BRCA1 and BRCA2 mutations in males with familial breast and ovarian cancer syndrome. Results of a Spanish multicenter study

Article *in* Familial Cancer · May 2015

DOI: 10.1007/s10689-015-9814-z · Source: PubMed

28 authors, including:

Inmaculada De Juan Jimenez
Hospital Universitari i Politècnic la Fe
42 PUBLICATIONS **500** CITATIONS
SEE PROFILE

Sarai Palanca
Hospital Universitari i Politècnic la Fe
77 PUBLICATIONS **834** CITATIONS
SEE PROFILE

Lídia Feliubadaló
Catalan Institute of Oncology
118 PUBLICATIONS **3,096** CITATIONS
SEE PROFILE

Ana Osorio
Universidad Veracruzana
198 PUBLICATIONS **6,244** CITATIONS
SEE PROFILE

BRCA1 and *BRCA2* mutations in males with familial breast and ovarian cancer syndrome. Results of a Spanish multicenter study

Inmaculada de Juan[1], Sarai Palanca[1], Asunción Domenech[1], Lidia Feliubadaló[2], Ángel Segura[3], Ana Osorio[4], Isabel Chirivella[5], Miguel de la Hoya[6], Ana Beatriz Sánchez[7], Mar Infante[8], Isabel Tena[9], Orland Díez[10], Zaida Garcia-Casado[11], Ana Vega[12], Àlex Teulé[2], Alicia Barroso[4], Pedro Pérez[13], Mercedes Durán[8], Estela Carrasco[14], Mª José Juan-Fita[15], Rosa Murria[1], Marta Llop[1], Eva Barragan[1], Ángel Izquierdo[2], Javier Benítez[16], Trinidad Caldés[6], Dolores Salas[17], Pascual Bolufer[1]

1. Laboratory of Molecular Biology, Service of Clinical Analysis. University and Polytechnic Hospital La Fe; Valencia (Spain)
2. Hereditary Cancer Program. Catalan Institute of Oncology (ICO). L'Hospitalet de Llobregat; (Spain)
3. Unit of Genetic Counseling in Cancer. Hospital Universitario y Politécnico La Fe; Valencia (Spain)
4. Human Genetics Group, Human Cancer Genetics Programme, Spanish National Cancer Research Centre and Spanish Network on Rare Diseases (CIBERER); Madrid (Spain)
5. Unit of Genetic Counseling in Cancer. Hospital Clínico; Valencia (Spain)
6. Molecular Oncology Laboratory. IdISSC, Hospital Clínico San Carlos; Madrid (Spain)
7. Unit of Genetic Counseling in Cancer. Hospital General de Elche; Elche (Spain)
8. Cancer Genetic Group. Institute of Genetics and Molecular Biology (UVa-CSIC); Valladolid (Spain)
9. Unit of Genetic Counseling in Cancer. Hospital General de Castellón; Castellón (Spain)
10. Oncogenetics Laboratory. University Hospital Vall d'Hebron; Vall d'Hebron Institute of Oncology (VHIO) and Universitat Autònoma de Barcelona. Barcelona (Spain)
11. Laboratory of Molecular Biology. Valencian Institute of Oncology (IVO); Valencia (Spain)
12. Fundación Pública Galega de Medicina Xenómica-SERGAS. Grupo de Medicina Xenómica-USC, IDIS, CIBERER. Santiago de Compostela (Spain)
13. Department of Oncology. IdISSC, Hospital Clínico San Carlos; Madrid (Spain)
14. High Risk and Prevention Unit, University Hospital Vall d'Hebron. Barcelona (Spain)
15. Unit of Genetic Counseling in Cancer. IVO; Valencia (Spain)
16. Human Genetics Group and Genotyping Unit, Human Cancer Genetics Programme, Spanish National Cancer Research Centre and Spanish Network on Rare Diseases (CIBERER); Madrid (Spain)
17. General Directorate Public Health and Centre for Public Health Research (CSISP), Valencia Genetic Counseling in Cancer Programme, Valencia (Spain)

Corresponding author:

Inmaculada de Juan Jiménez,
Laboratory of Molecular Biology, University Hospital La Fe,
Escuela de Enfermería 7ª planta. Avd. Campanar 21, 46009 Valencia (Spain)
TLF/FAX numbers: 34 961973351
Email address: dejuan_inm@gva.es

ABSTRACT

Background: Male breast cancer (MBC) is a rare disease that represents less than 1% of all breast cancers (BCs). We analyze the results of a multicenter study performed in Spanish familial MBC including family history of hereditary breast and ovarian cancer syndrome (HBOCS) and clinicopathological features. We also study the relationship between *BRCA1/ BRCA2* mutational status in male relatives affected with cancer (MAC) and, family history and tumor types.

Methods: The study included 312 men index cases (ICs) with family history of HBOCS and 61 MAC *BRCA1/2* mutation-carriers. Family history, histological grade (HG), clinicopathological and immunohistochemistry data were collected. *BRCA1/2* mutation analyses were performed by direct sequencing or screening methods and the large rearrangements by multiplex ligation dependent probe amplification.

Results: We found 49 mutation-carriers (15.7%), 95.9% with *BRCA2* mutations. *BRCA2* mutation-carriers were associated with families with at least one MBC and one BC in female (type II; p=0.05). Strong association were found between the presence of pathogenic mutations in MBCs and the advanced HG (p=0.003). c.658_659delTG, c.2808_2811delACAA, c.6275_6276delTT and c.9026_9030delATCAT were the most prevalent mutations. In 61 MAC we found 20 mutations in *BRCA1* and 41 in *BRCA2*. For MAC we show that mutational status was differentially associated with family history (p=0.018) and tumor type, being *BRCA2* mutations linked with BC and prostatic cancer (p=0.018).

Conclusions: MBC caused by *BRCA1/2* mutations define two types of MBCs. The most frequent caused by *BRCA2* mutation linked to type II families and the rarest one attributed to *BRCA1* mutation. Tumor associated with MAC suggest that only *BRCA2* mutations have to do with a specific type of cancer (BC and prostatic cancer); but the linkage to tumors is questionable for *BRCA1* mutations.

Key words: Familial male breast cancer, hereditary breast and ovarian cancer syndrome, *BRCA1*, *BRCA2* mutations

Introduction

Male breast cancer (MBC) is a poorly understood and a rare disease that accounts for less than 1% of all breast cancers (BCs). Compared with female BCs, MBCs are usually more advanced diseases with larger tumor size and lymph node involvement [1]. MBCs have later onset than female BCs (67 *vs.* 61 years), with an unimodal incidence distribution that reaches a maximum at 71 years, while BCs in women show two peaks, at 52 and 71 years [1]. Clinically, MBCs resemble postmenopausal female BCs and the dominant histopathological type is also the invasive ductal carcinoma [1]. However, compared with female BCs, hormone receptors show higher incidence in MBCs.

Around 15-20% of MBCs present BC and/or ovarian cancer (OC) family history and only 10% of them can be attributed to a known genetic origin. The most frequent genetic causes of MBCs with family history are *BRCA2* mutations [2, 3, 4, 5], being less frequent *BRCA1* [6], *PTEN*, *CHEK2* or *TP53* [7, 8, 9] mutations. *BRCA2* mutations confer around 5-10% of MBCs cumulative risk throughout life compared with 0.1% of general population [10]. *BRCA2* mutations also confer an increased risk of other cancers, particularly prostate cancer (PC) [RR = 4.65 (95% CI: 3.48-6.22)] [10]. In fact, 2% of *BRCA2* mutations have been detected in PCs with age of onset $\leq$ 55 years [11]. Furthermore, *BRCA2* mutations also confer an increased risk of malignant melanoma [RR = 2.58 (95% CI: 1.28-5.17)], pancreatic cancer [RR = 3.51 (95% CI: 1.87-6.58)] and gallbladder cancer [RR = 4.97 (95% CI: 1.50-16.52)] [10]. Likewise it has also been reported that *BRCA1* mutations are associated with an increased risk of colorectal (CRC), pancreatic, gastric and fallopian tube cancer, with regard to normal population [6].

One of the most recent studies on the implications of *BRCA1* and *BRCA2* (*BRCA1/2*) mutations in MBC was reported by Ottini *et al* (2012) [12]. This was an Italian multicenter study in which 50 mutation-carriers were identified among 382 MBCs (13.1%). The study pointed out clear differences between sporadic MBCs and hereditary MBCs (HMBCs), mainly due to *BRCA2* mutations. Mutation-carriers presented BC/OC family history, more aggressive tumors, advanced tumor stage, high histological grade (HG), lower proportion of hormonal markers and HER2 amplification.

MBC is a rare disease, especially HMBC; therefore, it has been difficult to understand its pathogenic process, making it difficult to design appropriate therapeutic strategies. Most published studies are limited to short series from single institutions. Therefore, cooperative studies are mandatory in order to get a better insight of this rare disease.

In the present study, we analyze the clinicopathological parameters, family history and mutational status of familial MBC (FMBC) in a Spanish multicenter cohort. We also analyze the relationship between *BRCA1/ BRCA2* mutation status in male relatives affected with cancer (MAC) and family history and tumor types.

Material and methods

Patients

The study included 312 male index cases (ICs) selected from 11,812 families with hereditary breast and ovarian cancer syndrome (HBOCS) collected between 1995 and 2014 by eight genetic counseling units in cancer located throughout Spain. Furthermore, we included 61 MAC with *BRCA1/2* mutations.

The following laboratories participated in the study: Molecular Biology laboratory of the University hospital La Fe (Valencia), Molecular Biology laboratory of the Valencian Institute of Oncology (Valencia), Spanish National Cancer Research Centre, CNIO (Madrid), Molecular Diagnostics Unit of ICO (L'Hospitalet, Barcelona), Cancer Genetics Laboratory, Institute of Molecular Biology and Genetics (Valladolid), Oncogenetics Laboratory of University Hospital Vall d'Hebron, (Barcelona), Laboratory of Molecular Oncology of Hospital Clínico San Carlos (Madrid), Galician Public Foundation Genomic Medicine (Santiago de Compostela).

All ICs tested for *BRCA1/2* mutations were MBCs who belonged to families that accomplished at least one of the following criteria: (Ia) families with BC&OC; (Ib) families with BC&OC in the same relative; (II) families with at least one BC in a male and another in a female; (III) families with BC in three or more members, at least two of them first degree relative; (IV) families with two cases of BC in first degree relatives (at least one of them before 50 years or bilateral) and (V) one BC under 30 years.

All patients signed an informed consent elaborated by their respective centers or hospitals according to the recommendations of the Declaration of Human Rights, the Conference of Helsinki [13] and institutional regulations that was approved by the Hospital Ethics Committee.

For each case, we collected the age at the onset of the tumor, inclusion criteria, tumor types, clinicopathological parameters [histology, histological grade (HG) and node involvement], immunohistochemical (IHC) parameters (ER, PR and HER2) and *BRCA1/2* mutational status.

Tumor types of MAC mutation-carriers were classed in three groups: a) **BC:** BC or bilateral BC as primary tumor alone or combined with a secondary tumor (colorectal, prostate, lung, bladder, etc.); b) **PC** as primary tumor either alone or combined with a secondary tumor (colorectal, bladder, pancreatic, etc.) and c) **Others** including single tumors such: colorectal, pancreatic, melanoma, skin cancer, lung or gastric cancer.

Molecular studies

BRCA1/2 mutation analysis was performed by the participating laboratories. Briefly, all coding exons and exon–intron boundaries of *BRCA1/BRCA2* were amplified by PCR followed in some laboratories by direct sequencing. The remaining laboratories performed a *BRCA1/2* mutation pre-screening based on abnormal pattern detection using either conformation sensitive gel electrophoresis (CSGE) [14], heteroduplex analysis by capillary array electrophoresis (HA-CAE) [15], conformation sensitive capillary electrophoresis (CSCE) [16], high performance liquid chromatography (HPLC) or high resolution melting (HRM) [17, 18] methods followed by sequencing of the abnormal patterns. Large genomic rearrangements were studied by multiplex ligation dependent probe amplification (MLPA; MRC Holland, Amsterdam, The Netherlands) [19]. In the last years, three laboratories have replaced Sanger sequencing by next generation sequencing (NGS).

Immunohistochemistry

Clinicopathological parameters, tumor histology, HG and IHC parameters (ER, PR and HER2) were collected from medical and pathological reports. IHC was performed on formalin-fixed paraffin embedded tumor blocks. The slides were incubated with primary antibodies against ER, PR or HER2 and counter-stained with haematoxylin-eosin. ER and PR expression was evaluated according to the allred scoring system [20]. HER2 expression was scored according to Hercep Test interpretation criteria [21].

Statistical analysis

Chi-square test was applied to compare the qualitative data between the two groups and Z test was used to compare proportions from 2 independent samples. These analyses were performed using SPSS v.19 package. *P-values* < 0.05 were considered as statistically significant.

Results

Clinicopathological features, mutational spectrum and family history of index cases

We found 49 mutations in 312 ICs (15.7%; Table 1), two in *BRCA1* and 47 in *BRCA2*. The median age of tumor onset of the 312 ICs was 59.5 years (range: 24-88 years), while for mutation-carriers was 60 (range: 34-79 years). These differences were not statistically significant.

The invasive ductal BC was the most frequent histological type (87.1% of all MBC, Table 1), followed by ductal carcinoma *in situ* and papillary carcinoma, that together represent 11.2% of MBC.

Most of the mutation-carriers, 44 out of 49 (89.8%), belonged to type II families, which represents 94.5% of all families studied ($p = 0.011$; Table 1).

We did not find association between tumor size (T) and *BRCA1/2* mutational status, although we observed a higher proportion of $T \geq 3$ MBCs in mutation-carriers (21.9% *vs.* 12.7%; Table 1). Eighty-six per cent of tumors were N0 or N1, and no associations were found between nodal involvement and mutational status. Metastases were only detected in six BCs (3.2%, Table 1).

HGs 2 and 3 were most frequent in MBC representing 83.8% of all tumors (Table 1). HG showed significant association with mutational status, where the mutation-carriers presented HGs > 2 in 95% of cases ($p = 0.003$, Table 1).

Ninety-six per cent and 88.9% of the total MBCs were ER+ and PR+, respectively, and only 13.1% presented HER2 amplification (Table 1). However, no association between IHC parameters and mutational status was found.

We detected 29 different mutations in *BRCA2*; four of them with prevalence > 6.3% (Table 2). These four mutations represented around 40% of all mutations. But the most prevalent by far was c.9026_9030delATCAT, representing 14.6% of all mutations.

For ICs we observed that family types were associated with *BRCA1/2* mutational status. Most of *BRCA1/2* mutation-carriers belonged to type II families (at least one MBC and one BC in female) that harbored 44 of 49 mutations (89.8%, Table 2).

Clinicopathological and mutational status in male relatives affected with cancer and *BRCA1/2* mutation-carriers

We studied 61 MACs, affected by different tumor types who harbored the ICs family mutation. The median age of cancer presentation among them was 58 years (range 22-88 years).

Family histories III and IV were predominant, accounting for 62.3% of all family types (38 out of 61 families; Table 3). We found an association between *BRCA2* mutation-carriers and type II and III families (p = 0.018; Table 3).

Tumor types were also associated with *BRCA2* mutations, particularly with BC and PC (p = 0.018, Table 3), whereas the other tumor types were not linked with *BRCA1/2* mutational status.

Twenty MACs carried 17 different mutations in *BRCA1*, of which c.211A>G and c.212+1G>A were recurrent (Table 4). In *BRCA2*, we identified 28 different mutations among 41 MACs, of which c.2808_2811delACAA, c.3264dupT and c.9026_9030delATCAT showed a prevalence ≥ 7.3% and represented 31.7% of all *BRCA2* mutations.

Discussion

In this study, we show that 15.7% of MBC ICs present *BRCA1/2* pathogenic mutations. This prevalence is slightly higher than that reported by Ottini *et al* (13.1%) in Italian population series [12]. Our higher mutational prevalence could be explained by the different inclusion criteria; Ottini *et al* (2012) included all MBCs while we exclusively included patients with HBOCS history. However, in other study performed in 115 MBCs, 105 cases without family history, 18 *BRCA2* pathogenic mutations were detected, with 15.6% prevalence [22], similar to the present study.

The low relevance of family history on the mutational prevalence in MBC justifies that in the Guidelines from the National Cancer Comprehensive Network® Version 1.2014 NCCN [23] was considered MBC as testing criteria for mutation study, regardless of family history.

BRCA2 mutations were present in 95.9% of HMBC ICs, whereas *BRCA1* only accounted for 4.1%, in agreement with previous reports [12, 24, 25]. Since the inheritance of *BRCA1* and *BRCA2* mutations is not linked to gender, male and female should have the same probabilities of harboring them. Therefore, it can be hypothesized that *BRCA2* mutations should be more penetrant in males than *BRCA1*; which could be explained by the different modulatory effects of the endocrine environment in men as compared with women.

In the present study we have not found differences between *BRCA1/2* mutation-carriers and no-carriers regarding the age of cancer onset or the mutated gene. This may be attributed to the wide scatter in the age of onset in our series (range 24 to 88 years).

Unlike Ottini *et al* (2012) [12], we did not find associations between *BRCA1/2* mutational status and TNM, hormonal receptors or HER2. However, like them we found association between advanced HG (2 and 3) and high mutation-carrier prevalence.

Sixteen mutations detected in ICs, one in *BRCA1* and 15 in *BRCA2*, previously reported in the Spanish population [26, 27, 28, 29, 39] (Table 2). In addition, prevalent *BRCA2* mutations have been reported in previous Spanish population studies. Particularly, the most prevalent *BRCA2* mutation, c.9026_9030delATCAT, was initially reported by Neuhausen *et al* [32] in French families. This mutation is the most prevalent in the Mediterranean Spanish area [27] although it has also been detected in families from Almeria [33] and Basque Country.

The association of MAC tumor type with *BRCA1/2* mutations was particularly remarkable. None of the 20 *BRCA1* mutation-carriers presented BC, while 11 out of 41 *BRCA2* mutation-carriers (26.8%) developed BC. These results could suggest that contrarily to women, *BRCA1* mutations could have lower penetrance to develop BC in men.

MAC associated tumors indicate that *BRCA2* mutations are also correlated with PC but are not related with the other cancers. These results are in agreement with those reported by The Breast Cancer Linkage Consortium [10] and Edwards *et al* [11].

MAC exhibit a broader mutational spectrum as compared to ICs, particularly for *BRCA1* (Table 4). Three *BRCA2* recurrent mutations were identified (c.2808_2811delACAA, c.3264dupT and c.9026_9030delATCAT), representing 21.3% of the total mutations (Table 4). These recurrent mutations have also been found in ICs and most of them have already been reported in studies carried out in Spanish population [26, 27, 28, 29, 35, 36, 37, 38, 39] or included in mutation databases [30, 31] (Table 4).

In summary, it could be concluded that HMBC caused by *BRCA1/2* mutations define two types of MBCs. *BRCA2* mutation-carriers MBCs are, by far, the most frequent MBCs, and are linked to type II families, whereas MBCs attributed to *BRCA1* mutations are the rarest. The tumors developed by MACs suggest that only *BRCA2* mutations are linked to specific types of cancer (BC and PC); whereas the role of *BRCA1* mutations is unknown.

Competing interests

The author(s) declare that they have no conflict of interest.

Acknowledgments

All authors manifest their gratitude to the families who participated in the research studies described in this article and to the researchers José Antonio López Guerrero (IVO; Valencia) and Conxi Lázaro (Laboratori de Recerca Translacional del ICO; Hospitalet).

We also would like to thank to the Health Research Institute La Fe for having granted fellowships to Rosa Murria Estal, Marta Llop García and Gema Pérez Simó which have made possible their participation in the study in the laboratory of Molecular Biology La Fe; the ICO authors wish to thank to the ICO Hereditary Cancer Program team; the Institute of Genetics and Molecular Biology of Valladolid authors wish to thank the Cancer Prevention Program of the Regional Government of Castilla y León.

This study has been supported, in part, by grants from the Asociación Española Contra el Cáncer, Spanish Health Research Fund; Carlos III Health Institute; Catalan Health Institute and Autonomous Government of Catalonia; Mutua Madrileña Foundation (FMMA); Spanish Association against Cancer (AECC08); FMM Foundation given to AV and the following projects: ISCIIIRETIC; RD06/0020/1051; RD12/0036/008; PI10/01422; PI10/00748; PI13/00285; 2009SGR290; RTICC 06/0020/1060; FISPI12/00070; 10PXIB 9101297PR and ISCIII (PI13/01711)

References

[1] Larissa A. Korde, Jo Anne Zujewski, Leah Kamin, et al. **Multidisciplinary Meeting on Male Breast Cancer**: Summary and Research Recommendations. J Clin Oncol. 2010;28:2114-2122.

[2] Couch FJ, Farid LM, DeShano ML, et al. **BRCA2 germline mutations in male breast cancer cases and breast cancer families**. Nat Genet. 1996;13:123-125

[3] Haraldsson K, Loman N, Zhang QX, et al. **BRCA2 germ-line mutations are frequent in male breast cancer patients without a family history of the disease**. Cancer Res. 1998;58:1367-1371

[4] Thorlacius S, Tryggvadottir L, Olafsdottir GH, et al. Linkage to BRCA2 region in hereditary male breast cancer. Lancet. 1995; 346:544-545

[5] Wooster R, Bignell G, Lancaster J, et al. **Identification of the breast cancer susceptibility gene BRCA2**. Nature. 1995;378:789-792

[6] Brose MS, Rebbeck TR, Calzone KA, et al. **Cancer risk estimates for BRCA1 mutation carriers identified in a risk evaluation program**. J Natl Cancer Inst. 2002;94:1365-1372

[7] Anelli A, Anelli TF, Youngson B, et al. Mutations of the p53 gene in male breast cancer. Cancer. 1995;75:2233-2238

[8] Fackenthal JD, Marsh DJ, Richardson AL, et al: **Male breast cancer in Cowden syndrome patients with germline PTEN mutations**. J Med Genet. 2001;38:159-164

[9] Meijers-Heijboer H, van den Ouweland A, Klijn J, et al. **Low-penetrance susceptibility to breast cancer due to CHEK2(*)1100delC in noncarriers of BRCA1 or BRCA2 mutations**. Nat Genet. 2002; 31:55-59

[10] **Cancer risks in BRCA2 mutation carriers**: The Breast Cancer Linkage Consortium. J Natl Cancer Inst. 1999;91:1310-1316.

[11] Edwards SM, Kote-Jarai Z, Meitz J, et al. The Cancer Research UK/British Prostate Group UK Familial Prostate, Cancer Study Collaborators, British Association of Urological Surgeons Section of Oncology,Doug F. Easton, and Rosalind A. Eeles. **Two Percent of Men with Early-Onset Prostate Cancer Harbor Germline Mutations in the BRCA2 Gene.** Am. J. Hum. Genet. 2003;72:1–12.

[12] Ottini L, Silvestri V, Rizzolo P, et al. **Clinical and pathologic characteristics of BRCA-positive and BRCA-negative male breast cancer patients: results from a collaborative multicenter study in Italy**. Breast Cancer Res Treat. 2012;134:411–418.

[13] World Medical Association Declaration of Helsinki. Ethical Principles for Medical Research Involving Human Subjects [http://www.wma.net/e/policy/pdf/17c.pdf]

[14] Ganguly A, Rock MJ, Prockop DJ. **Conformation-sensitive gel electrophoresis for rapid detection of single-base differences in double-stranded PCR products and DNA fragments: evidence for solvent-induced bends in DNA heteroduplexes.** Proc Natl Acad Sci USA. 1993;90:10325–10329

[15] Velasco E, Infante M, Durán M, et al. **Heteroduplex analysis by capillary array electrophoresis for rapid mutation detection in large multiex genes**. Nat Protoc. 2007;2(1):237-46.

[16] Mattocks CJ, Watkins G, Ward D, et al. **Interlaboratory Diagnostic Validation of Conformation-Sensitive Capillary Electrophoresis for Mutation Scanning**. Clinical Chemistry. 2010;56(4):593-602

[17] van der Stoep N1, van Paridon CD, Janssens T, Krenkova P, Stambergova A, Macek M, Matthijs G, Bakker E. **Diagnostic guidelines for high-resolution melting curve (HRM) analysis: an**

interlaboratory validation of BRCA1 mutation scanning using the 96-well LightScanner. Hum Mutat. 2009 Jun;30(6):899-909. doi: 10.1002/humu.21004.

[18] de Juan I, Esteban E, Palanca S, Barragan E, Bolufer P. **High-resolution melting analysis for rapid screening of BRCA1 and BRCA2 Spanish mutations.** Breast Cancer Res Treat. 2009;115:405–414

[19] Schouten JP et al. **Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification.** Nucleic Acids Res. 2002;30(12):e57.

[20] Hammond ME, Hayes DF, Dowsett M, et al. **American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer.** Arch Pathol Lab Med. 2010;134(7):e48-72.

[21] Milanezi F, Carvalho S, Schmitt FC. **EGFR/HER2 in breast cancer: a biological approach for molecular diagnosis and therapy**. Expert Rev Mol Diagn. 2008;8(4):417-434.

[22] Yuan Chun Ding, Linda Steele, Chih-Jen Kuan, Scott Greilac, Susan L. Neuhausen. **Mutations in BRCA2 and PALB2 in male breast cancer cases from the United States.** Breast Cancer Res Treat. 2011; 126(3):771–778. doi:10.1007/s10549-010-1195-2.

[23] **Hereditary Breast and/or ovarian syndrome.** National Comprenhensive Cancer Network NCCN Guidelines Version 1.2014.HBOC-1

[24] Deb S, Jene N, kConFab investigators and Fox SB. **Genotypic and phenotypic analysis of familial male breast cancer shows under representation of the HER2 and basal subtypes in BRCA-associated Carcinomas**. BMC Cancer. 2012;12:510. doi:10.1186/1471-2407-12-510

[25] Loughrey M, Provan PJ, Byth K, Balleine RL. **Histopathological features of 'BRCAX' familial breast cancers in the kConFab resource.** Pathology. 2008;40(4):352–358.

[26] Díez O, Osorio A, Durán M, et al. **Analysis of BRCA1 and BRCA2 genes in Spanish breast/ovarian cancer patients: a high proportion of mutations unique to Spain and evidence of founder effects**. Hum Mutat. 2003;22(4):301-12

[27] Esteban Cardeñosa E, Bolufer Gilabert P, de Juan Jimenez I, et al. **Broad BRCA1 and BRCA2 mutational spectrum and high incidence of recurrent and novel mutations in the eastern Spain population**. Breast Cancer Res Treat.2010;121:257–260

[28] Diez O, Gutiérrez-Enríquez S, Balmaña J. **Heterogeneous prevalence of recurrent BRCA1 and BRCA2 mutations in Spain according to the geographical area: implications for genetic testing**. Familial Cancer.2010;9:187–191

[29] de Juan Jiménez I, García Casado Z, Palanca Suela S, et al. **Novel and recurrent BRCA1/BRCA2 mutations in early onset and familial breast and ovarian cancer detected in the Program of Genetic Counseling in Cancer of Valencian Community (eastern Spain)**. Relationship of family phenotypes with mutation prevalence. Fam Cancer. 2013;12(4):767-77.

[30] **Breast Cancer Information Core**: BIC: [http://research.nhgri.nih.gov/bic/]

[31] **The Universal Mutation Database**: UMD: [http://www.umd.be/]

[32] Neuhausen SL, Godwin AK, Gershoni-Baruch R et al. **Haplotype and phenotype analysis of nine recurrent BRCA2 mutations in 111 families: results of an international study**. Am J Hum Genet. 1998;62:1381–1388

[33] Llort G, Yagüe C, Peris M et al. **Low frequency of recurrent BRCA1 and BRCA2 mutations in Spain**. Hum Mutat. 2002;19:307

[34] Beristain E, Martınez-Bouzas C, Guerra I et al. **Differences in the frequency and distribution of BRCA1 and BRCA2 mutationsin breast/ovarian cancer cases from the Basque country withrespect**

to the Spanish population: implications for genetic counselling. Breast Cancer Res Treat. 2007;106:255–262

[35] Gutierrez-Enrique S, de la Hoya M, Martínez-Bouzas C, et al. **Screening for large rearrangements of the BRCA2 gene in Spanish families with breast/ovarian cancer**. Breast Cancer Res Treat. 2007;103:103–107

[36] Menéndez M, Castellsagué J, Mirete M, et al. **Assessing the RNA effect of 26 DNA variants in the BRCA1 and BRCA2 genes**. Breast Cancer Res Treat. 2012;132(3):979-92.

[37] del Valle J, Feliubadaló L, Nadal M, et al. **Identification and comprehensive characterization of large genomic rearrangements in the BRCA1 and BRCA2 genes.** Breast Cancer Res Treat.2010; 122(3):733-43.

[38] The BRCA1 Exon 13 Duplication Screening Group. **The Exon 13 Duplication in the BRCA1 Gene Is a Founder Mutation Present in Geographically Diverse Populations**. Am J Hum Genet. 2000;67(1):207–212.

[39] Vega A, Torres M, Martínez JI, et al. **Analysis of BRCA1 and BRCA2 in breast and breast/ovarian cancer families shows population substructure in the Iberian Peninsula**. Ann Hum Genet. 2002;66:29-36.

Table 1: Demographic, family history, clinicopathological and immunohistochemistry characteristics of index cases

Parameter; χ^2 (p)	**BRCAX** N (%)	***BRCA1/2+*** N (%)	**TOTAL** N (%)
Male breast cancer	263	49	312
Family history types; χ^2=11.12 (p=0.01)			
Ia. Families with BC & OC	3 (1.2)	1 (2.0)	4 (1.3)
II. Families with at least one BC in male and in female	252 (95.8)	44 (89.8)	295 (94.5)
III. Families with ≥ 3 BC	5 (1.9)	4 (8.2)	10 (3.2)
IV. Families with < 3 BC	3 (1.2)	0	3 (1.0)
Histology classification; ns			
Invasive ductal carcinoma (IDC)	171 (85.1)	38 (97.4)	209 (87.1)
Ductal carcinoma in situ (DCIS)	17 (8.4)		17 (7.0)
Papillary carcinoma	9 (4.5)	1 (2.6)	10 (4.2)
Invasive lobular carcinoma (ILC)	3 (1.5)		3 (1.3)
undifferentiated	1 (0.5)		1 (0.4)
T; ns			
Tis	8 (5.1)		8 (4.2)
1	75 (47.5)	13 (40.6)	88 (46.3)
2	55 (34.8)	12 (37.5)	67 (35.5)
≥3	20 (12.7)	7 (21.9)	27 (14.2)
N; ns			
0	97 (57.1)	16 (44.4)	113 (54.9)
1	50 (29.4)	14 (38.9)	64 (31.1)
2	15 (8.8)	4 (11.1)	19 (9.2)
3	8 (4.7)	2 (5.6)	10 (4.9)
M; ns			
0	148 (96.1)	31 (100)	179 (96.8)
1	6 (3.9)		6 (3.2)
Grade; χ^2=11.54 (p=0.003)			
0	4 (2.9)		4 (2.5)
1	21 (15)	1 (5)	22 (13.8)
2	78 (55.7)	6 (30)	84 (52.5)
3	37 (26.4)	13 (65)	50 (31.3)
ER; ns			
Negative	7 (4.2)	1 (2.9)	8 (4)
Positive	159 (95.8)	33 (97.1)	192 (96)
PR; ns			
Negative	18 (10.9)	4 (11.8)	22 (11.1)
Positive	147 (89.1)	30 (88.2)	177 (88.9)
HER2; ns			
Negative	108 (85.7)	25 (92.6)	133 (86.9)
Positive	18 (14.3)	2 (7.4)	20 (13.1)

BC: breast cancer; OC: ovarian cancer; ns: non-significant

Table 2: Mutations, family histories and tumor types of index cases mutation-carriers

Genes	Mutations	N. Cases (%)	Family Type (N*)	References
BRCA1 N=2	c.1961delA	1 (50)	II	26-28
	c.3869_3870delAA	1 (50)	II	30
BRCA2 N= 47	c.145G>T	1 (2.1)	II	29
	c.262_263delCT	1 (2.1)	II	27, 29
	c.370delA	1 (2.1)	II	29
	c.658_659delGT	3 (6.3)	II (3)	30, 31
	c.682-2A>G	1 (2.1)	III	ND
	c.1368_1369dupGA	1 (2.1)	II	ND
	c.2808_2811delACAA	5 (10.4)	II (4)	26-29
	c.3264dupT	2 (4.2)	II (2)	26-29
	c.3922G>T	1 (2.1)	II	26-29
	c.4936_4939delGAAA	1 (2.1)	II	26-29
	c.5042_5043delTG	1 (2.1)	II	30
	c.5073dupA	1 (2.1)	II	30, 31
	c.5116_5119delAATA	1 (2.1)	II	26-29
	c.5146_5149delTATG	2 (4.2)	II, III	26, 28, 29
	c.5722_5723delCT	1 (2.1)	II	30, 31
	c.6209_6212delAAAG	1 (2.1)	II	30, 31
	c.6275_6276delTT	4 (8.3)	Ia,II (3)	26-29
	c.7109_7110delAA	1 (2.1)	II	30
	c.8067_8068insTT	1 (2.1)	II	26
	c.8490 G>A	1 (2.1)	III	30
	c.8695C>T	1 (2.1)	II	31
	c.8978_8991del14	1 (2.1)	II	26-29
	c.8988_8990delATAinsTT	1 (2.1)	II	27, 29
	c.9018C>A	1 (2.1)	II	26-29
	c.9026_9030delATCAT	7 (14.6)	II (6),III	26-29
	c.9117G>A (r.8954_9117del)	1 (2.1)	II	30, 31
	c.9376C>T	1 (2.1)	II	30
	DelEx.1-24	1 (2.1)	II	37
	DelEx.2	2 (4.2)	II (2)	ND

N*: indicate the number of cases when is >1; the most recurrent mutations are underlined; ND: Not described

Table 3: Inclusion criteria and tumor type of male relatives affected with cancer and *BRCA1/2* mutation-carriers

Parameter; χ^2 (p)	*BRCA1+* N (%)	*BRCA2+* N (%)	TOTAL N (100%)
Family history types; χ^2=11.85 (p=0.018)			
Ia. Families with BC & OC	7 (58)	5 (41.6)	12
Ib. Index case with BC & OC	1 (100)	0	1
II. Families with at least one BC in male and in female	0	8 (100)	8
III. Families with ≥ 3 BC	5 (21.7)	18 (78.2)	23
IV. Families with < 3 BC	7 (46.6)	8 (53.3)	15
V. One BC < 30	0	2 (100)	2
Tumor types; χ^2=8.83 (p=0.018)			
BC	0	11 (100)	11
PC	3 (23)	10 (77)	13
CPC/Others	17 (46)	20 (54)	37

BC: breast cancer; OC: ovarian cancer; PC: prostate cancer; CRC/others: colorectal or other cancers (bladder, pancreatic, melanoma, skin cancer, lung or gastrointestinal); ns: non-significant

Table 4: Mutations, family histories and tumor types of male relatives affected with cancer and *BRCA1/2* mutation-carriers

Genes	Mutations	N. Cases (%)	Family Type (N*)	Tumor type (N*)	References
BRCA1 N=20	c.68_69delAG	1 (5)	IV	CRC/Others	26-29
	c.211A>G	3 (15)	Ia(3)	CRC/Others(3)	26-29, 36, 39
	c.212+1G>A	2 (10)	III(2)	PC, CRC/Others	26-29, 36
	c.431dupA	1 (5)	III	CRC/Others	ND
	c.981_982delAT	1 (5)	Ia	CRC/Others	29
	c.1121_1123delCACinsT	1 (5)	IV	CRC/Others	29
	c.1961delA	1 (5)	Ia	CRC/Others	26-28
	c.3257T>G	1 (5)	IV	CRC/Others	29
	c.3770_3771delAG	1 (5)	IV	CRC/Others	26-29
	c.4484+1G>T	1 (5)	III	CRC/Others	36
	c.5027_5030delTAAC	1 (5)	III	CRC/Others	30
	c.5123C>A	1 (5)	Ib	PC	26-29
	c.5152+5 G>A	1 (5)	IV	CRC/Others	27, 29
	c.5154G>A	1 (5)	IV	CRC/Others	27, 29
	c.5266dupC	1 (5)	Ia	PC	31
	DelEx.8-13	1 (5)	IV	CRC/Others	29
	DupEx.13	1 (5)	Ia	CRC/Others	29, 38
BRCA2 N=41	c.145G>T	1 (2.4)	III	PC	27, 29
	c.262_263delCT	2 (4.9)	III(2)	BC, CRC/Others	27, 29
	c.538_539delAT	1 (2.4)	III	PC	31
	c.1368_1369delGA	1 (2.4)	II	BC	27, 29
	c.1813delA	1 (2.4)	IV	PC	30, 31
	c.2451_2452dup	1 (2.4)	III	PC	ND
	c.2701delC	1 (2.4)	III	CRC/Others	29
	c.2808_2811delACAA	5 (12.2)	III(3),IV,V	CRC/Others(5)	26-29
	c.3264dupT	3 (7.3)	Ia, III(2)	CRC/Others(3)	26-29
	c.3860deA	1 (2.4)	III	PC	30, 31
	c.3922G>T	1 (2.4)	III	CRC/Others	26-29
	c.4797delT	1 (2.4)	Ia	PC	26, 27
	c.6244G>T	1 (2.4)	Ia	CRC/Others	ND
	c.6275_6276delTT	2 (4.9)	II,III	BC(2)	26-29
	c.6405_6409delCTTAA	1 (2.4)	Ia	BC	30, 31
	c.6445_6446delAT	1 (2.4)	IV	CRC/Others	30
	c.6486_6489delACAA	2 (4.9)	Ia,II	BC	29
	c.6656C>G	1 (2.4)	II	BC	30, 31
	c.7234insG	1 (2.4)	III	CRC/Others	27, 29
	c.7480C>T	1 (2.4)	III	PC	27
	c.8695C>T	1 (2.4)	II	BC	31
	c.8946delA	1 (2.4)	II	PC	30
	c.9018C>A	1 (2.4)	III	PC	26-29
	c.9026_9030delATCAT	5 (12.2)	III,IV(4)	BC, CRC/Others(4)	26-29, 36
	c.9154C>T	1 (2.4)	I	PC	30, 31
	c.9310_9311delAA	1 (2.4)	II	BC	30
	c.9376C>T	1 (2.4)	II	BC	30
	DupEx.21	1 (2.4)	IV	CRC/Others	35

N*: indicate the number of cases when is >1; ND: not described; BC: breast cancer; OC: ovarian cancer; PC: prostate cancer; CRC/Others: colorectal or other cancers (bladder, pancreatic, melanoma, skin cancer, lung or gastrointestinal); the most recurrent mutations are underlined

Table 1
Click here to download Table: Table 1.docx

Table 1: Demographic, family history, clinicopathological and immunohistochemistry characteristics of index cases

Parameter; χ^2 (p)	**BRCAX** N (%)	***BRCA1/2+*** N (%)	**TOTAL** N (%)
Male breast cancer	263	49	312
Family history types; χ^2=11.12 (p=0.01)			
Ia. Families with BC & OC	3 (1.2)	1 (2.0)	4 (1.3)
II. Families with at least one BC in male and in female	252 (95.8)	44 (89.8)	295 (94.5)
III. Families with ≥ 3 BC	5 (1.9)	4 (8.2)	10 (3.2)
IV. Families with < 3 BC	3 (1.2)	0	3 (1.0)
Histology classification; ns			
Invasive ductal carcinoma (IDC)	171 (85.1)	38 (97.4)	209 (87.1)
Ductal carcinoma in situ (DCIS)	17 (8.4)		17 (7.0)
Papillary carcinoma	9 (4.5)	1 (2.6)	10 (4.2)
Invasive lobular carcinoma (ILC)	3 (1.5)		3 (1.3)
undifferentiated	1 (0.5)		1 (0.4)
T; ns			
Tis	8 (5.1)		8 (4.2)
1	75 (47.5)	13 (40.6)	88 (46.3)
2	55 (34.8)	12 (37.5)	67 (35.5)
≥3	20 (12.7)	7 (21.9)	27 (14.2)
N; ns			
0	97 (57.1)	16 (44.4)	113 (54.9)
1	50 (29.4)	14 (38.9)	64 (31.1)
2	15 (8.8)	4 (11.1)	19 (9.2)
3	8 (4.7)	2 (5.6)	10 (4.9)
M; ns			
0	148 (96.1)	31 (100)	179 (96.8)
1	6 (3.9)		6 (3.2)
Grade; χ^2=11.54 (p=0.003)			
0	4 (2.9)		4 (2.5)
1	21 (15)	1 (5)	22 (13.8)
2	78 (55.7)	6 (30)	84 (52.5)
3	37 (26.4)	13 (65)	50 (31.3)
ER; ns			
Negative	7 (4.2)	1 (2.9)	8 (4)
Positive	159 (95.8)	33 (97.1)	192 (96)
PR; ns			
Negative	18 (10.9)	4 (11.8)	22 (11.1)
Positive	147 (89.1)	30 (88.2)	177 (88.9)
HER2; ns			
Negative	108 (85.7)	25 (92.6)	133 (86.9)
Positive	18 (14.3)	2 (7.4)	20 (13.1)

BC: breast cancer; OC: ovarian cancer; ns: non-significant

Table 2
Click here to download Table: Table 2.docx

Table 2: Mutations, family histories and tumor types of index cases mutation-carriers

Genes	Mutations	N. Cases (%)	Family Type (N*)	References
BRCA1 N=2	c.1961delA	1 (50)	II	26-28
	c.3869_3870delAA	1 (50)	II	30
BRCA2 N= 47	c.145G>T	1 (2.1)	II	29
	c.262_263delCT	1 (2.1)	II	27, 29
	c.370delA	1 (2.1)	II	29
	<u>c.658_659delGT</u>	<u>3 (6.3)</u>	II (3)	30, 31
	c.682-2A>G	1 (2.1)	III	ND
	c.1368_1369dupGA	1 (2.1)	II	ND
	<u>c.2808_2811delACAA</u>	<u>5 (10.4)</u>	II (4)	26-29
	c.3264dupT	2 (4.2)	II (2)	26-29
	c.3922G>T	1 (2.1)	II	26-29
	c.4936_4939delGAAA	1 (2.1)	II	26-29
	c.5042_5043delTG	1 (2.1)	II	30
	c.5073dupA	1 (2.1)	II	30, 31
	c.5116_5119delAATA	1 (2.1)	II	26-29
	c.5146_5149delTATG	2 (4.2)	II, III	26, 28, 29
	c.5722_5723delCT	1 (2.1)	II	30, 31
	c.6209_6212delAAAG	1 (2.1)	II	30, 31
	<u>c.6275_6276delTT</u>	<u>4 (8.3)</u>	Ia,II (3)	26-29
	c.7109_7110delAA	1 (2.1)	II	30
	c.8067_8068insTT	1 (2.1)	II	26
	c.8490 G>A	1 (2.1)	III	30
	c.8695C>T	1 (2.1)	II	31
	c.8978_8991del14	1 (2.1)	II	26-29
	c.8988_8990delATAinsTT	1 (2.1)	II	27, 29
	c.9018C>A	1 (2.1)	II	26-29
	<u>c.9026_9030delATCAT</u>	<u>7 (14.6)</u>	II (6),III	26-29
	c.9117G>A (r.8954_9117del)	1 (2.1)	II	30, 31
	c.9376C>T	1 (2.1)	II	30
	DelEx.1-24	1 (2.1)	II	37
	DelEx.2	2 (4.2)	II (2)	ND

N*: indicate the number of cases when is >1; the most recurrent mutations are underlined; ND: Not described

Table 3
Click here to download Table: Table 3.docx

Table 3: Inclusion criteria and tumor type of male relatives affected with cancer and *BRCA1/2* mutation-carriers

Parameter; χ^2 (p)	*BRCA1+* N (%)	*BRCA2+* N (%)	TOTAL N (100%)
Family history types; χ^2=11.85 (p=0.018)			
Ia. Families with BC & OC	7 (58)	5 (41.6)	12
Ib. Index case with BC & OC	1 (100)	0	1
II. Families with at least one BC in male and in female	0	8 (100)	8
III. Families with ≥ 3 BC	5 (21.7)	18 (78.2)	23
IV. Families with < 3 BC	7 (46.6)	8 (53.3)	15
V. One BC < 30	0	2 (100)	2
Tumor types; χ^2=8.83 (p=0.018)			
BC	0	11 (100)	11
PC	3 (23)	10 (77)	13
CPC/Others	17 (46)	20 (54)	37

BC: breast cancer; OC: ovarian cancer; PC: prostate cancer; CRC/others: colorectal or other cancers (bladder, pancreatic, melanoma, skin cancer, lung or gastrointestinal); ns: non-significant

Table 4: Mutations, family histories and tumor types of male relatives affected with cancer and *BRCA1/2* mutation-carriers

Genes	Mutations	N. Cases (%)	Family Type (N*)	Tumor type (N*)	References
BRCA1 N=20	c.68_69delAG	1 (5)	IV	CRC/Others	26-29
	c.211A>G	3 (15)	Ia(3)	CRC/Others(3)	26-29, 36, 39
	c.212+1G>A	2 (10)	III(2)	PC, CRC/Others	26-29, 36
	c.431dupA	1 (5)	III	CRC/Others	ND
	c.981_982delAT	1 (5)	Ia	CRC/Others	29
	c.1121_1123delCACinsT	1 (5)	IV	CRC/Others	29
	c.1961delA	1 (5)	Ia	CRC/Others	26-28
	c.3257T>G	1 (5)	IV	CRC/Others	29
	c.3770_3771delAG	1 (5)	IV	CRC/Others	26-29
	c.4484+1G>T	1 (5)	III	CRC/Others	36
	c.5027_5030delTAAC	1 (5)	III	CRC/Others	30
	c.5123C>A	1 (5)	Ib	PC	26-29
	c.5152+5 G>A	1 (5)	IV	CRC/Others	27, 29
	c.5154G>A	1 (5)	IV	CRC/Others	27, 29
	c.5266dupC	1 (5)	Ia	PC	31
	DelEx.8-13	1 (5)	IV	CRC/Others	29
	DupEx.13	1 (5)	Ia	CRC/Others	29, 38
BRCA2 N=41	c.145G>T	1 (2.4)	III	PC	27, 29
	c.262_263delCT	2 (4.9)	III(2)	BC, CRC/Others	27, 29
	c.538_539delAT	1 (2.4)	III	PC	31
	c.1368_1369delGA	1 (2.4)	II	BC	27, 29
	c.1813delA	1 (2.4)	IV	PC	30, 31
	c.2451_2452dup	1 (2.4)	III	PC	ND
	c.2701delC	1 (2.4)	III	CRC/Others	29
	c.2808_2811delACAA	5 (12.2)	III(3),IV,V	CRC/Others(5)	26-29
	c.3264dupT	3 (7.3)	Ia, III(2)	CRC/Others(3)	26-29
	c.3860deA	1 (2.4)	III	PC	30, 31
	c.3922G>T	1 (2.4)	III	CRC/Others	26-29
	c.4797delT	1 (2.4)	Ia	PC	26, 27
	c.6244G>T	1 (2.4)	Ia	CRC/Others	ND
	c.6275_6276delTT	2 (4.9)	II,III	BC(2)	26-29
	c.6405_6409delCTTAA	1 (2.4)	Ia	BC	30, 31
	c.6445_6446delAT	1 (2.4)	IV	CRC/Others	30
	c.6486_6489delACAA	2 (4.9)	Ia,II	BC	29
	c.6656C>G	1 (2.4)	II	BC	30, 31
	c.7234insG	1 (2.4)	III	CRC/Others	27, 29
	c.7480C>T	1 (2.4)	III	PC	27
	c.8695C>T	1 (2.4)	II	BC	31
	c.8946delA	1 (2.4)	II	PC	30
	c.9018C>A	1 (2.4)	III	PC	26-29
	c.9026_9030delATCAT	5 (12.2)	III,IV(4)	BC, CRC/Others(4)	26-29, 36
	c.9154C>T	1 (2.4)	I	PC	30, 31
	c.9310_9311delAA	1 (2.4)	II	BC	30
	c.9376C>T	1 (2.4)	II	BC	30
	DupEx.21	1 (2.4)	IV	CRC/Others	35

N*: indicate the number of cases when is >1; ND: not described; BC: breast cancer; OC: ovarian cancer; PC: prostate cancer; CRC/Others: colorectal or other cancers (bladder, pancreatic, melanoma, skin cancer, lung or gastrointestinal); the most recurrent mutations are underlined