symposium article

High prevalence of *BRCA1* deletions in BRCAPRO-positive patients with high carrier probability

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Mutation screening of the *BRCA1* and *BRCA2* genes in probands with familial breast/ovarian cancer has been greatly improved by the multiplex ligation-dependent probe amplification (MLPA) assay able to evidence gene rearrangements not detectable by standard screening methods. However, no criteria for selection of cases to be submitted to the MLPA test have been reported yet. We used the BRCAPro software for the selection of familial breast/ovarian cancer probands investigated with the MLPA approach after negative *BRCA1/2* conventional mutation screening. One hundred and seventy-seven probands were investigated for germline *BRCA1/2* mutations after assessment of genetic risk using BRCAPro. Probands were classified as BRCAPro positive ($n = 67$) when the carrier probability (CP) was >10% and as BRCAPro negative ($n = 110$), when the CP was <10%. Conventional mutational analyses of the *BRCA1/2* genes and, in one case, of *p53* identified 22 pathogenetic germline mutations, 12 in *BRCA1*, 9 in *BRCA2* and 1 in *p53*, in 22/177 (12.4%) probands. All the mutations except one were detected in BRCAPro-positive patients. In the 46 BRCAPro-positive cases that resulted negative by *BRCA1/2* mutation, screening analysis of rearrangements within *BRCA1/2* by MLPA was carried out. Three patients with a very high CP showed *BRCA1* deletions, consisting of deletions of exons 1–2 in two probands and of exon 24 in the third proband. In one case, the exons 1–2 deletion was shown to cosegregate with disease in the family. No *BRCA2* rearrangements were detected, but one patient showed the 1100delC of the *CHEK2* gene, whose probe is present in the *BRCA2* kit. In our series, the highest carrier detection rate of mutation screening plus MLPA analysis (52.3%) was in patients with a BRCAPro CP >50%.
Key words: *BRCA1*, *BRCA2*, BRCAPro, breast cancer, MLPA, ovarian cancer

introduction

Breast cancer (BC) and ovarian cancer (OC) are among the most frequent malignancies in women, and 5%–10% of all BC/OC cases are attributed to hereditary predisposition [1]. Point mutations in the *BRCA1* and *BRCA2* genes account for a large portion of the putative hereditary BC/OC cases, with variable prevalences among different populations [2, 3]. It has been indicated that genomic *BRCA1/2* rearrangements, not detectable by the techniques commonly used for the screening of point mutations, could account for at least a portion of the *BRCA1/2* mutation-negative cases [4]. The search for genomic rearrangements within the *BRCA1/2* genes has been considerably improved by the introduction of multiplex ligation-dependent probe amplification (MLPA), an assay able to detect deletions–duplications within specific genes in a simple, fast and efficient way [5]. Using MLPA, an early study

reported a *BRCA1* deletions/duplications detection rate of >20% in northern Italian *BRCA1/2* mutation-negative BC/OC families. This indicated that screening by MLPA should be routinely included in *BRCA1* mutational analysis [6]. However, subsequent studies carried out in patient series from different populations reported lower detection rates [7–13]. Differences in genetic background and case selection criteria could account for discrepancies in *BRCA1* deletions/duplications detection rates between studies.

The identification of specific criteria for the selection of cases to be submitted to MLPA analysis is therefore important. BRCAPro, a statistical software using Mendelian genetics and Bayesian updating, calculates the carrier probability (CP) of *BRCA1/2* mutations on the basis of the individual and familial cancer history [14, 15]. In a previous study, we demonstrated the usefulness of this software in the selection of cases for *BRCA1/2* mutation screening [16]. In the present study, we report on the use of BRCAPro in the selection of probands with personal and/or familial BC/OC history to be analyzed by MLPA for the *BRCA1/2* genes.

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patients and methods

patients

A total of 177 probands with personal and/or family history of BC and/or OC were enrolled at two centers in Italy, Pescara Hospital, Pescara [115 probands, Central Italian Group (CIG)] and the 'Regional Reference Center for the Characterization and Genetic Screening of Hereditary Tumors', University of Palermo, Palermo [62 probands, Southern Italian Group (SIG)]. At Pescara, 115 unrelated CIG probands who underwent genetic counseling for BC/OC between 2000 and 2006 were selected using previously described criteria [16]. CIG probands included 92 cancer-affected females, of which 78 with BC (mean age at diagnosis 44.3 years; six cases had bilateral BC, one also developed OC), 13 with OC (mean age at diagnosis 41.4 years, one subsequently developed BC) and 1 with thyroid cancer (a 54-year-old with BC family history). The remaining 17 female probands were healthy first-degree relatives of BC/OC patients (not available for mutational analysis). In addition, CIG cases comprised six males, of which three diagnosed with BC (at ages 33, 31 and 77 years, respectively) and three with BC family history (of which two were cancer unaffected and one diagnosed with prostate cancer at age 51 years).

At Palermo, 62 unrelated SIG probands who underwent genetic counseling between 1999 and 2005 were selected for this study. SIG probands included 58 females, of which 55 affected with BC (mean age at diagnosis: 42.9 years; eight had bilateral BC and four also OC) and three with OC (<40 years of age). In addition, SIG cases included four male BC patients, aged 47, 60, 62 and 62 years.

CIG and SIG probands were enrolled after approval of the study by the local Ethical Committee. Written informed consent was always obtained before enrollment in the study.

assessment of genetic risk using BRCAPro

For each CIG and SIG case, we calculated the CP of BRCA1/2 mutations by means of the BRCAPro (CAGene) program. We classified probands as BRCAPro positive or -negative when CP was >10% or <10%, respectively. Of the total 177 probands selected for genetic analyses, 67 were BRCAPro positive (37.8%). These included 21 probands with CP >50% (range 51.5%–100%) and 46 probands with CP <50% (range 11.2%–45.2%).

mutation screening

Genomic DNA from peripheral blood was extracted using the QIAamp blood kit (Qiagen Inc., Chatsworth, CA) for both the CIG and SIG case groups. Conventional mutational analysis of a subset of 68 CIG probands was previously described [16]. For the other 47 CIG probands, exons 1, 3–10 and 12–24 of BRCA1 and exons 2–9 and 12–27 of BRCA2 were screened by Denaturing High Performance Liquid Chromatography (DHPLC) analysis using the Wave®Nucleic Acid Fragment Analysis System (Transgenomic Inc., San Jose, CA) as previously described [17]. Samples showing altered patterns by DHPLC or truncated peptides by Protein Truncation Test (PTT) were sequenced using an ABI Prism 310 Genetic Analyzer (Applied Biosystems, Forster City, CA). For CIG-110, in addition to BRCA1/2 screening, exons 5–8 of the p53 gene were examined by direct sequencing using reported PCR primers [18]. In the 62 SIG cases, the entire coding region and splicing boundaries of BRCA1 and of BRCA2 were examined by direct sequencing (BRCA1), or by DHPLC followed by sequencing (BRCA2), as previously described [19].

MLPA analysis

Altogether 46 BRCAPro-positive probands negative to BRCA1/2 mutation screening (CIG: 31 cases; SIG: 15 cases) were further tested for BRCA1/2 genomic rearrangements using the MLPA method (Table 1). MLPA was carried out using the SALSA P002B-BRCA1 and P045-BRCA2 (lot 0905) MLPA kits (MRC-Holland, Amsterdam, The Netherlands). The P045-BRCA2 kit, in addition to specific probes for all the BRCA2 exons, contains also three probes for the low penetrance BC susceptibility gene CHEK2 [20]. Reaction products were run using an ABI 310 automated sequencer (Applied Biosystems). As the MLPA signals, particularly for BRCA1 exons 1a, 1b and 2, may be low when large amounts of DNA and/or contaminants interfere with denaturation, MLPA results were always confirmed in three independent experiments, using only 40 ng of test DNA. Moreover, the MLPA kit includes DNA quantity (DQ) and DNA denaturation (DD) controls. Obtained MLPA data were analyzed by GeneScan Analysis software and copied to Excel files (Windows). Final results were calculated using Coffalyser software (MRC-Holland). Sample results are displayed as a ratio between reference/experimental samples. After Coffalyzer analysis, dosage alterations were considered significant if sample values of peaks area deviated >30% from the values of at least two normal controls (healthy individuals with no history of familiar cancer). Two samples with single exon deletion in BRCA1 provided from RESNOVA S.r.l. (Rome, Italy) were included in the analysis as positive controls.

results

mutation screening

Conventional mutational analyses of the BRCA1/2 genes and, in one case, of p53 identified 22 pathogenetic germline mutations, 12 in BRCA1, nine in BRCA2 and one in p53, in 22/177 (12.4%) CIG and SIG probands (Table 2). The p53 mutation [880G>T (E294X), Table 2] was detected in CIG-110 (CP 90.5%), whose pedigree, with an osteosarcoma-affected 15-year-old son, satisfied the Li–Fraumeni syndrome criteria [21].

MLPA analysis

MLPA analysis was carried out in the 46 CIG and SIG probands with CP >0.1 which resulted BRCA1/2 mutation negative by conventional analyses. MLPA allowed the detection of germline BRCA1 rearrangements in three patients (CIG-70, CIG-75 and CIG-111) with BRCA1 CPs of 99.2%, 85.2% and 70.8%, respectively. In the first two cases the rearrangements consisted of a deletion involving exons 1a, 1b and 2, while the third case showed a deletion of exon 24. For CIG-70 it was possible to investigate the segregation of the BRCA1 deletion with disease in the family (Figures 1 and 2). Direct sequencing of the ligation sites of the MLPA probes for BRCA1 exons 1a, 1b and 2 excluded the presence of mutations/polymorphisms that could have interfered with probe hybridization. Attempts to identify deletion break points by long range PCR were unsuccessful due to the complex structure of the BRCA1 promoter region [22, 23]. The rearrangements were confirmed by Coffalyser software analysis (Figure 3). In some cases, deletions of BRCA1 exon 24 produce only 25% reductions in the signal of the specific probe. In our patient, Coffalyzer analysis carried out based on three different experiments revealed ratios ranging from 0.85 to 0.59. Although a ratio >0.7 should indicate normal peak size, in CIG-111 all carried out analyses showed a reduction in exon 24 peak size of at least 25% and

Table 1. Characteristics of the 46 probands with CP > 0.1 selected for MLPA analysis

Probands	Sex/age	Cancer	Age at diagnosis	CP BRCA1	CP BRCA2	Total CP
CIG-2	F/54	bilBC	54	0.118	0.173	0.292
CIG-3	F/22	BC	22	0.256	0.038	0.295
CIG-10	F/41	BC	40	0.028	0.896	0.923
CIG-15	F/35	BC	35	0.300	0.030	0.330
CIG-25	F/45	bilBC	44	0.237	0.028	0.266
CIG-33	F/38	BC	38	0.175	0.092	0.268
CIG-38	F/41	BC	38	0.181	0.013	0.195
CIG-42	F/30	BC	29	0.150	0.024	0.175
CIG-46	F/60	OC	60	0.110	0.016	0.127
CIG-55	F/35	cu	/	0.381	0.040	0.422
CIG-60	F/30	cu	/	0.222	0.098	0.320
CIG-66	F/26	cu	/	0.158	0.014	0.172
CIG-69	F/45	BC	42	0.711	0.163	0.874
CIG-70	F/66	bilBC/OC	33–48	0.992	0.007	1.000
CIG-71	F/37	BC	37	0.493	0.065	0.559
CIG-75	F/33	BC	33	0.852	0.025	0.877
CIG-77	F/47	BC	47	0.593	0.060	0.654
CIG-80	F/52	bilBC	52	0.425	0.089	0.515
CIG-81	F/39	BC	39	0.107	0.002	0.117
CIG-84	M/33	BC	33	0.131	0.140	0.272
CIG-89	F/58	OC	57	0.237	0.240	0.527
CIG-91	F/29	cu	/	0.390	0.038	0.431
CIG-93	F/45	BC	44	0.128	0.191	0.32
CIG-94	M/37	BC	31	0.195	0.228	0.424
CIG-95	F/49	BC	49	0.440	0.230	0.67
CIG-96	F/50	bilBC	49	0.122	0.030	0.153
CIG-99	F/63	BC	46	0.464	0.070	0.535
CIG-108	F/50	bilBC	47	0.396	0.31	0.708
CIG-111	F/46	bilBC	37–44	0.708	0.109	0.817
CIG-112	F/50	BC	49	0.094	0.208	0.303
CIG-113	F/39	BC	38	0.827	0.025	0.853
SIG-25BC	F/56	BC	50	0.106	0.047	0.153
SIG-37BC	F/57	bilBC	49–54	0.104	0.035	0.139
SIG-38BC	F/70	cu	66–70	0.036	0.127	0.162
SIG-41BC	F/36	BC	36	0.121	0.011	0.132
SIG-55BC	F/36	BC	31	0.086	0.027	0.113
SIG-64BC	F/35	BC	35	0.0271	0.027	0.298
SIG-71BC	F/33	BC	30	0.373	0.032	0.405
SIG-93BC	M/70	BC	57	0.044	0.129	0.173
SIG-107BC	F/77	bilBC	50–60	0.076	0.040	0.117
SIG-134BC	F/56	BC/OC	50–54	0.319	0.133	0.452
SIG-149BC	F/54	bilBC	52–52	0.104	0.035	0.139
SIG-62SI	F/50	BC	48	0.086	0.027	0.112
SIG-71SI	F/40	BC	40	0.216	0.027	0.243
SIG-73SI	F/35	BC	34	0.395	0.080	0.475
SIG-77SI	F/78	bilBC/OC	42–77/77	0.052	0.136	0.188

CP, carrier probability; MLPA, multiplex ligation-dependent probe amplification; CIG, Central Italian Group; bilBC: bilateral breast cancer; BC: breast cancer; OC: ovarian cancer; cu: cancer unaffected; SIG, Southern Italian Group; /, in "age at diagnosis" indicates the absence of pathology, as shown in "cancer".

were interpreted as indicative of a deletion. The search for break points using long-range PCR primers is in progress [24].

MLPA analysis for *BRCA2* did not show any deletion. However, a male proband (CIG-84) showed the specific peak of the *CHEK2* 1100delC pathogenetic mutation, identified by the 1772-L1336 probe comprised in the MRC-Holland P045-*BRCA2* MLPA kit.

mutation detection rate

Taken together, conventional mutational screening and MLPA analysis identified 26 carriers of deleterious germline mutations

Table 2. Characteristics of the 22 probands with mutations in *BRCA1/2* or *p53* identified by conventional methods

Proband	Sex/age	Cancer	Age at diagnosis	CP total	BRCA1	BRCA2	p53
CIG-1	F/55	bilBC	46–54	0.16	c.2596 C>T (R866C)[a] c.2612C>T (P871L)[a]	–	NA
CIG-17	F/40	BC	39	0.74	c.4053insT (1351fsX1355)[a]	–	NA
CIG-28	F/33	BC	33	0.95	–	c.6468_6469delTC (2156fsX2174)[a]	NA
CIG-43	F/54	BC	46	0.44	–	c.8961_8964del4 (2987fsX2999)[a]	NA
CIG-49	F/39	OC	39	0.74	c.1380insA (461fsX479)[a]	–	NA
CIG-51	F/34	OC	34	0.33	c.3477_3480del4 (1159fsX1208)[a]	–	NA
CIG-52	M/51	PC	51	0	–	c.5217_5220del4 (1739fsX1739)[a]	NA
CIG-62	M/41	cu	/	0.43	c.5277 + 60dup12[a]	–	NA
CIG-90	F/30	cu	/	0.18	c.5035_5039del5[a] (1679fsX1680)	–	NA
CIG-92	F/52	OC/BC	49–52	0.76	c.4117G>T (E1373X)[a]	–	NA
CIG-102	F/35	BC	35	0.98	–	c.8487 + 1G>A	NA
CIG-110	F/43	bilBC	33–41	0.93	–	–	880G>T (E294X)
SIG-51BC	M/64	BC	60	0.19	c.4327C>T[a]		NA
SIG-68BC	F/70	BC/OC	59–60	0.16		c.6079_6082del4 (1951fsX1961)	NA
SIG-79BC	F/34	BC	33	0.18		c.6352C>T	NA
SIG-85BC	F/42	BC	40	0.21		c.7070-19delAT	NA
SIG-108BC	F/50	bilBC/OC	33–43/50	1.0	c.797_798delTT[a]		NA
SIG-128BC	F/58	BC	54	0.39		c.9254_9258de5 (3009fsX3016)	NA
SIG-133BC	F/43	BC	37	0.16	c.797_798delTT[a]		NA
SIG-146BC	F/42	BC	36	0.13	c.303T>G[a]		NA
SIG-65SI	F/42	OC	39	0.92	c.514delC[a]		NA
SIG-82SI	F/39	BC	38	0.17		c.6310_6314del5 (2028fsX2046)	NA

[a]Previously reported [16, 19].

CP, carrier probability; CIG, Central Italian Group; bilBC, bilateral breast cancer; NA, not analyzed; BC, breast cancer; OC, ovarian cancer; PC, prostate cancer; cu, cancer unaffected; SIG, Southern Italian Group.
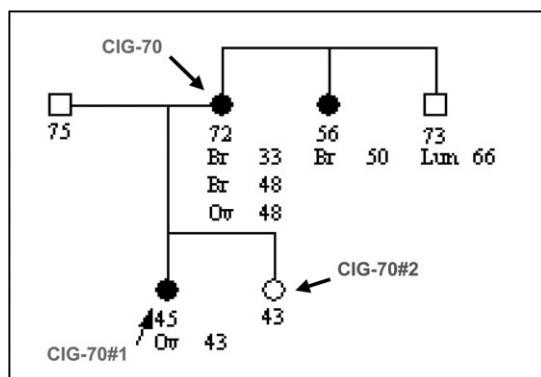

Figure 1. Pedigree of proband CIG-70, a 72 years-old woman found to be carrier of a *BRCA1* exons 1–2 deletion identified by MLPA. CIG-70 had bilateral breast cancer (at ages 33 and 48 years) and ovarian cancer (at age 48). Of the two daughters, the one diagnosed with ovarian cancer at age 43 (CIG-70#1) inherited the mutation, the other one (CIG-70#2), cancer unaffected at age 43, resulted negative (Br: breast cancer; Ov: ovarian cancer; Lun: lung cancer).

in 177 investigated probands (14.6%). In detail, we detected 12 *BRCA1* point mutations, nine *BRCA2* point mutations, three *BRCA1* deletions, one *p53* mutation and one *CHEK2* mutation. When considering only patients with CP >10% (BRCAPro positive), we detected 25 mutations in 67 probands (37.3%). The only case with CP <10% (BRCAPro-negative) in which

a pathogenetic mutation (in *BRCA2*) was detected was a prostate cancer-affected male with BC family history. In this case, the BRCAPro software most likely failed to correctly estimate CP.

When considering patients with CPs >50%, we detected mutations in 11/21 probands (52.3%). The 11 mutations detected in this very high-risk group included all three *BRCA1* deletions, five *BRCA1* point mutations, two *BRCA2* point mutations and one *p53* mutation. In the group of patients with CP <50%, we detected 14 mutations in 46 patients (30.4%). The detected mutations included seven *BRCA1* point mutations, six *BRCA2* point mutations and the 1100delC *CHEK2* mutation.

discussion

Despite the high sensitivity of the techniques employed for *BRCA1/2* mutation screening in a large number of patients, no deleterious point mutations within the *BRCA1/2* genes are detected [1, 4]. In recent years, it has been demonstrated that at least a portion of the cases negative for *BRCA1/2* point mutations carry genomic rearrangements of *BRCA1* or *BRCA2*, not detectable by conventional mutation screening, or mutations in other BC susceptibility genes [4]. Several reports demonstrated the usefulness of MLPA in the detection of *BRCA1/2* rearrangements [6–13]. However, criteria for the identification of cases to be submitted to MLPA analysis are undefined.
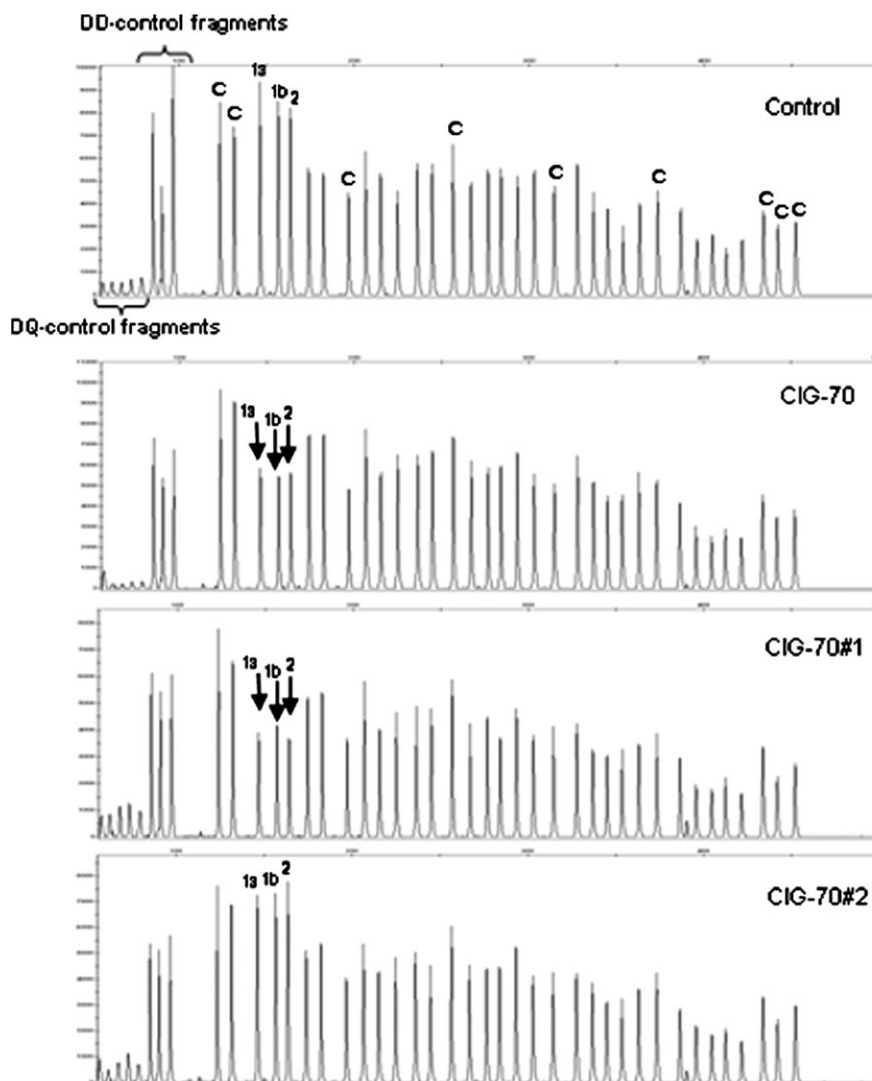
Figure 2. Multiplex ligation-dependent probe amplification electropherograms from proband CIG-70, her two daughters, CIG-70#1 and CIG-70#2 and a healthy control (top). Arrows point to the deletion of exons 1–2 of *BRCA1* in CIG-70 and in CIG-70#1. The DNA quantity- and DNA denaturation-control fragments indicate a good amount of DNA and complete denaturation of all samples.

In this study, we carried out MLPA analysis of 67 BRCAPro-positive probands with personal and/or familial BC/OC history that resulted negative after conventional *BRCA1/2* mutation screening. MLPA allowed to detect genomic *BRCA1* rearrangements in 3/21 patients with BRCAPro values >50% (14.2%). The *BRCA1* CP values in the three carriers of *BRCA1* rearrangements were very high: 99.2%, 85.2% and 70.8%. In contrast, no *BRCA1/2* alterations were evidenced by MLPA in 46 BRCAPro-positive probands with CP <50%. This demonstrates that BRCAPro CP values can importantly contribute to the identification of probands to be submitted to MLPA analysis for the detection of rearrangements within *BRCA1*.

The *BRCA1* deletions detected by MLPA involved in two cases exons 1 and 2 and in the third case exon 24. We could not define the break points of these deletion(s). Rearrangements involving *BRCA1* exons 1 and 2 were previously reported in BC/OC patients of different geographic origin [6, 8, 22, 23]. In one of our two cases, the exons 1–2 deletion cosegregated with disease in the family and we assume a pathogenic role. A *BRCA1* exon 24 genomic rearrangements has been reported in a BC–OC patient from Greece, geographically close to Italy [24]. Mutations in the *BRCA1* COOH-terminal region may interfere with important protein functions as well as with messenger RNA stability and are known to be pathogenic [24–26].

MLPA analysis of BRCA2 did not evidence any deletion, which is in agreement with literature data reporting a lower frequency of *BRCA2* rearrangements as compared to *BRCA1* [27, 4]. This could also be due to the presence of BRCAPro CP values lower for *BRCA2* than for *BRCA1*. Anyway, in one male patient with *BRCA2* CP 14%, MLPA analysis showed the presence of the 1100delC *CHEK2* mutation. This mutation is considered to produce a two-fold increase in the risk of BC in carriers [19]. Thus, despite the low detection rate of *BRCA2* deletions, the use of the MLPA *BRCA2* kit can be considered useful for detecting also the *CHEK2* 1100delC mutation.

Gene	chr pos.	Length (bp)	Distance to next BRCA2 probes	calculated ratio	Ratio
BRCA1 probe 0763-L0268, exon 1A	17-038.5	148	0.3 Kb	1	0.49
BRCA1 probe 0764-L0269, exon 1B	17-038.6	157	1.0 Kb	2	0.530
BRCA1 probe 0765-L0270, exon 2	17-038.7	166	8.3 Kb	3	0.510
BRCA1 probe 0826-L0341, exon 3	17-038.8	175	9.3 Kb	4	1.070
BRCA1 probe 0767-L0272, exon 5	17-038.9	184	1.6 Kb	5	0.990
BRCA1 probe 0827-L0342, exon 6	17-038.5	208	0.8 Kb	6	1.100
BRCA1 probe 0769-L0274, exon 7	17-038.6	217	4.4 Kb	7	1.070
BRCA1 probe 1004-L0569, exon 8	17-038.7	226	2.6 Kb	8	1.080
BRCA1 probe 1005-L0581, exon 9	17-038.8	235	1.3 Kb	9	1.160
BRCA1 probe 0772-L0277, exon 10	17-038.9	244	1.2 Kb	10	1.03
BRCA1 probe 0830-L0345, exon 11	17-038.5	268	2.8 Kb	11	1.09
BRCA1 probe 0774-L0279, exon 11	17-038.6	277	1.0 Kb	12	1.04
BRCA1 probe 0775-L0280, exon 12	17-038.7	286	8.5 Kb	13	1.060
BRCA1 probe 2603-L2074, exon 13 NEW	17-038.8	295	5.9 Kb	14	1.020
BRCA1 probe 0833-L0349, exon 14	17-038.9	304	2.1 Kb	15	0.980
BRCA1 probe 0778-L0347, exon 15	17-038.5	328	3.2 Kb	16	1.040
BRCA1 probe 0779-L0003, exon 16	17-038.6	337	3.5 Kb	17	0.970
BRCA1 probe 0780-L0283, exon 17	17-038.7	346	3.7 Kb	18	1.060
BRCA1 probe 0781-L0284, exon 18	17-038.8	355	0.6 Kb	19	1.04
BRCA1 probe 0782-L0285, exon 19	17-038.9	364	6.3 Kb	20	0.960
BRCA1 probe 0783-L0356, exon 20	17-038.5	388	6.0 Kb	21	0.97
BRCA1 probe 0784-L0287, exon 21	17-038.6	397	1.9 Kb	22	1.00
BRCA1 probe 0785-L0288, exon 22	17-038.7	406	1.5 Kb	23	0.98
BRCA1 probe 0786-L0289, exon 23	17-038.8	415	2.0 Kb	24	0.97
BRCA1 probe 0787-L0290, exon 24	17-038.9	424	0 kb	25	0.860
Control probe 0797-L0093, chromosome 5q31	05-132.0	127	c	26	1.030
Control probe 0662-L0158, chromosome 6p21	06-031.6	136	c	27	1.04
Control probe 2946-L3265	17-?	198	c	28	1.10
Control probe 0518-L0098, chromosome 2q14.	02-113.3	256	c	29	1.070
Control probe 0495-L0303, chromosome 12p12.	12-025.1	316	c	30	1.000
Control probe 0655-L0304, chromosome 4q26.	04-123.7	376	c	31	0.990
Control probe 0596-L0083, chromosome 11p13.	11-033.9	436	c	32	1.00
Control probe 0678-L0124, chromosome 12p13.	12-006.4	445	c	33	0.910
Control probe 0673-L0117, chromosome 3p21.	03-041.2	454	c	34	0.980

Figure 3. Final *BRCA1* Coffalyser software analysis window for proband CIG-70, with information about probes of the multiplex ligation-dependent probe amplification mix (gene name, chromosomal position, cloned probe length, mapview location) and sample results, displayed as a ratio between reference/experimental samples. The deletions of *BRCA1* exons 1a, 1b and 2 were confirmed by a ratio <0.7 for each probe (light gray).

In conclusion, selection of probands using the BRCAPro software confirmed to be a powerful tool for the identification of carriers of both point mutations and deletions in the *BRCA1/2* genes, and of mutations in two other genes related to cancer susceptibility, *p53* and *CHECK2*.

Selection of patients based on CPs obtained by BRCAPro allowed to obtain mutation detection rates of 37.3% for CPs between 10% and 50% and of 52.3% for CPs >50%. On the other hand, the mutation detection rate in the entire series, independently from CP-based selection, was significantly lower (14.6%). Of particular interest is the ability of BRCAPro to indicate patients who should be submitted to MLPA analysis. In fact, *BRCA1* deletions were detected only in patients showing upper CP values. Previous studies indicated that the BRCAPro software overestimates the carrier frequency in cases with CP >70% [15, 28]. Our results confirm this assumption only for *BRCA1/2* point mutations, which did not show higher frequency in patients with CP >50% as compared to those with CP between 10% and 50%. However, a completely different trend was observed for *BRCA1* deletions, which were detected exclusively in patients with CP >50%. This indicates that the overestimation of the carrier frequency in patients with CP >70% may reflect lack of detection of genomic rearrangements.

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