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Mutations in *BRCA2* and *PALB2* in male breast cancer cases from the United States

Yuan Chun Ding,

Department of Population Sciences, Beckman Research of Institute at the City of Hope, 1500 East Duarte Road, Duarte, CA 91010, USA

Linda Steele,

Department of Population Sciences, Beckman Research of Institute at the City of Hope, 1500 East Duarte Road, Duarte, CA 91010, USA

Chih-Jen Kuan,

Formerly at University of California Irvine, Irvine, CA 92657, USA

Scott Greilac, and

PrimeGen Biotech LLC, Irvine, CA CA 92618, USA

Susan L. Neuhausen

Department of Population Sciences, Beckman Research of Institute at the City of Hope, 1500 East Duarte Road, Duarte, CA 91010, USA

Abstract

Male breast cancer (MBC) is an uncommon disease with a frequency of approximately one in 1000. Due to the rarity of MBC, it is understudied and its etiology is poorly understood. Our objectives are to determine the frequency of pathogenic mutations in *BRCA2* and *PALB2* in MBC cases and to investigate the correlations between mutation status and cancer phenotypes. Single strand conformation polymorphism analysis, direct sequencing, and multiplex ligation-dependent probe amplification were employed to screen for mutations in the *BRCA2* gene, followed by direct sequencing of the *PALB2* gene in *BRCA2*-negative MBC cases. Pathogenic *BRCA2* mutations were identified in 18 of the 115 MBC cases, including four of the ten cases (40%) from breast cancer families and 14 of the 105 cases (13%) unselected for family history of breast cancer. The difference in *BRCA2*-mutation frequencies between cases with and without family history of breast cancer was not statistically significant ($P = 0.145$), suggesting that family history is not a strong predictor of carrying a mutation in males. We observed a highly significant association of carrying a pathogenic *BRCA2* mutation with high tumor grade ($P < 0.001$) and a weak association with positive lymph nodes ($P < 0.02$). Of the 97 *BRCA2*-negative MBC cases, we identified one *PALB2* mutation with confirmed pathogenicity and one mutation predicted to be pathogenic, a prevalence of pathogenic *PALB2*-mutation of 1–2%. Based on our results and previous studies, genetic testing for *BRCA2* should be recommended for any diagnosed MBC case, regardless of family history of breast cancer.

Keywords

Male breast cancer; *BRCA2*; *PALB2*; Genetic testing

Introduction

Male breast cancer (MBC) is uncommon, affecting less than 0.1% of males in the United States [1,2]. However, the incidence rate of MBC is increasing, having increased 0.9% yearly from 1975–2006 [3]. The treatment of MBC is the same as female breast cancer (FBC), as there are too few males to assess whether there are differences in treatment response or outcome. Risk factors for MBC include lifestyle factors (obesity, alcohol consumption, and estrogen intake), occupational exposure to high ambient temperature and exhaust emissions, medical conditions (testicular damage, liver damage, and radiotherapy to the chest), and genetic factors (Klinefelter's syndrome, Cowden syndrome, and family history of breast cancer) [4]. Several genes have been identified which confer more than a twofold increased risk to develop FBC including *BRCA1*, *BRCA2*, *CHEK2*, *PALB2*, *BRIP1*, *PTEN*, *ATM*, and *TP53* [5]. *BRCA1* and *BRCA2* mutations account for approximately 15% of familial FBC, and the others combined account for less than 10% [5]. In MBC cases, *BRCA2* mutations are much more common than *BRCA1* mutations [4] and confer a lifetime relative risk to develop the disease of approximately 8.9% [6]. In 16 previously published studies, the frequency of *BRCA2* mutations in MBC varied from 4 to 40% [7–22]. The wide range in mutation frequency may be due to small sample sizes, mutation screening methods with varying sensitivity, and missense variants that were not classified as pathogenic or benign across the different studies. *PALB2* plays crucial roles in the localization and stabilization of *BRCA2* in nuclear chromatin, which is essential for *BRCA2* to function in double strand break (DSB) repair by homologous recombination [23,24]. Truncating mutations in *PALB2* have been identified in familial FBC cases, and although they are rare (approximately 1%), they confer an estimated 2–4 fold risk to develop breast cancer [23,25–28]. In view of its close relationship to *BRCA2* and the risk for FBC, it is plausible that *PALB2* may also confer significant risk to develop MBC. In this study, we screened 115 MBC cases for mutations in *BRCA2*, followed by direct sequencing of *PALB2* to look for pathogenic *PALB2* mutations in MBC cases negative for *BRCA2* mutations. We also investigated the association of *BRCA2* mutation status and clinical–pathological features of the cancer.

Materials and methods

Subjects

A total of 115 MBC cases were recruited from state cancer registries (83 cases) in Utah and the surrounding intermountain states (Colorado, Idaho and Wyoming), an online male support group (18 cases) and referrals from physicians (four cases), and family members (ten cases). The participants were enrolled under Institutional Review Board approval and all signed informed consent. The ten cases referred by family members were from breast cancer families and the remaining 105 cases were unselected for age or family history of breast cancer. Of the 115 MBC cases, 107 cases were non-Hispanic Caucasian, three were Hispanic, and five were Ashkenazi Jewish ancestry. The average age at diagnosis was 60 years with a range from 28 to 93 years. From questionnaires, medical records and pathology reports, we abstracted information on race/ethnicity ($N = 115$), family history and personal history of breast cancer ($N = 114$), histopathologic type ($N = 110$), TNM stage ($N = 108$), tumor size ($N = 106$), histopathology grade ($N = 102$), regional lymph node involvement ($N = 101$), estrogen receptor (ER) status ($N = 65$), and progesterone receptor (PR) status ($N = 61$).

Mutation screening

DNA was extracted from blood by standard phenol/chloroform procedures. Primers were designed to screen for mutations in all *BRCA2* exons and intron/exon boundaries (up to 200 bp of each intron). Mutation detection was initially performed by single strand conformation polymorphism analysis (SSCP) on the 115 DNA samples. Exons 2, 3, 10, 11, 14, 16, 18, 22, 23, 25, and 27 were PCR amplified into 2, 2, 6, 19, 3, 2, 2, 2, 2, and 7 overlapping amplicons, respectively, with the remaining exons covered in single amplicon. PCR amplicons with size ranging from 120 to 300 bp were denatured and run on non-denaturing gels prepared with 0.5× mutation detection enhancement (MDE) (AT Biochem) in 0.6× TBE buffer at 6 W for 12 to 16 h at room temperature, depending on amplicon size. Samples with aberrant bands were selected for sequencing. All samples for which no pathogenic *BRCA2* mutations were detected by SSCP were then directly sequenced for entire *BRCA2* gene. The size of the PCR amplicons for sequencing varied from 400 to 1200 bp. Each exon was amplified by one PCR amplicon with the exception of exons 10 and 11 which were amplified into two and eight overlapping amplicons, respectively. Briefly, for sequencing, the PCR was conducted in 25 μ l volumes containing 100 ng DNA following the manufacturer's protocols. After PCR, Shrimp Alkaline Phosphatase (SAP) and Exonuclease I (EXO I) were used to clean-up the amplified fragments. The ABI Prism BigDye Terminator cycle sequencing kit 3.1 (PE Applied Biosystems) was used for the cycle sequencing reaction. Each amplicon was sequenced in both directions. Sequences were aligned using Sequencer software (GeneCode Corporation, MI), as well as manually inspected if needed. For the samples without *BRCA2* pathogenic mutations detected by SSCP and direct sequencing, the SALSA P045 *BRCA2* MLPA kit (MRC-Holland) was used to screen for large insertions and deletions in *BRCA2* according to the manufacturer's instructions. Briefly, 100 ng of genomic DNA was used in the ligation reaction. Then multiplex PCR amplification (33 cycles) was performed on a PTC-225 tetrad PCR machine (MJ Research). After PCR amplification, DNA fragments were run on an ABI 3130 sequencer. Data including information of peak size, height, and area were exported from the sequencer to an Excel macro-spread-sheet for identification of peak gain and loss. A peak height differing more than 30% from the mean of references was considered altered. For the samples without pathogenic *BRCA2* mutations, *PALB2* was screened by direct sequencing as described above. Sixteen pairs of previously published primers were used to amplify the 13 exons and the intron-exon boundaries [29].

Statistical analysis

Fisher's exact test was used to test the associations between mutation status and clinical and pathological features. The statistical significance of differences between groups in age at diagnosis was determined by a two-tailed *t*-test, with *P* value <0.05 considered significant. The confidence interval for the proportion of MBC cases attributable to pathogenic *BRCA2* mutations was calculated based on the Wilson method [30]. Statistical analyses were performed using SAS 9.1.3.

Results

Of the 114 MBC cases with family history information, 55 cases (48%) had a minimum of one first- or second-degree relative affected with breast cancer. Of the 114 MBC cases with personal history information on other cancers, 26 cases had at least one other cancer (23%), excluding basal skin cancers. Four cases had two other cancers including two cases with prostate and colon cancers, one with melanoma and brain cancer, and one with colon cancer and melanoma. Of those cases with one other cancer, there were six prostate cancers, five bladder cancers, four melanomas, two colorectal cancers, two lip cancers, one pancreatic, one parotid cancer, and one nasal papillary adenocarcinoma. Of 110 MBC cases with tumor

type, the majority of cases (79.1%) were invasive ductal carcinoma, with the remaining being in situ ductal (3.6%), lobular (1.8%), invasive ductal and lobular (0.9%), medullary (0.9%), and other types not specified (13.7%). The distribution of histopathologic grade from the 102 MBC cases with data was 26.5% Grade 1 (G1), 48.0% G2, and 25.5% G3. For tumor size at diagnosis, 72 of 106 tumors (67.9%) were smaller than 2 cm. Regional lymph nodes were present in 32 of 101 cases (31.7%). Of the 65 cases with ER status, 60 cases (92.3%) were ER positive. PR positive tumors were also predominant and detected in 50 of 61 cases (82.0%). In addition, one MBC case was reported to have Klinefelter's syndrome.

The SSCP, direct sequencing and MLPA were employed to screen for mutations in *BRCA2*. A total of 45 germline variants in *BRCA2* were detected in 115 MBC cases (Table 1). Of the 45 variants, 36 variants were reported in the Breast Information Core (BIC) database (<http://research.nhgri.nih.gov/projects/bic>). Of those 36 variants in BIC, 11 were classified as pathogenic, 12 as neutral, and 13 as variants of unknown significance (VUS). Mutations 2158delA, 8804delA, and 8822insT, which were not listed in BIC, are pathogenic as they cause truncation of more than seven exons of the *BRCA2* protein sequence. Fourteen missense variants and intervening-sequence (IVS or intronic) variants, classified as VUS in BIC or not included in the BIC, were assessed for clinical significance as described by Easton et al. [31]. D2723H was classified as pathogenic, IVS21+ 4 A > G was likely pathogenic (posterior probability of 0.90), G1529R and I2285 V were neutral; T1505 was likely neutral; and the remaining nine VUS could not be classified as pathogenic or neutral. Thus, a total of 16 pathogenic variants were identified, including 6174 delT detected in two unrelated cases of Ashkenazi descent and D2723H in two unrelated Caucasians cases. One MBC of Ashkenazi descent had a pathogenic mutation (4075delGT) that was not the founder 6174delT mutation. Two of the three Hispanic MBC cases had mutations (8822insT and 3036delA). Five of sixteen mutations were not found by SSCP and were identified by direct sequencing. No large insertions or deletions were detected by MLPA. In total, 18 of 115 MBC cases carried pathogenic variants, resulting in a *BRCA2*-mutation prevalence of 16%, (95% CI 11–24). Of the ten MBC cases referred by family members in high-risk breast cancer families, 4 (40%) had a mutation. Excluding those ten cases referred by family members, the *BRCA2*-mutation prevalence is 13% (14 of 105, 95% CI, 6–18).

We tested the association between *BRCA2* mutation status and family history of breast cancer, diagnosis age, tumor size, regional lymph node status, histological grade, and ER and PR status (Table 2). Excluding the ten MBC cases referred by family members, *BRCA2* mutations were identified in nine of 45 cases (20%) with a family history of breast cancer and five of 59 cases with no family history (8.5%) ($P = 0.145$; OR 2.7, 95% CI 0.8–8.7). Positive regional lymph nodes were observed in 59% of *BRCA2* mutation carriers compared to 26% of non-carriers ($P < 0.02$). Sixty percent of carriers presented with highgrade tumors (G3) compared to 20% of non-carriers ($P < 0.001$). The distributions of diagnosis age, tumor size, ER, and PR status were similar between carriers and non-carriers.

The DNA samples from the 97 MBC cases without a pathogenic *BRCA2* mutation were sequenced for *PALB2*. Fourteen germline variants were identified (Table 3). The truncating mutation, Y1183X, was classified as pathogenic according to Reid et al. [32] and Oliver et al. [33]. The amino acid substitution prediction methods, Polyphen [34] and PMUT [35], were used to assess whether missense variants could affect protein function. Of the six missense variants, Y28C and G998E were predicted to likely affect *PALB2* protein function by both the Polyphen prediction and the PMUT prediction (Table 3).

Discussion

In this study, we screened for mutations in *BRCA2* and identified 18 of 115 MBC cases carrying pathogenic mutations. SSCP did not detect five pathogenic base substitution mutations (Table 1) including three splicing and two missense mutations, consistent with previous reports that SSCP is not sufficiently sensitive to detect single base changes [36,37]. In 12 previous studies screening MBC cases for mutations in *BRCA2*, SSCP or methods with similar sensitivities including the protein truncation test (PTT) and conformation sensitive gel electrophoresis (CSGE) were used [7,9–18,22] (Table 4). Of 57 *BRCA2* pathogenic mutations reported in those studies, only two were base substitutions, suggesting that pathogenic base substitution mutations may have been missed by the screening methodology. The frequency of mutations in this dataset is higher than reported in many of the previous studies. One reason may be that we were able to identify pathogenic missense mutations that were missed in studies using less sensitive detection methods.

In our study, a highly significant association ($P < 0.001$) was observed between pathogenic *BRCA2* mutations and high tumor grade (G3) (Table 2), consistent with the previous studies. Ottini et al. [18] in their study of 108 MBC cases from central Italy found a significant association of *BRCA2* mutation status and high-grade tumors ($P < 0.005$) and Kwiatkowska et al. [15] reported that 59% of *BRCA2* carriers had high-grade tumors (G3) compared to 29% in non-carriers. We also observed significantly higher frequencies of positive nodes among *BRCA2* carriers ($P = 0.02$). *BRCA2* is a key component involved in DSB repair. We speculate that the high frequency of high-grade tumors may be related to the defective function of DSB repair in cells with *BRCA2* mutations.

For FBC, a strong family history of breast cancer is a criterion for genetic testing for *BRCA1* and *BRCA2*. In non-familial FBC cases unselected for age, the frequency of pathogenic *BRCA2* mutations is less than 0.5% [38,39]. There are no published criteria for genetic testing of males with breast cancer. In our study, of the 18 cases with *BRCA2* mutations, 13 had a family history. If we exclude the ten MBC cases recruited through referrals from family members in breast cancer families of whom 4 (40%) had *BRCA2* mutations, *BRCA2* mutations were identified in nine of 45 cases (20%) with a family history of breast cancer and five of 59 cases (8.5%) with no family history ($P = 0.145$; OR 2.7, 95% CI 0.8–8.7). Our results suggest that family history is not as strong a predictor of carrying a mutation in males as it is in females and are consistent with results from other studies. Ottini et al. [18] reported that 50% of *BRCA2* pathogenic mutations were in MBC cases without a family history of breast cancer. In a study from Sweden [14], 86% (six of seven) of the MBC cases carrying *BRCA2* mutations had no family history of breast cancer. In a study from Hungary, Csokay et al. [10] found that none of the six MBC cases with pathogenic *BRCA2* mutations had a family history of breast cancer. Kwiatkowska [15] et al. identified four pathogenic *BRCA2* mutations in 37 MBC cases from Poland and only one of four mutation carriers had a second-degree relative with breast cancer. The only exception was the study of Couch et al. [9] who screened *BRCA2* mutations in 50 MBC cases and found that six of seven (85%) mutation carriers had a family history of breast cancer. However, given that 80% of the tested MBC cases had a family history, the proportion of cases with a family history and a mutation is the same as those without a mutation. Our results and those of previous studies would suggest genetic testing for *BRCA2* in any diagnosed MBC case with no criterion of a family history.

Truncating mutations in the *PALB2* gene were first identified in Fanconi Anemia subtype FA-N [23,32]. Subsequently, a number of studies found that truncating mutations in *PALB2* predisposed to FBC [23,25–28]. We screened the 97 MBC cases without a pathogenic *BRCA2* mutation and identified 14 germline variants (Table 2). The Y1183X results in the

loss of the last four amino acids in the C terminus of PALB2. This mutation had been previously detected in both FBC cases and individuals with FA-N [25,32]. By X-ray crystallography, Oliver et al. [33] demonstrated that the C terminus of PALB2 played an important role in stabilizing the PALB2 protein and removal of the last four codons by the Y1183X mutation resulted in an incompletely folded protein that degraded rapidly. The Y1183X carrier was diagnosed with breast cancer at age 54 and melanoma at age 58 and had one second-degree relative with FBC. Interestingly, one of three FBC cases with the Y1183X mutation identified by Rahman et al. [25] also developed melanoma. A Y28C mutation was identified (Table 3). A coiled-coil domain (from residues 9 to 42) in the N terminus of *PALB2* is involved in the process of oligomerization and accumulation of PALB2 protein at double-strand breakpoints (DSB), which further facilitates the recruitments of BRCA2 and RAD51 to DSB for DNA repair[33,40]. As Y28C is in the coiled-coil domain, we used the SIFT [41], PMUT [35], and POLYPHEN [34] programs to examine whether the amino acid change in codon 28 affected PALB2 protein function. All three programs predicted that Y28C was a pathogenic mutation. The Y28C mutation carrier was diagnosed with breast cancer at age 46, and had one first-degree and two second-degree female relatives diagnosed with breast cancer. He had an invasive ductal carcinoma of 3.8 cm with high tumor grade and positive regional lymph nodes. Further lab experiments need to be done to confirm the pathogenicity of Y28C. Rahman et al. [25] detected nine truncating *PALB2* mutations in 908 families in the UK with familial FBC and one truncating mutation in 15 UK families with both MBC and FBC. The prevalence of truncating mutations was higher in families with both MBC and FBC cases (6.7%) than in families with only FBC cases (1%), suggesting that *PALB2* might be important in MBC. Garcia et al. [28] screened 95 Spanish breast cancer families and identified one truncating *PALB2* mutation in a family with both FBC and MBC. Recently, Sauty de Chalon et al. [42] screened 25 MBC cases from 25 families in kConFab for *PALB2* and did not identify any pathogenic mutations. In our study of MBC, the frequency of pathogenic *PALB2* mutation was 1–2%, as we identified only one *PALB2* mutation with confirmed pathogenicity and one mutation predicted to be pathogenic.

In conclusion, in this study of 115 MBC cases from the United States, *BRCA2* accounted for 16% and *PALB2* accounted for 1–2% of the breast cancers. These results and previous studies suggest that males who develop breast cancer should be screened for mutations in *BRCA2*, and possibly in *PALB2*. Testing may be particularly important given the development of targeted therapies [43] including the Poly (ADP-Ribose) polymerase (PARP) inhibitors now being tested in cancer patients carrying mutations in genes involved in the homologous recombination repair pathway.

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Table 1Rare mutations and polymorphisms in *BRCA2* gene

Mutation^a	Location	Frequency^b	Clinical importance
203G>A	Exon 2	41/210	Unknown
279delAC	Exon 2	1/230	Pathogenic
E49X	Exon 3	1/230	Pathogenic
L74L	Exon 3	1/210	Unknown
IVS2-7 T < A	Intron 3	1/210	Unknown
<u>IVS4 + 67 G>T</u>	Intron 4	7/210	Unknown
<u>IVS7-69 T>C</u>	Intron 7	1/210	Unknown
IVS7 + 2 T>G	Intron 7	1/210	Pathogenic
IVS8 + 56 C>T	Intron 8	42/210	Unknown
<u>I247I</u>	Exon 9	1/210	Unknown
<u>C279C</u>	Exon 10	1/210	Unknown
N289H	Exon 10	7/210	Neutral
N372H	Exon 10	58/210	Neutral
S455S	Exon 10	7/210	Neutral
<u>2158delA</u>	Exon 11	1/230	Pathogenic
3036del4	Exon 11	1/210	Pathogenic
Q961Q	Exon 11	1/210	Unknown
N991D	Exon 11	9/210	Neutral
K1132 K	Exon 11	60/210	Neutral
V1269 V	Exon 11	36/210	Neutral
4075delGT	Exon 11	1/230	Pathogenic
4359ins6	Exon 11	1/230	Pathogenic
D1420Y	Exon 11	1/210	Neutral
4706del4	Exon 11	1/230	Pathogenic
T1505A	Exon 11	1/210	Likely neutral
G1529R	Exon 11	1/210	Neutral
5950delCT	Exon 11	1/230	Pathogenic
T1915 M	Exon 11	3/210	Unknown
6174 del T	Exon 11	2/230	Pathogenic
R2034C	Exon 11	2/210	Unknown
I2285 V	Exon 12	1/210	Neutral
S2414S	Exon 14	37/210	Neutral
IVS15 + 1G > A	Intron 15	1/210	Pathogenic
IVS16-14 T>C	Intron 16	78/158	Neutral
D2723H	Exon 18	2/210	Pathogenic
V2728I	Exon 18	2/210	Neutral
<u>IVS18-154 T>C</u>	Intron 18	1/210	Unknown
Q2859X	Exon 20	1/210	Pathogenic
<u>8804delA</u>	Exon 20	1/230	Pathogenic

Mutation ^a	Location	Frequency ^b	Clinical importance
<u>8822insT</u>	Exon 20	1/230	Pathogenic
IVS21 + 4 A > G	Intron 21	1/210	Probably pathogenic
A2951T	Exon 22	1/210	Neutral
<u>IVS24-113 T>G</u>	Intron 24	1/210	Unknown
IVS24-16 T>C	Intron 24	3/210	Unknown
K3326X	Exon 27	2/230	Neutral

^a Underlined variants were not included in BIC database; pathogenic mutations in bold font were missed by SSCP and detected by direct sequencing

^b Frequency was calculated by $n/2N$ (n is the number of rare alleles, N is number of samples screened)

Table 2Associations between *BRCA2* mutation status and clinical/pathologic features

	# samples	# Carriers	# Non-Carriers	P value
<i>Dx_age</i>				
Mean	60	62	60	0.538
<60 (years)	53 (46.1%)	8	45	
>60 (years)	62 (53.9%)	10	52	0.805
Family history ^a				
Yes	45 (39.5%)	9	36	
No	59 (60.5%)	5	54	0.145
Tumor size				
T1	72 (67.9%)	12	60	
T2	26 (24.5%)	4	22	
T3	4 (3.7%)	1	3	
T4	4 (3.7%)	1	3	0.734
Lymph nodes				
Negative	69 (68.3%)	7	62	
Positive	32 (31.7%)	10	22	0.019
Grades				
G1	27 (26.5%)	0	27	
G2	49 (48.0%)	6	43	
G3	26 (25.5%)	9	17	0.001
PR status				
Positive	50 (82.0%)	9	41	
Negative	11 (18.0%)	4	7	0.225
ER status				
Positive	60 (92.3%)	13	47	
Negative	5 (7.7%)	1	4	1.000

^aExcluding the ten MBC cases referred by family members in high-risk breast cancer families

Table 3

Rare mutations and polymorphisms in PALB2 gene

Mutation ^a	Location	Frequency ^b	Polyphen prediction	PMUT prediction
-159 G>C	Promoter	1/194	-	
103 G>A	5'-UTR	6/194	-	
Y28C	Exon 2	1/194	Probably damaging	Pathological
IVS3-58 A>C	Intron3	3/194		
L337S	Exon 4	5/194	Possibly damaging	Neutral
S524S	Exon 4	4/194		
Q559R	Exon 4	25/194	Benign	Neutral
E672Q	Exon 5	3/194	Benign	Neutral
E814E	Exon 5	1/194		
V932 M	Exon 8	1/194	Benign	Neutral
G998E	Exon 9	3/194	Probably damaging	Pathological
T1100T	Exon 12	3/194		
S1165S	Exon 13	1/194		
Y1183X	Exon 13	1/194		

^aNucleotide position +1 corresponding to the A of the ATG translation initiation site of genomic reference sequence downloaded from the human genome project

^bFrequency was calculated by $n/2N$ (n is the number of rare alleles, N is number of samples screened)

Table 4

Published mutation screening studies of *BRCA2* in MBC

Reference	Population	# Samples	# Mutations (%)	Screening strategy	Screening method
7	UK	94	5 (5%)	Entire gene	SSCP
8	Slovenia	25	4 (16%)	Founder mutation	PTT + DGGE
9	US	50	7 (14%)	Entire gene	CSGE
10	Hungary	18	6 (33%)	Entire gene	SSCP
11	Spain	17	3 (18%)	Entire gene	SSCP + PTT
12	UK	37	11 (30%)	Entire gene	SSCP + PTT
13	US	54	2 (4%)	Entire gene	SSCP + PTT
14	Sweden	34	7 (21%)	Entire gene	SSCP + PTT
15	Poland	43	8 (19%)	Entire gene	SSCP
16	UK	28	2 (7%)	Entire gene	SSCP
17	Italy	25	3 (12%)	Entire gene	SSCP
18	Italy	108	8 (7%)	Entire gene	SSCP + PTT
19	Ashkenazi	89	13 (15%)	Founder mutation	Gene Scan
20	Finland	34	2 (6%)	Entire gene	DHPLC + PTT
20	Finland	120	8 (7%)	Founder mutation	SBE
21	Iceland	30	12 (40%)	Founder mutation	DPG
22	Canada	14	2 (14%)	Entire gene	SSCP + PTT

CSGE Conformation sensitive gel electrophoresis; SSCP Single strand conformation polymorphism; PTT Protein truncation test; DPG Denaturing polyacrylamide gel; DHPLC Denaturing high performance liquid chromatography; SBE Single base extension