselling has been offered to cancer families at the Hospital de Sant Pau in Barcelona since 1996. All women who undergo surgery and have an increased risk of BC based on family history are offered screening.

After cloning BRCA2, we sought to establish the frequency of BRCA2 mutations in families with no BRCA1 mutation at the start of the study and at least two cases of BC/OC, one of them diagnosed in a patient under 50 years of age. Here we present the results of 87 families, including those of nine families with previously reported male BC cases [11]. The study was approved by the local ethics committee. Blood samples were obtained from each affected proband once the patient had given her informed consent to participate. Patients were interviewed on any family history of cancer in order to gather information about cancer profiles and dates of diagnoses of all individuals, including first- and second-degree relatives of the proband.
Single strand conformation polymorphism analysis

Genomic DNA was obtained from peripheral blood lymphocytes using the salting-out procedure [12]. Amplicons corresponding to BRCA2 exons (except exons 10 and 11) were obtained by PCR reactions performed by using pairs of primers as described elsewhere [13]. The samples were cycled at 94°C for 30 seconds, annealing at the optimal temperature of each primer pair for 30 seconds, and extension at 72°C for two minutes. The procedure was repeated 30 times.

Template preparation and DNA amplification

Amplified samples were diluted 1: 2 in formamide buffer, held at 95°C for 5 min, and then held on ice for 5 min. For each sample, 5 μl was loaded onto a non-denaturing polyacrylamide SSCP gel and was run at 4°C for 5 min. and then held on ice for 5 min. For each sample, 5 ul was amplified from genomic DNA In each case, the forward primer consisted of the T7 promoter sequence and a consensus translation-initiation sequence immediately preceding the BRCA2 nucleotides

Protein truncation test

Exon 10 and four overlapping fragments (1.5—1.8 kb) of exon 11 were amplified from genomic DNA. In each case, the forward primer consisted of the T7 promoter sequence and a consensus translation-initiation sequence immediately preceding the BRCA2 nucleotides

Sequencing

PCR products with a variant band in either SSCP or PTT were sequenced. Sequencing was performed in an ABI Prism 310 automated fluorescence-based cycle sequencer (Perkin Elmer, Foster City, California) and a dye terminator system. All mutations were confirmed by direct sequencing of an independently amplified PCR product from the patient.

Results

Mutation analysis

Fifteen BRCA2 truncating mutations were identified (Table 2). We also detected 11 sequence variations: 5’UTR-203G>A, 5’UTR-75C>G, 1VS8 910-7T>C, His372Asn (exon 10), Lys1132Lys, Ser1528Ser, Arg2034Cys (exon 11), Ser2414Ser (exon 14), Thr2515Ile (exon 15), IVS25 9729+9A>C, and Arg3370Arg (exon 27). Most of them are known polymorphisms previously described in BIC studies of European/American families, and found in control chromosomes. Four variants were located in non-coding regions of the gene, presumably with no pathological consequences, but further research is required to ascertain whether some of them are disease-associated mutations or neutral polymorphisms. The mutations and polymorphisms described were deposited in the BIC database [14].

Phenotype in BRCA2 mutation families

The ages of diagnosis as well as the number and types of cancer in each family are shown in Table 2. A total of three male and 43 female BC cases (five with bilateral BC, and two with OC) were observed in the 15 families with BRCA2 mutations (mean age at onset in female BC = 42 years; median = 40 years; range = 28—82 years). The mutation status in all family members is not known. Three of the families included OC (three cases; mean age at onset = 61 years).

The mutations in families 18, 71, 120 and 127 were within the OC-cluster region (OCCR), ranging between nucleotides 3035 and 6629 [15]. However, these families included only one woman affected with BC and OC (family 120), and there was one case of OC in family 121, with the mutation 3034del4bp, localised in the 5’ limit of the OCCR region. Three families with BC/OC or OC cases carried mutations outside the OCCR region in exon 11, and in exons 18, and 23.

The phenotypes of families with BRCA2 mutations varied with respect to cancer types and ages at diagnosis. An incomplete penetrance of the mutations was observed in three families. In family 18, the eldest of the eight sisters (four of them with BC or uterine cancer) remains healthy at 65 years of age and had a daughter who developed BC at 44 years of age. In family 119, a 64-year-old female carrier of the mutation is cancer free, whereas her sister had OC at 62, and three nieces
Table 2. Phenotypes in families with BRCA2 mutations. In parenthesis years of age at diagnosis.

<table>
<thead>
<tr>
<th>Fam</th>
<th>Exon</th>
<th>Mutation</th>
<th>Proband*</th>
<th>Affected relatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>94</td>
<td>10</td>
<td>1825delA</td>
<td>BC bilat (38/41)</td>
<td>BC (33), BC (28)</td>
</tr>
<tr>
<td>113</td>
<td>10</td>
<td>1825delA</td>
<td>BC (40), brain (76)</td>
<td>BC (41)<em>, BC (42), uter (40)</em>, prostate (69)*</td>
</tr>
<tr>
<td>121</td>
<td>11</td>
<td>3034del4bp</td>
<td>BC (38)</td>
<td>BC (50), OC (68)</td>
</tr>
<tr>
<td>122</td>
<td>11</td>
<td>3034del4bp</td>
<td>BC (82)</td>
<td>BC (36), BC (33), BC (30), lung (48)*</td>
</tr>
<tr>
<td>71</td>
<td>11</td>
<td>3374delA</td>
<td>BC bilat (32/44)</td>
<td>Male BC (55)*, brain (48)</td>
</tr>
<tr>
<td>18</td>
<td>11</td>
<td>3492insT</td>
<td>BC (44)</td>
<td>BC (60)<em>, BC (33), BC (50) + uter (63)</em>, uter (52), uter (58), colon (50)</td>
</tr>
<tr>
<td>120</td>
<td>11</td>
<td>4082delA</td>
<td>BC (48) + OC (61)</td>
<td>Uter (50), leukemia</td>
</tr>
<tr>
<td>127</td>
<td>11</td>
<td>6076delA</td>
<td>BC (34)</td>
<td>BC (35), BC (36)</td>
</tr>
<tr>
<td>23</td>
<td>11</td>
<td>6857delAA</td>
<td>BC (29)</td>
<td>BC (51)<em>, BC (45) and cervix (45)</em>, uter (40) + BC bilat (50/50), BC (40), BC (30), brain (62), rectum (68), stomach (60)</td>
</tr>
<tr>
<td>54</td>
<td>11</td>
<td>6857delAA</td>
<td>BC and cervix and lung (30)</td>
<td>Male BC</td>
</tr>
<tr>
<td>76</td>
<td>18</td>
<td>8297insT</td>
<td>BC (45) and OC (53)</td>
<td>BC (43)<em>, BC (39), BC (35), prostate (67)</em>, prostate (73), prostate (72)</td>
</tr>
<tr>
<td>4</td>
<td>23</td>
<td>9254del5bp</td>
<td>BC (58) and melanoma (49)</td>
<td>BC (30)<em>, OC (53)</em></td>
</tr>
<tr>
<td>37</td>
<td>23</td>
<td>9254del5bp</td>
<td>BC (49) and OC (53)</td>
<td>Male BC (74)*</td>
</tr>
<tr>
<td>119</td>
<td>23</td>
<td>9254del5bp</td>
<td>BC (45)</td>
<td>BC (39)<em>, BC (37)</em>, OC (60)*, BC (57)</td>
</tr>
<tr>
<td>33</td>
<td>25</td>
<td>E3096X</td>
<td>BC bilat (41/60)</td>
<td>BC bilat (48/48)*, BC (48), BC (46)</td>
</tr>
</tbody>
</table>

* carriers. ** non carriers.

presented BC at 45, 39, and 37, respectively. A similar situation occurred in family 121 in which both the proband, with BC at 38 years, and her mother, still healthy at 69 years, were carriers of a mutation. We also observed considerable phenotypic variations with respect to the age of diagnosis. In family 122, there was a striking difference between the age of BC diagnosis of the proband (82 years) and the other three cases, diagnosed at 33, 36, and 30 years of age. Furthermore, in families 4, 18, 23 and 119, the ranges between the lowest and the highest age at diagnosis were 28, 27, 21 and 18 years, respectively.

In family 113, there was an affected woman with BC at 41 who is a non carrier, and there was another possible phenocopy in family 23. In this family, the grandmother had been diagnosed with BC at 65 and pancreatic cancer at 70. The presence of three BC and other tumours on the paternal side of the family suggests that the mutation was inherited through this branch, and that the grandmother's BC is a sporadic case. There was no available sample to confirm this hypothesis.

Discussion

All coding regions and splice boundaries of the BRCA2 gene were screened for germ-line mutations in 87 Spanish BC/OC families. Direct sequencing of the whole coding region of the gene is the most reliable approach, having the highest specificity and sensitivity. However, because of the large size of BRCA2, this method is time consuming and expensive. As an alternative, we used a combination of PTT for large exons 10 and 11, and SSCP for the remaining part of the gene. Although the PTT method does not detect missense mutations, it is considered to be quick and efficient, and has been widely used to detect truncating mutations in the BRCA genes. The SSCP technique is estimated to have 75%-80% sensitivity in detecting single-base-pair substitutions; and is inexpensive and suitable for screening large series of patients. However, there is no technique available to date that can guarantee the identification of all cancer-predisposing mutations.

We detected 15 (17.2%) disease-causing frame-shift or nonsense mutations that are predicted to prematurely truncate the protein and to severely disrupt normal function.

The BRCA2 gene has in fact been reported to be one of the major BC susceptibility genes, accounting for about 70% of the inherited BC not linked to BRCA1 [8]. In our series, the percentage of BRCA2 mutations was low (17.2%). This percentage is, however, consistent with recent reports [6, 16, 17] and agrees with results obtained in Spanish families [18]. Furthermore, the proportion of families with mutations varies according the population analysed and the type of the family selected [9, 10, 19].

In our study, the greatest proportion of mutations (12 of 15) was found in families with three or more cases of BC/OC or male BC. However, the presence of a mutation was not limited only to families with several cancer cases, e.g. family 120 was made up only of one woman with both BC and OC and a second-degree relative with uterine carcinoma. For families with a reduced number of cases, the study conducted by Martin et al. [20] of 100 families with at least one BC and one OC showed that the strongest predictors of a mutations in BRCA1 or BRCA2 were a young average age at BC diagnosis in the family and the presence of a single family member with both BC and OC. Therefore, the presence of this type of patient in a BC/OC family should be considered as a basis for risk evaluation and genetic testing.

Leaving aside the families with male BC cases, the BRCA2 mutation frequency in BC/OC families is higher
(5 of 23: 21.7%) than in BC families (7 of 55: 12.7%). Seven (58%) of the 12 mutations were found in site-specific BC families, and five (42%) mutations in families with OC cases. Although preliminary studies suggested that most hereditary BC/OC were explained by BRCA1 mutations, our findings agree with some earlier reports [4, 5, 21] in which OC was present in up to 48% of families with BRCA2 cancer-predisposing mutations.

Four mutations were recurrent, and 5 out of 15 mutations are novel. The recurrent 3034del4bp mutation found in families 121 and 122 had been identified in earlier reports as a founder mutation in European countries [14]. The mutations 1825delA, 3374delA, 4082delA, 6857delAA, and 8297insTT are, to date, unique to Spain. The mutations 3492insT, 6076del4bp, and E3096X have already been registered once in BIC, identified in American and Latin/Caribbean families. The recurrent mutation 9254del5bp in exon 23 had previously been described in French families and in one Spanish family. Haplotype studies are in the process of ascertaining the potential founder effect of these mutations.

Gayther et al. [15] have reported that BRCA2 truncating mutations in families with a high proportion of OC appear to be clustered in a region of 3.3 kb in exon 11, between nucleotides 3035 and 6629. Data from our families do not provide support for this clustering. The four families with mutations in this 3.3 kb region contain 10 BC cases (one of them male BC) and one BC/OC case, compared with 3 OC and 33 BC cases in 11 families with mutations elsewhere. Earlier studies on the common 6174delT and 999del5 mutations have suggested with mutations elsewhere. Earlier studies on the common 6174delT and 999del5 mutations have suggested that the mutations in the OCCR are less penetrant for BC. In our cohort, there were no significant differences in age at diagnosis of BC in accordance with the location relative to OCCR, 42 years being the mean age at diagnosis inside and outside this region.

We observed a number of tumours other than breast and ovary in the BRCA2 families. This finding is in agreement with earlier studies that suggest that mutations in BRCA2 confer an increased risk for different types of cancer. An earlier work from the Breast Cancer Linkage Consortium [22] shows that pancreatic and prostate cancers are the most frequent in BRCA2 families. In our series, although we found four families with prostate cancer (three cases in family 76), and four families with six cases of uterine cancer, the significance of this is unclear since the presence of the mutant allele in such cases was not studied. There are several case reports of uterine carcinoma in women with BRCA1 mutations, and this has led to speculation regarding the effect of these genes on the risk of uterine cancer. However, retrospective studies of women with endometrial carcinoma have not shown an increased lifetime risk of this type of cancer in individuals with germline BRCA mutations [23].

The majority of families remained negative for mutations in the BRCA2 gene even though many showed a clear predisposition for BC or OC. Further work is warranted to investigate other mutation mechanisms which may have been missed, such as large genomic rearrangements, big deletions or duplications, and regulatory mutations in other parts of the gene (like the promoter region), and which could also account for the low detection frequency found. Such research will more accurately determine the mutation spectrum in Spanish BC/OC families. Moreover, since the sensitivity of the mutation-detection methods is not complete, some mutations could also have remained undetected. Finally, the families studied are quite heterogeneous in terms of the number of cases of BC and/or OC per family. It is, therefore, conceivable that some of these families include mutations conferring lower BC risks in gene(s) other(s) than BRCA1 or BRCA2.

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