Breast Cancer Res Treat (2012) 131:217–222
DOI 10.1007/s10549-011-1718-5

EPIDEMIOLOGY

Characteristics of double heterozygosity for BRCA1 and BRCA2 germline mutations in Korean breast cancer patients

Jae Myoung Noh · Doo Ho Choi · Seok Jin Nam · Jeong Eon Lee · Jong Won Kim · Sung-Won Kim · Eunyoung Kang · Min Hyuk Lee · Sei Hyun Ahn · Ku Sang Kim · Sue K. Park · Bruce G. Haffty · Korea Breast Cancer Study Group

Received: 31 July 2011 / Accepted: 1 August 2011 / Published online: 17 August 2011

Abstract To investigate clinical, pathological, and familial characteristics of Korean patients with double heterozygosity for BRCA1 and BRCA2 mutations, six breast tumors of five patients who carried deleterious mutations in both of the genes were included. Medical records of the patients were reviewed and genetic testing by direct sequencing was undertaken to detect mutations in BRCA1 and BRCA2. Seven frameshift and three nonsense mutations were identified, and four mutations are novel in the Breast Cancer Information Core. There were no Ashkenazi founder mutations detected. The mean age at diagnosis for breast cancer was 33 years. All six tumors were infiltrating ductal carcinoma and poorly differentiated. Pathologic stage was I or II, and immunohistochemistry showed negative immunoreactivity for estrogen receptor and Her-2/neu in all tumors. Positive immunoreactivity for progesterone receptor was found only in one tumor. Three patients had familial history of breast, ovarian or other cancers. One patient who was diagnosed for breast cancer at the age of 26 had two maternal family members of metachronous bilateral breast cancer. Another patient who experienced metachronous bilateral breast cancer had maternal history of ovarian and esophageal cancer. In summary, Korean patients with double heterozygosity for

J. M. Noh · D. H. Choi (✉)
Department of Radiation Oncology, Samsung Medical Center, Sungkyunkwan University School of Medicine, 50 Irwon-dong, Gangnam-gu, Seoul 135-710, Korea
e-mail: doho.choi@samsung.com

S. J. Nam · J. E. Lee
Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

J. W. Kim
Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

S.-W. Kim · E. Kang
Department of Surgery, Seoul National University College of Medicine, Seoul, Korea

M. H. Lee
Department of Surgery, Soonchunhyang University Hospital, Seoul, Korea

S. H. Ahn
Department of Surgery, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

K. S. Kim
Department of Surgery, Ajou University School of Medicine, Suwon, Korea

S. K. Park
Department of Preventive Medicine, Seoul National University College of Medicine, Seoul, Korea

S. K. Park
Department of Biomedical Science, Seoul National University Graduate School, Seoul, Korea

S. K. Park
Cancer Research Institute, Seoul National University, Seoul, Korea

B. G. Haffty
Department of Radiation Oncology, Robert Wood Johnson Medical School, Cancer Institute of New Jersey, New Brunswick, NJ, USA

Korea Breast Cancer Study Group
Korea Breast Cancer Society, Seoul, Korea

BRCA1 and BRCA2 were young at diagnosis of breast cancer. Tumors were early stage, high grade, and almost triple-negative phenotype. All familial history of breast, ovary or other cancer was maternal. Close surveillance and accurate risk assessment should be provided for the patients with mutations in the both of the genes.

Keywords Breast cancer · BRCA1 gene · BRCA2 gene · Heterozygote

Introduction

Younger age at onset of breast cancer has been a distinctive characteristic in Korea. The peak age at onset of breast cancer occurs in the fifth decade of life and about two-third of cases occur in premenopausal women younger than 50 years [1–3]. Hereditary breast cancer accounts for only 5–10% of total cases. It has been known that mutations in BRCA1 and BRCA2 genes are associated with the development of early-onset breast cancer [4–7]. There have been several reports regarding BRCA1 and BRCA2 mutations in Korean patients [8–14]. The prevalence of BRCA1 and/or BRCA2 mutations was reported to 2.5–3.1% in sporadic breast cancers [10, 12, 13]. It strikingly increased to 19.4–22.1% in patients with family history of breast cancer [10, 14]. Younger age of onset was also related to increased frequency of BRCA1/2 mutations ranging 8.1–18.3% [10, 11, 14, 15]. The frequency of deleterious mutations in BRCA1/2 of young Korean breast cancer patients was similar to that of African-American and Caucasian women [15].

Patients with deleterious mutations in both BRCA1 and BRCA2 are extremely rare and it is remarkable that two patients in a relatively small series had mutations in both BRCA1 and BRCA2, who were reported previously [16]. Most studies dealing with double heterozygosity for BRCA1 and BRCA2 mutations have been come from western countries, including Ashkenazi Jewish population [17–21]. But there has been few reports regarding double heterozygosity for the genes in Asia. In Korea, the Korean Hereditary Breast Cancer (KOHBRA) study has been conducted to examine the prevalence of BRCA1/2 mutation and the prevalence of ovarian cancer among the high-risk group of hereditary breast cancer patients and their families [22]. During the prospective study, two more patients have been discovered to have mutations in both BRCA1 and BRCA2 genes. And one patient with metachronous bilateral breast cancer was discovered to have mutations in both of the genes at Samsung Medical Center.

In this study, we describe the clinical, pathological, and familial characteristics of the five Korean patients with double heterozygosity for BRCA1 and BRCA2 mutations.

Methods

Five Korean patients with breast cancer who carried deleterious mutations in both BRCA1 and BRCA2 were included in this study. Patients with a known deleterious mutation in one BRCA site and unknown significance in the other site were excluded. Two patients were found to have the double heterozygosity during the course of a hospital-based study as previously reported [16]. Another two patients were found during the course of KOHBRA study. And the last patient was referred to genetic counseling because of metachronous bilateral breast cancer with an interval of 6 years, and then mutations in both of the genes were detected.

Genomic DNA was extracted and purified from patients' peripheral blood. For the two patients from KOHBRA study, fluorescence-based conformation sensitive gel electrophoresis or denaturing high performance liquid chromatography was preceded as screening test, followed by direct sequencing to confirm the mutations [22]. Samples from the other three patients were examined by direct sequencing. All mutations are described according to the Breast Cancer Information Core (BIC) nomenclatures.

Clinical, pathological, and familial history data were collected from medical records and informed consent was obtained from the patients. Because the last patient had metachronous bilateral breast cancer, pathological characteristics were described for the overall six tumors.

Results

The data on the patients' mutations of BRCA1 and BRCA2 are given in Table 1. By mutation type, seven frameshift and three nonsense mutations were identified. Four mutations are novel in the BIC. Figure 1 demonstrates mutations at 509C > A in BRCA1 and 3246delA in BRCA2 of patient No. 4. Four patients were diagnosed to have breast cancer at 35 years or younger (Table 1). The mean age at diagnosis for breast cancers was 33 years.

Three patients had familial history of breast, ovarian or other cancers. Patient No. 2 had maternal history of stomach cancer at the age of 62 years. Patient No. 3 from the KOHBRA study had two maternal relatives with a history of metachronous bilateral breast cancer. Her mother was diagnosed to have breast cancer at the age of 43 years, followed by contralateral breast cancer 7 years later. Her maternal aunt had also metachronous bilateral breast cancer with an interval of 3 years. Patient No. 5 had two familial members with history of cancer. Her maternal grandmother experienced ovarian cancer and maternal aunt experienced esophageal cancer. All the tumors were infiltrating ductal carcinoma and poorly differentiated

Table 1 Genetic mutations for both BRCA1 and BRCA2 in Korean breast cancer patients

No.	Age at diagnosis	Gene	Mutation	Site (Exon)	Citation No. in BIC	Familial history of cancer
1	26	BRCA1	1623del5	11	28	–
		BRCA2	3026delCA	11	Novel	
2	33	BRCA1	E1661X	16	Novel	Stomach
		BRCA2	6714del4	11	21	
3	26	BRCA1	3746_3747insA	11	6	Breast
		BRCA2	6952_6953delGA	11	Novel	
4	45	BRCA1	509C > A	7	1	–
		BRCA2	3246delA	11	Novel	
5	35	BRCA1	5149delCTAA	17	17	Ovary
		BRCA2	1627A > T	10	2	Esophagus

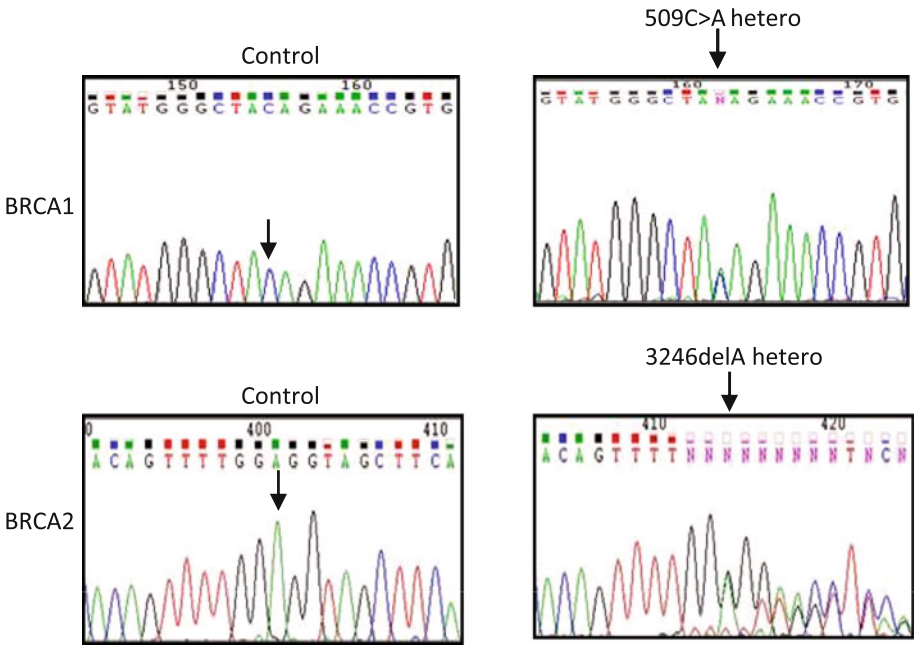

Fig. 1 Direct sequencing data of the BRCA1 and BRCA2 genes demonstrating mutations at 509C > A in BRCA1 and 3246delA in BRCA2 (patient 4)

(Table 2). The tumors of the patient with metachronous bilateral breast cancer were both pathologic stage I, and that of the others were stage II. Immunohistochemistry showed negative immunoreactivity for estrogen receptor and Her-2/neu in all tumors. Only one tumor had positive immunoreactivity for progesterone receptor.

Discussion

At present, five Korean cases of double heterozygosity for BRCA1 and BRCA2 mutations have been described. Ashkenazi founder mutations, 185delAG and 5382insC in BRCA1 gene and the 6174delT mutation in BRCA2 gene, did not appear in these cases. In Korea, there has been no report presenting Ashkenazi founder mutations and no established founder mutations in the genes [9, 12, 14]. It is interesting that 1627A > T with BRCA2 gene of patient No. 5 was found seven times in previous report [9, 13, 14]. This could be a possible candidate for founder mutations in Korea next to 7708C > T and 3972del4.

Recently, the interim report of KOHBRA study has been published [23]. Among 853 probands, a total of 167 (19.6%) mutations carriers identified. The prevalence of BRCA mutation was 24.8% for breast cancer patients with a familial history of breast/ovarian cancer and 11.3% for patients with early-onset (<35 years) breast cancer without a familial history. For patients with bilateral breast cancer, the prevalence was 22.1%. They identified 33 types of BRCA1 mutations in 68 cases and 35 types of BRCA2 mutations in 80 cases. The most frequent mutations in BRCA1 gene were 509C > A and 5615_5625del11insA, each of them was found 11 times, respectively. In BRCA2 gene, 7708C > T was most frequently identified, which

Table 2 Pathological characteristics of the tumors

Characteristic	No. of tumors ($N = 6$)
Tumor histology	
Infiltrating ductal	6
Location	
Right	2
Left	4
T-stage	
1	4
2	2
N-Stage	
0	3
1	3
Histologic grade	
Poorly differentiated	6
Nuclear grade	
Low	2
Intermediate	1
High	3
Lymphovascular invasion	3
Extensive intraductal component	1
Immunohistochemistry	
Estrogen receptor	0
Progesterone receptor	1
Her-2/neu	0
p53	3

was found 18 times. The 1627A > T with BRCA2 gene was found eight times, too. The mutations of patient No. 3, 3746_3747insA in BRCA1 and 6952_6953delGA in BRCA2, were also detected in other patients from the KOHBRA study. As accumulation of data regarding BRCA1 and 2 mutations, founder mutations in Korean patients possibly might be established.

The prevalence of double heterozygosity for BRCA1 and BRCA2 mutations is 1.8–1.85% among carriers of one of the three Jewish Ashkenazi BRCA gene mutations [18, 21]. But there has been few reports regarding the prevalence among non-Ashkenazi carriers. One study from Italy reported the prevalence as 0.62%, which was lower than that of Jewish Ashkenazi carriers [24]. According to the interim report of the KOHBRA study, the prevalence could be estimated as 1.2% (2/167) among Korean mutation carriers [23].

It is important to determine whether double heterozygosity for the genes has an effect on phenotypic expression of breast cancer or not. In the review of 34 cases with the double heterozygosity by Leegte et al. [19], the phenotypic expression was comparable to the severe end of the spectrum of BRCA1 mutation. The mean age at diagnosis for breast cancers was 41.1 years and the cumulative incidence proportion of cancer in double heterozygosity at age 70 years was 80% for breast cancer. Four cases from Italy showed more severe phenotype than the above series, since three of them developed both a breast and an ovarian cancer [24]. The tumors of the four patients were all high grade, stage II, mostly negative for hormonal receptor and/or Her-2/neu expression, which was nearly the same as the tumors of the five Korean cases. There have been no known histopathological features that distinguish BRCA2 from sporadic tumors. But tumors of BRCA1 mutation carriers usually exhibit high histologic grade and triple-negative phenotype [25]. Like the above series from Italy, Korean patients with double heterozygosity showed similar histopathological features with BRCA1 mutation carriers. Given that BRCA1 patients typically have triple negative cancers, while BRCA2 patients typically have hormonally positive cancers, it appears that in those patients with both BRCA1 and BRCA2 mutations, the BRCA1 genotype tends to drive the phenotype from a pathology perspective. The mean age at onset of breast cancer was about 10 years younger than that of the 34 patients from the series mentioned above.

Although the histopathologic features of the double heterozygosity are similar to that of patients with BRCA1 mutation, increased risk of cancer is related to not only BRCA1 but also BRCA2 [26–28]. In addition to breast and ovarian cancer, increased risk of cancer of pancreas, stomach, biliary tract, and melanoma is associated with BRCA2 mutation. When the mutation of BRCA1 detected, comprehensive analysis should be carefully considered to detect second mutation of BRCA2 [29].

There were three patients who had familial history of breast, ovarian or other cancer. It is interesting that all of the familial history were maternal. The 34 patients in the study of Leegte al. [19] also showed more frequent maternal history of breast and/or ovarian cancer than paternal history. Another distinguishable feature of the present cases is that one patient had metachronous bilateral breast cancer. The risk of contralateral breast cancer in BRCA1 and BRCA2 carriers was estimated in a recently published study [30]. The 15-year actuarial risk of contralateral breast cancer was 36.1% for women with a BRCA1 mutation and 28.5% for women with a BRCA2 mutation. Women younger than 50 years of age at the time of breast cancer diagnosis and who had two or more first-degree relatives with early-onset breast cancer were at high risk of contralateral breast cancer. The Korean patient with contralateral breast cancer was 35 years old at diagnosis of first breast cancer, so she had been also at high risk of developing contralateral breast cancer. It is interesting that another patient from the KOHBRA study had two maternal familial members with metachronous bilateral breast cancer. The age at diagnosis of first breast cancer of her mother and maternal aunt were 43 and 35 years old,

respectively. In addition to the familial history, her age at diagnosis of breast cancer was 26 years old. According to the recent study, she is at very high risk of contralateral breast cancer [30]. The authors suggested that both oophorectomy and contralateral mastectomy should be discussed as a component of the treatment plan to reduce the risk of contralateral breast cancer in high-risk women. This suggestion could be an effective treatment option to the high-risk patient.

In summary, Korean patients with double heterozygosity for BRCA1 and 2 genes were younger at diagnosis of breast cancer than Jewish Ashkenazi carriers. The histopathologic features, high grade and triple-negative phenotype were similar to that of BRCA1 mutation carriers. All familial history of breast, ovary or other cancer was maternal. Close surveillance and surgical options to reduce risk of secondary cancer should be provided for the patients with mutations in both BRCA1 and BRCA2 genes.

Acknowledgments This study was partly supported by a grant from the National R&D Program for Cancer Control, Ministry for Health, Welfare, and Family Affairs, Republic of Korea (#1020350).

Conflict of interest None.

References

1. Ahn SH, Yoo KY (2006) Chronological changes of clinical characteristics in 31, 115 new breast cancer patients among Koreans during 1996–2004. Breast Cancer Res Treat 99:209–214. doi:10.1007/s10549-006-9188-x
2. Ko SS (2008) Chronological changing patterns of clinical characteristics of Korean breast cancer patients during 10 years (1996–2006) using nationwide breast cancer registration on-line program: biannual update. J Surg Oncol 98:318–323. doi:10.1002/jso.21110
3. Son BH, Kwak BS, Kim JK, Kim HJ, Hong SJ, Lee JS, Hwang UK, Yoon HS, Ahn SH (2006) Changing patterns in the clinical characteristics of Korean patients with breast cancer during the last 15 years. Arch Surg 141:155–160. doi:10.1001/archsurg.141.2.155
4. Ford D, Easton DF, Peto J (1995) Estimates of the gene frequency of BRCA1 and its contribution to breast and ovarian cancer incidence. Am J Hum Genet 57:1457–1462
5. Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W et al (1994) A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 266:66–71
6. Robson M, Gilewski T, Haas B, Levin D, Borgen P, Rajan P, Hirschaut Y, Pressman P, Rosen PP, Lesser ML, Norton L, Offit K (1998) BRCA-associated breast cancer in young women. J Clin Oncol 16:1642–1649
7. Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregory S, Gumbs C, Micklem G (1995) Identification of the breast cancer susceptibility gene BRCA2. Nature 378:789–792. doi:10.1038/378789a0
8. Oh JH, Noh DY, Choe KJ, Kang SB, Kim LS, Ro MS (1995) Germline mutation of BRCA1 gene in Korean breast and ovarian cancer patients. J Korean Cancer Ass 27:1061–1069
9. Kang HC, Kim IJ, Park JH, Kwon HJ, Won YJ, Heo SC, Lee SY, Kim KH, Shin Y, Noh DY, Yang DH, Choe KJ, Lee BH, King SB, Park JG (2002) Germline mutations of BRCA1 and BRCA2 in Korean breast and/or ovarian cancer families. Hum Mutat 20:235. doi:10.1002/humu.9059
10. Ahn SH, Hwang UK, Kwak BS, Yoon HS, Ku BK, Kang HJ, Kim JS, Ko BK, Ko CD, Yoon KS, Cho DY, Son BH (2004) Prevalence of BRCA1 and BRCA2 mutations in Korean breast cancer patients. J Korean Med Sci 19:269–274
11. Choi DH, Lee MH, Bale AE, Carter D, Haffty BG (2004) Incidence of BRCA1 and BRCA2 mutations in young Korean breast cancer patients. J Clin Oncol 22:1638–1645. doi:10.1200/JCO.2004.04.179
12. Seo JH, Cho DY, Ahn SH, Yoon KS, Kang CS, Cho HM, Lee HS, Choe JJ, Choi CW, Kim BS, Shin SW, Kim YH, Kim JS, Son GS, Lee JB, Koo BH (2004) BRCA1 and BRCA2 germline mutations in Korean patients with sporadic breast cancer. Hum Mutat 24:350. doi:10.1002/humu.9275
13. Han SH, Lee KR, Lee DG, Kim BY, Lee KE, Chung WS (2006) Mutation analysis of BRCA1 and BRCA2 from 793 Korean patients with sporadic breast cancer. Clin Genet 70:496–501. doi:10.1111/j.1399-0004.2006.00717.x
14. Ahn SH, Son BH, Yoon KS, Noh DY, Han W, Kim SW, Lee ES, Park HL, Hong YJ, Choi JJ, Moon SY, Kim MJ, Kim KH, Kwak BS, Cho DY (2007) BRCA1 and BRCA2 germline mutations in Korean breast cancer patients at high risk of carrying mutations. Cancer Lett 245:90–95. doi:10.1016/j.canlet.2005.12.031
15. Haffty BG, Choi DH, Goyal S, Silber A, Ranieri K, Matloff E, Lee MH, Nissenblatt M, Toppmeyer D, Moran MS (2009) Breast cancer in young women (YBC): prevalence of BRCA1/2 mutations and risk of secondary malignancies across diverse racial groups. Ann Oncol 20:1653–1659. doi:10.1093/annonc/mdp051
16. Choi DH, Lee MH, Haffty BG (2006) Double heterozygotes for non-Caucasian families with mutations in BRCA-1 and BRCA-2 genes. Breast J 12:216–220. doi:10.1111/j.1075-122X.2006.00245.x
17. Friedman E, Bar-Sade Bruchim R, Kruglikova A, Risel S, Levy-Lahad E, Halle D, Bar-On E, Gershoni-Baruch R, Dagan E, Kepten I, Peretz T, Lerer I, Wienberg N, Shushan A, Abeliovich AD (1998) Double heterozygotes for the Ashkenazi founder mutations in BRCA1 and BRCA2 genes. Am J Hum Genet 63:1224–1227
18. Frank TS, Deffenbaugh AM, Reid JE, Hulick M, Ward BE, Lingenfelter B, Gumpper KL, Scholl T, Tavtigian SV, Pruss DR, Critchfield GC (2002) Clinical characteristics of individuals with germline mutations in BRCA1 and BRCA2: analysis of 10, 000 individuals. J Clin Oncol 20:1480–1490
19. Leegte B, van der Hout AH, Deffenbaugh AM, Bakker MK, Mulder IM, ten Berge A, Leenders EP, Wesseling J, de Hullu J, Hoogerbrugge N, Ligtenberg MJ, Ardern-Jones A, Bancroft E, Salmon A, Barwell J, Eeles R, Oosterwijk JC (2005) Phenotypic expression of double heterozygosity for BRCA1 and BRCA2 germline mutations. J Med Genet 42:e20. doi:10.1136/jmg.2004.027243
20. Spannuth WA, Thaker PH, Sood AK (2007) Concomitant BRCA1 and BRCA2 gene mutations in an Ashkenazi Jewish woman with primary breast and ovarian cancer. Am J Obstet Gynecol 196:e6–e9. doi:10.1016/j.ajog.2007.01.026
21. Lavie O, Narod S, Lejbkowicz F, Dishon S, Goldberg Y, Gemer O, Rennert G (2010) Double heterozygosity in the BRCA1 and BRCA2 genes in the Jewish population. Ann Oncol 22:964–966. doi:10.1093/annonc/mdq460

22. Kim EK, Kim KS, Park SK, Ahn SH, Lee MH, Kim SW (2007) The Korean Hereditary Breast Cancer (KOHBRA) study: protocol review. J Breast Cancer 10:241–247
23. Han SA, Park SK, Hyun Ahn S, Hyuk Lee M, Noh DY, Kim LS, Noh WC, Jung Y, Sang Kim K, Kim SW, Korean Breast Cancer Study Group (2011) The Korean Hereditary Breast Cancer (KOHBRA) Study: protocols and interim report. Clin Oncol (R Coll Radiol) 23:434–441. doi:10.1016/j.clon.2010.11.007
24. Zuradelli M, Peissel B, Manoukian S, Zaffaroni D, Barile M, Pensotti V, Cavallari U, Masci G, Mariette F, Benski AC, Santoro A, Radice P (2010) Four new cases of double heterozygosity for BRCA1 and BRCA2 gene mutations: clinical, pathological, and family characteristics. Breast Cancer Res Treat 124:251–258. doi:10.1007/s10549-010-0853-8
25. Phillips KA (2000) Immunophenotypic and pathologic differences between BRCA1 and BRCA2 hereditary breast cancers. J Clin Oncol 18:107S–112S
26. Levine DA, Argenta PA, Yee CJ, Marshall DS, Olvera N, Bogomolniy F, Rahaman JA, Robson ME, Offit K, Barakat RR, Soslow RA, Boyd J (2003) Fallopian tube and primary peritoneal carcinomas associated with BRCA mutations. J Clin Oncol 21:4222–4227. doi:10.1200/JCO.2003.04.131
27. The Breast Cancer Linkage Consortium (1999) Cancer risks in BRCA2 mutation carriers. The Breast Cancer Linkage Consortium. J Natl Cancer Inst 91:1310–1316
28. Thompson D, Easton DF (2002) Cancer Incidence in BRCA1 mutation carriers. J Natl Cancer Inst 94:1358–1365
29. Augustyn AM, Agostino NM, Namey TL, Nair S, Martino MA (2011) Two patients with germline mutations in both BRCA1 and BRCA2 discovered unintentionally: a case series and discussion of BRCA testing modalities. Breast Cancer Res Treat. doi:10.1007/s10549-011-1597-9
30. Metcalfe K, Gershman S, Lynch HT, Ghadirian P, Tung N, Kim-Sing C, Olopade OI, Domchek S, McLennan J, Eisen A, Foulkes WD, Rosen B, Sun P, Narod SA (2011) Predictors of contralateral breast cancer in BRCA1 and BRCA2 mutation carriers. Br J Cancer 104:1384–1392. doi:10.1038/bjc.2011.120