A novel PALB2 truncating mutation in an Italian family with male breast cancer

MARIÀ TERESA VIETRI, GEMMA CALIENDO, AMELIA CASAMASSIMI, MICHELE CIOFFI, MARIA LAURA DE PAOLA, CLAUDIO NAPOLI and ANNA MARIA MOLINARI

Department of Biochemistry, Biophysics and General Pathology, School of Medicine, Second University of Naples, I-80138 Naples, Italy

Received May 12, 2014; Accepted July 21, 2014

DOI: 10.3892/or.2014.3685

Abstract. Male breast cancer (MBC) is a rare disease, accounting for ~1% of all breast cancer cases worldwide. Although other genes are also involved, predisposing genetic factors to MBC include germline mutations in the BRCA genes (BRCA2). Among the other genes, partner and localizer of BRCA2 (PALB2) is considered a moderate-penetrance breast cancer susceptibility gene that may also play a role in MBC predisposition. Thus, the aim of the present study was to determine the PALB2 gene status in 8 MBC cases selected from a cohort of 181 hereditary breast and/or ovarian cancer probands. We performed PALB2 mutational analysis by direct sequencing of 13 exons and adjacent intronic regions. This study showed the presence of a PALB2 truncating mutation in 1/8 (12.5%) cases. This novel mutation was named c.1285_1286delAinsTC (p.I429SfsX12) and is localized in exon 4 of PALB2, in the region encoding for the ChAM motif which is important for the efficient association of PALB2 to chromatin and for recruitment of the BRCA complex to accumulate RAD51 at double-strand break sites. Our findings indicate that PALB2 could be added to the list of breast cancer susceptibility genes also in families with MBC cases.

Introduction

Breast cancer in men is a rare disease, accounting for ~1% of all breast cancer cases worldwide (1). An important predisposing genetic factor for male breast cancer (MBC) includes germline mutations in the BRCA genes, particularly in BRCA2, although other genes are implicated (2). Partner and localizer of BRCA2 (PALB2) is the third hereditary breast cancer susceptibility gene; it is mutated in approximately 1-2% of cases, with higher rates in certain populations (3,4). PALB2 is also the second most commonly mutated gene noted in hereditary pancreatic cancer (5). Indeed, deleterious germline PALB2 mutations have been reported in families with breast and pancreatic cancer from the USA (6) and Europe (7,8).

Other studies have reported that various PALB2 mutations also predispose to hereditary prostate cancer (9,10).

The role of PALB2 in MBC predisposition still remains to be clarified. Several studies have reported truncating mutations of PALB2 in cases of MBC (11-14). In contrast, no evidence of PALB2 pathogenic mutations in MBC was found in other studies (15-17).

Thus, the aim of the present study was to determine the prevalence of PALB2 mutations in Italian families with at least one male breast cancer case.

Patients and methods

Patients. We selected 8 patients affected with MBC, or who had at least one MBC case in the pedigree, from a group of 181 patients affected with hereditary breast and/or ovarian cancer from Campania, a region of southern Italy. Of the patients, 5 were males with breast cancer and 3 were females with breast cancer who had a first-degree relative with MBC. Patients were selected according to the selection criteria for hereditary breast cancer based on the Breast Cancer Linkage Consortium (18).

Ethics committee approval was obtained for the study. Informed consent for molecular analysis was obtained from all subjects, and the main clinical and histopathological data were generated by genetic counseling. Peripheral blood samples were collected from all patients. All patients were previously screened for BRCA1 and BRCA2 mutations.

Mutation analysis. Genomic DNA was isolated from peripheral blood lymphocytes, using the Wizard Genomic DNA purification kit (Promega Corporation, Madison, WI, USA) according to the manufacturer's instructions. PALB2 mutational analysis was conducted by direct sequencing of 13 exons and adjacent intronic regions. One set
of primers was used to amplify each exon, except for exons 4 and 5, which were amplified in four and two PCR products, respectively.

The primers used to amplify the coding exon-intron boundaries of PALB2, were previously reported (19). All PCR products were sequenced on both strands using the ABI Prism di-Deoxy Terminator Cycle Sequencing kit in the ABI 9700 thermal cycler and an ABI Prism 3100 automatic sequencer (both from Life Technologies, Carlsbad, CA, USA). The results were analyzed using Mutation Surveyor® software, version 3.24 (Softgenetics, State College, PA, USA).

GenBank reference sequences used for naming the novel mutation were NM_024675.3 and NT_010393.15. The sequence variant was named and referred to in the text according to the nomenclature used by the human Genome Variation Society (hgVS; http://www.hgvs.org), using the descriptions suggested by den Dunnen and Antonarakis (20).

RNA analysis. The patient with the novel mutation was subjected to a second peripheral blood sample, to confirm the presence of the mutation also in mRNA.

Total-RNA was isolated from peripheral blood lymphocytes using TRIzol reagent and reverse transcribed with SuperScript First-Strand Synthesis System (both from Life Technologies) according to the manufacturer's protocol. From our set of primers used for the mutation analysis, we selected a forward primer in exon 4 and a reverse primer in exon 5 to amplify from total cDNA the region spanning the mutation. The RT-PCR product was electrophoresed on agarose gel and then sequenced.

Results
We observed 8 cases of MBC in a cohort of 181 hereditary breast and/or ovarian cancer probands. The prevalence of MBC in our group of patients was 4.4%.

Mutation analysis of the PALB2 gene showed the presence of a mutation in 1/8 (12.5%) breast cancer patients. This mutation, not previously described, was named c.1285_1286delAinsTC (p.I429SfsX12). It was localized within exon 4 and consisted of an A deletion and TC insertion at position c.1285_1286, which shifted the reading frame at the 429 codon and led to a premature termination 12 codon downstream, at codon 441 (Fig. 1).

The presence of the mutation was confirmed by resequencing a second DNA sample from the patient. RNA analysis showed the same mutation.

In Table I, we document the clinical and histopathological characteristics of the 8 analyzed patients.

The c.1285_1286delAinsTC (p.I429SfsX12) mutation was identified in a 30-year-old woman affected with hereditary breast cancer, with one MBC case in the family. The pedigree is reported in fig. 2. This family showed multiple affected members of the same disease. Breast cancer was diagnosed in the patient at the age of 29, her father at the age of 60 and two sisters at the age of 31 and 34 years, respectively; in addition a paternal aunt with breast cancer died at 40 years of age.

The Finnish founder c.1952delT mutation increased the risk of breast cancer 6-fold by the age of 70 years (22), and five PALB2 mutations reported in the UK confer a 2.3-fold increased risk of breast cancer (23).

We found a novel PALB2 truncating mutation in an Italian family with an MBC case. Few studies have analyzed the PALB2 status in MBC. Particularly, in Italy, only one study has been conducted (16). In this study, based on a series of 108 MBC cases, of which 97 cases were BRCA1/2-negative,
no PALB2 mutation was found (16). In contrast, we found a mutation in 1/8 (12.5%) patients. The high frequency of the PALB2 mutation observed in our study was similar to that described in a US study that reported two truncating mutations in 13 proband males (16%) (12), whereas other authors showed a lower rate, ranging from 1 to 9% (11,13,14). In agreement with these studies, we confirmed the presence of the PALB2 mutations in MBC cases, suggesting that PALB2 might play an important role in hereditary MBC.

Most of the truncating mutations previously reported were mapped to exons 4 and 5 of the PALB2 gene, probably as they are the largest two exons in PALB2 (24). Importantly, all of the mutations discovered in a Chinese population occurred in exons 4 of PALB2, suggesting a potential hotspot (25). The c.1285_1286delAinsTC (p.I429SfsX12) mutation is localized in exon 4, whereas other Italian studies reported the PALB2 mutations localized in exons 4 and 5 (26,27), as well as in exon 2 and 13 (8), suggesting no mutational hot spot in the PALB2 gene for the Italian population.

To map the BRCA1-interacting region in PALB2, 13 internal deletion mutants of PALB2 were generated with the P1 mutant, deleted for amino acids 6-90, that failed to associate with BRCA1 (28). These results showed that PALB2 protein has a coiled-coil motif at the N terminus, required for interaction with BRCA1 (fig. 3). Other biochemical studies showed that PALB2 binds DNA via two separate regions in the N-terminus of the protein, called PALB2 truncation 1 (P2T1) and PALB2 truncation 3 (P2T3), respectively (29,30). Particularly, P2T3 contains an evolutionarily conserved PALB2 motif, named chromatin-association motif (ChAM), which is

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Gender</th>
<th>Age at diagnosis (years)</th>
<th>Diagnosis</th>
<th>Receptor status</th>
<th>BRCA1/2 mutation</th>
<th>PALB2 mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>65</td>
<td>MBC</td>
<td>ER+/PR+/HER2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>83</td>
<td>MBC</td>
<td>ER+/PR+/HER2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>69</td>
<td>MBC</td>
<td>ER+/PR+/HER2</td>
<td>UV in BRCA2</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>40</td>
<td>MBC</td>
<td>ER+/PR+/HER2</td>
<td>UV in BRCA2</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>58</td>
<td>MBC</td>
<td>ER+/PR+/HER2</td>
<td>UV in BRCA2</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>29</td>
<td>BC (father MBC)</td>
<td>ER+/PR+/HER2</td>
<td>-</td>
<td>c.1285_1286delAinsTC (p.I429SfsX12)</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>29</td>
<td>BC (grandfather MBC)</td>
<td>ER+/PR+/HER2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>38</td>
<td>BC (grandfather MBC)</td>
<td>ER+/PR+/HER2</td>
<td>5382insC in BRCA1</td>
<td>-</td>
</tr>
</tbody>
</table>

F, female; M, male; MBC, male breast cancer; BC, breast cancer; UV, unclassified variant; ER, estrogen receptor; PG, progesterone receptor; HER2, human epidermal growth factor receptor 2.
localised at amino acid region 395-446. ChAM is important for the efficient association of PALB2 to chromatin and for recruitment of the BRCA complex to accumulate RAD51 at double-strand break sites (31). Furthermore, the amino acidic region 611-764 of PALB2 interacts with mortality factor 4 like protein 1 (MORF4L1) which is important for promoting the function of the BRCA complex. PALB2 interacts with RAD51 by two regions (amino acids 101-184 and 853-1186) and has a C-terminal domain, containing four WD repeats which mediate the interaction with BRCA2 (32).

The novel mutation c.1285_1286delAinsTC (p.I429SfsX12) was found to be localized in exon 4 of PALB2, in the region that encodes for the ChAM motif (Fig. 3). It introduces a premature stop at codon 441 position; therefore, it may be expected that this mutation reduces the capacity of the ChAM motif for mediating PALB2 chromatin association thus altering DNA damage repair processes. In addition, this mutation induces the lack of interaction between MORF4L1 and BRCA2.

The prevalence of MBC in our patients was 4.4% in agreement with other studies that reported frequencies ranging from 4.1 to 4.6% (1). Out of 7 breast cancer patients, negative for PALB2 mutations, one was a carrier of the 5382insC mutation in BRCA1, as previously reported in our study (33). Instead, 3 MBC cases were carriers of unclassified variants in BRCA2 (Table I). However, the patients with the BRCA1 mutation or with unclassified variants in BRCA2 were additionally tested for a PALB2 mutation in order to not underestimate a possible condition of double heterozygosity (DH) in the BRCA and PALB2 genes. In a recent study, Pern et al described DH for BRCA1 and PALB2 mutations in one German patient with triple-negative breast cancer (34).

On the basis of our findings, PALB2 could be added to the list of breast cancer susceptibility genes, not only in families with recurring breast and pancreatic cancers but also in families with MBC.

Acknowledgements

The authors thank Mrs. Anna Cuomo for technical assistance in the mutation analysis.

References

