



Characteristics of *BRCA1/2* mutations carriers including large genomic rearrangements in high risk breast cancer patients

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Abstract

Purpose We investigated the prevalence of *BRCA1/2* small mutations and large genomic rearrangements in high risk breast cancer patients who attended a genetic counseling clinic.

Methods In total 478 patients were assessed for *BRCA1/2* mutations by direct sequencing, of whom, 306 were identified as non-carriers of *BRCA1/2* mutation and assessed for large rearrangement mutations by multiplex ligation-dependent probe amplification. Family history and clinicopathological characteristics of patients were evaluated.

Results Sixty-three mutation carriers (13.2%) were identified with *BRCA1* mutations (6.3%) and *BRCA2* mutations

(6.9%), respectively. Mutation frequency was affected by familial and personal factors. Breast cancer patients with family history of breast and ovarian cancer showed the highest prevalence of *BRCA1/2* mutations (67%), and triple-negative breast cancer (TNBC) patients showed high *BRCA1* mutation prevalence (25%). The three probands of *BRCA1* deletion (1%) represented both familial risk and personal or clinicopathological risk factors as two with TNBC and one with bilateral ovarian cancer.

Discussion This is the largest study assessing large genomic rearrangement prevalence in Korea and *BRCA1* deletion frequency was low as 1% in patients without *BRCA1/2* small mutations. For clinical utility of large genomic rearrangement testing needs further study.

Boyoung Park, Ji Yeon Sohn, Sun-Young Kong, and Eun Sook Lee have contributed equally to this work.

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Keywords *BRCA1/2* mutation · Large genomic rearrangements · Breast cancer · Genetic counseling · Family counseling

Introduction

Germline mutations in the *BRCA1/2* genes are the most important cause of hereditary breast cancer [1]. The average cumulative risk of breast cancer in female carriers above 70 years is estimated to be 57–65 and 45–49% for *BRCA1* and *BRCA2* mutation carriers, respectively [2, 3].

Risk of hereditary cancers is assessed by taking into account familial and personal factors or clinicopathological characteristics of cancers such as triple-negative breast cancers (TNBC), which do not express the genes for estrogen receptor (ER), progesterone receptor (PR), or HER2 receptor [4]. With this information, women with a high risk of developing hereditary cancers are recommended to be tested for mutations in *BRCA1/2* genes [5, 6]. Given that *BRCA1/2* mutation test targets a test targets a high-risk population and the further management plans are largely dependent on the results of *BRCA1/2* mutation testing are important.

Direct Sanger sequencing, conformation-sensitive gel electrophoresis, and denaturing high-performance liquid chromatography have been used to identify *BRCA1/2* mutations. However, these techniques cannot identify large genomic rearrangements, which are often reported in patients with negative results from conventional direct sequencing [7, 8]. This may lead to an underestimation of mutation prevalence and provide false-negative information to patients and their families. Previous studies showed varying results with the prevalence of large genomic rearrangements ranging from 0 to 30% [9–14]. This wide range might be caused by different genetic backgrounds and the different inclusion criteria of various studies [11]. Thus, we investigated the prevalence of *BRCA1/2* large genomic rearrangements using multiplex ligation-dependent probe amplification in high-risk breast cancer patients with negative results for *BRCA1/2* mutation by direct sequencing.

Materials and methods

Study process

All the patients who were referred to a genetic counseling clinic and screened for *BRCA1/2* mutation, at the National Cancer Center between April 2008 and 2015, were initially considered for this study. Among the total 523 patients

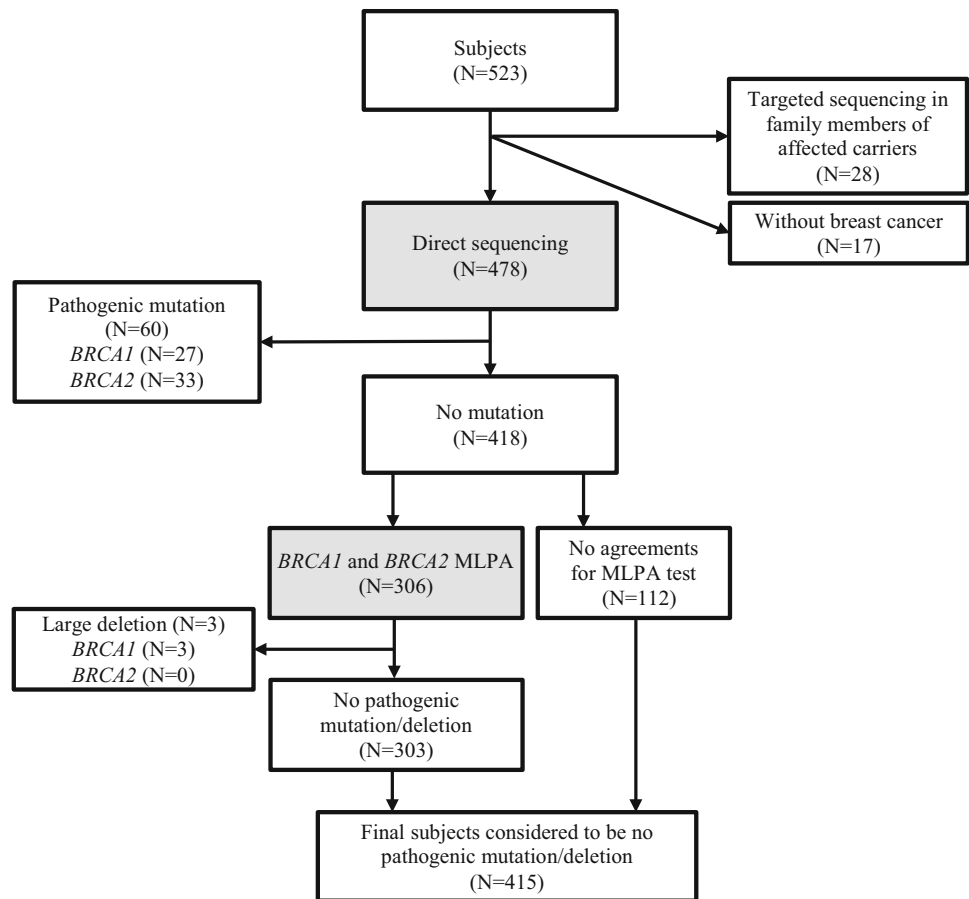
screened for *BRCA1/2* mutations, we excluded 28 family members of known *BRCA1/2* mutation carriers and 17 patients with other types of cancers. There were 478 probands with breast cancer included in this study. The referred criteria for breast cancer were: (1) breast cancer patients with a family history of breast or ovarian cancer; (2) breast cancer patients who were 40 years or younger during diagnosis, who had bilateral breast cancer or breast cancer with other primary malignancy, or male breast cancer patients, in accordance with the standard of National Medical Insurance Reimbursement in Korea. In the genetic counseling process, all patients' mutation probabilities were estimated by CaGene5.0 software [15] using pedigree information up to second-degree relatives and considering estimates by BRCAPRO [16] and Myriad [17]. Clinicopathological characteristics of cancer, such as the stage and the hormone receptor and human epidermal growth factor receptor 2 (HER2) status, were evaluated by reviewing medical records.

The initial *BRCA1/2* mutation test was performed by PCR amplification and direct sequencing covering all exons and flanking intronic sequences. A subset (73%) of the 418 cases designated as non-carriers by direct sequencing, agreed to participate in the study. Written informed consent was obtained from 306 patients, and they were screened for the presence of large genomic rearrangements using a multiplex ligation-dependent probe amplification (MLPA) assay. This study protocol was approved by the institutional review board of the National Cancer Center (NCC2015-0177). The selection of patients and the test process is presented in the Fig. 1.

Direct sequencing for mutation detection of *BRCA1* and *BRCA2*, and nomenclature

Genomic DNA was extracted from peripheral blood using the Chemagic DNA Blood 200 Kit (Chemagen, Baesweiler, Germany). Amplified products were sequenced on an ABI 3500xl analyzer (Applied Biosystems, Foster City, CA, USA), using the Bigdye Terminator v3.1 Cycle Sequencing Kit. Sequences were analyzed using Sequencher v4.10.1 software. The clinical significance of each sequence variation was determined according to the Breast Cancer Information Core database (BIC: <http://research.nhgri.nih.gov/bic/>) and the recommendations of the American College of Medical Genetics [18]. All the mutations are described according to HUGO-approved systematic nomenclature (<http://www.hgvs.org/mutnomen/>). GenBank accession sequences NM_007294.3 and NM_000059.3 were used as reference sequences for *BRCA1* and *BRCA2*, respectively. Traditional mutation nomenclatures of the BIC were used for description.

Fig. 1 The study flowchart outlining the number of subjects and the genetic testing approach used in the study. A total of 478 breast cancer patients were included and multiple ligation-dependent probe amplification (MLPA) analysis was performed for 306 patients who did not have small mutations in the *BRCA1* and *BRCA2* genes and agreed for this study



Multiplex ligation-dependent probe amplification

MLPA was performed to detect large genomic rearrangements using the SALSA P002-D1 *BRCA1* Kit (MRC Holland, Amsterdam, Holland) for *BRCA1* and P045-B3 *BRCA2* Kit (MRC Holland, Amsterdam, Holland) for *BRCA2*. PCR products were analyzed using an ABI 3500xl analyzer with GeneMarker v2.4.0 demonstration program (Softgenetics, State College, PA, USA). Peak heights were normalized, and a deletion or duplication was identified when the normalized peak ratio value was below 0.75 or above 1.30, respectively.

Risk factors and statistical analysis

The subjects were classified according to familial and personal factors. Familial factors taken into account included family history of breast cancer, number of family members with breast cancer, closest degree of family members with breast cancer, and family history of ovarian cancer. Forty-two of the 306 patients had a family history of ovarian cancer. Personal factors taken into account included early onset of breast cancer which is defined as the development of breast cancer before the age of 40,

bilateral breast cancer irrespective of age at onset, both breast and ovarian cancer irrespective of age at onset, multiple organ cancers defined as breast cancer patients with other primary organ cancer except ovarian cancer, and male cancer. The clinicopathological factors considered were age at diagnosis of breast cancer, stage, and the hormone receptor (including estrogen and PR) and HER2 status.

The frequencies of mutations were presented according to familial and personal factors and clinicopathological characteristics. All analyses were conducted using SAS 9.2 software (SAS Institute, Cary, NC).

Results

Mutational status according to subjects' characteristics

Pathogenic mutations in *BRCA1/2* genes, including large genomic rearrangements, were detected in 63 of 478 (13.2%) patients. In total, 30 *BRCA1* mutation carriers (6.3%) and 33 *BRCA2* mutation carriers (6.9%) were identified. Table 1 shows the frequency of *BRCA1/2*

Table 1 The frequencies of *BRCA1* and *BRCA2* mutations^a in high-risk breast cancer patients according to familial and personal risk factors (*N* = 478)

Risk category	Total <i>N</i> (% ^d)	<i>BRCA1</i> mutation <i>N</i> (% ^e)	<i>BRCA2</i> mutation <i>N</i> (% ^e)	<i>BRCA1/2</i> mutation <i>N</i> (% ^e)
<i>Family history</i>				
Breast cancer family only (without ovarian cancer) [§]	303 (63.4)	14 (4.6)	29 (9.6)	43 (14.2)
1 breast cancer family	253 (52.9)	11 (4.4)	17 (6.7)	28 (11.1)
2 ≤breast cancer families ^{*,§}	50 (10.5)	3 (6.0)	12 (24.0)	15 (30.0)
Breast cancer families in 1st degree relatives ^b	217 (45.4)	13 (6.0)	20 (9.2)	33 (15.2)
Breast cancer families in second/third degree relatives ^{b,§}	86 (18.0)	1 (1.2)	9 (10.5)	10 (11.6)
Ovarian cancer family ^b				
Without breast cancer [§]	29 (6.1)	6 (20.7)	0 (0.0)	6 (20.7)
With breast cancer [*]	13 (2.7)	4 (30.8)	2 (15.4)	6 (46.2)
Any of breast/ovarian cancer families ^{c,*}	345 (72.2)	24 (7.0)	31 (9.0)	55 (15.9)
No family history [*]	133 (27.8)	6 (4.5)	2 (1.5)	8 (6.0)
<i>Personal history</i>				
Early-onset breast cancer (age < 40)	199 (41.6)	12 (6.0)	11 (5.5)	23 (11.6)
Bilateral breast cancer	47 (9.8)	2 (4.3)	5 (10.6)	7 (14.9)
Multiple organ cancers ^f	27 (5.6)	0 (0.0)	2 (7.4)	2 (7.4)
Both breast and ovarian cancer [*]	6 (1.3)	3 (50.0)	1 (16.7)	4 (66.7)
Male breast cancer	4 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)
<i>Clinicopathological factor</i>				
Age at diagnosis				
<40	186 (38.9)	12 (6.5)	11 (6.8)	23 (14.3)
40–49	172 (36.0)	10 (5.8)	15 (8.7)	25 (14.5)
50–59	89 (18.6)	7 (7.9)	6 (6.7)	13 (14.6)
60–79	31 (6.5)	1 (3.3)	1 (3.3)	2 (6.5)
Stage [*]				
0	55 (11.5)	0 (0.0)	0 (0.0)	0 (0.0)
I	173 (36.2)	12 (6.9)	12 (6.9)	24 (13.9)
II	159 (33.3)	12 (7.6)	18 (11.3)	30 (18.9)
III+	88 (18.4)	5 (7.4)	2 (2.9)	7 (10.3)
Unknown	3 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)
Hormone receptor status ^{*,§}				
ER+ & PR+	307 (64.2)	5 (1.6)	25 (8.1)	20 (9.8)
ER+ & PR–	44 (9.2)	1 (2.3)	4 (9.1)	5 (11.4)
ER– & PR+	7 (1.5)	1 (14.3)	0 (0.0)	1 (14.3)
ER– & PR–	115 (24.1)	23 (20.0)	4 (3.5)	27 (23.5)
Unknown	5 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)
Subtype according to hormone receptor and HER2 status ^{*,§}				
HR+ & HER2–	252 (52.7)	6 (2.4)	19 (7.5)	25 (9.9)
HR– & HER2+	28 (5.9)	1 (3.6)	0 (0.0)	1 (3.6)
HR+ & HER2+	37 (7.7)	0 (0.0)	2 (5.4)	2 (5.4)
Triple-negative	76 (15.9)	19 (25.0)	3 (4.0)	22 (29.0)
Unclassifiable	85 (17.8)	4 (4.7)	9 (10.6)	13 (15.3)
Total	478 (100.0)	30 (6.3)	33 (6.9)	63 (13.2)

HR hormone receptor, HER2 human epidermal growth factor receptor 2

* *P* value <0.05 for *BRCA1/2* mutation prevalence between those included in each category and those not

§ *P* value <0.05 between *BRCA1* and *BRCA2* ratio in carriers

^a Including three patients with large genomic rearrangements in *BRCA1* gene

^b Among 42 patients who had family history of ovarian cancer, 40 had one family member with ovarian cancer history and 2 had two family members with ovarian cancer history

^c Closest degree of relatives with breast cancer

^d Percent among all subjects (column percent)

^e Percent among subjects with each risk category (row percent)

^f Multiple organ cancer was defined as breast cancer patients with other primary organ cancer except ovarian cancer

Table 2 Characteristics of the probands with *BRCA1* large genomic rearrangements

Pt	Personal factor				Familial factor			Mutation carrier risk BRCAPRO/ Myriad
	Early-onset BC (age at diagnosis)	Bilateral BC	Both BC and OC	TNBC	Family history of BC (number, closest degree)	Family history of OC (number, closest degree)	Other cancer	
A	No (51)	No	No	Yes	Yes (2, second degree)	No	Liver Cervix Stomach Thyroid Colon	0.8/5.3
B	Yes (35)	No	No	Yes	Yes (1, second degree)	Yes (1, 1st degree)	Thyroid	57.2/39.2
C	Yes (33)	Yes	Yes	No	Yes (1, first degree)	No	Lung	51.2/15.8

mutations according to familial and personal factors, and clinicopathological characteristics. *BRCA1/2* mutation prevalence in familial breast cancer cases was 15.9%, which was significantly higher in non-familial breast cancer cases (6.0%, $P = 0.004$). When personal factors were considered, *BRCA1/2* mutations were observed in 11.6% of early-onset breast cancer patients, 14.9% of bilateral breast cancer patients, and 66.7% of patients who were diagnosed with both breast and ovarian cancer. The prevalence of *BRCA1/2* mutations also differed significantly according to hormone receptor and HER2 status ($P = 0.003$ and <0.001 , respectively). The prevalence of *BRCA1/2* mutation according to the combinations of familial and personal factors with denominators and numerators is described in Appendix Table 4.

Patients with large genomic rearrangements

Three *BRCA1* deletion carriers were identified by MLPA from 306 patients *BRCA1/2* mutation negative by standard sequencing. These deletion mutations account for 10% of all *BRCA1* mutation carriers (Fig. 1). The characteristics of each patient are summarized in Table 2 and detailed explanation is as following. Patient A, diagnosed with ductal carcinoma in situ with TNBC at the age of 51, carried exons 5–8 deletion (Fig. 2a). She had a second- and a third-degree relative with breast cancer. Estimated mutation probabilities for *BRCA1/2* before mutation test were 0.8% by BRCAPRO and 5.3% by Myriad. Following genetic counseling about the *BRCA1* deletion, genetic testing was performed on her daughter and three sisters without breast cancer and her daughter was found to have the same deletion.

Patient B, diagnosed with stage III TNBC at the age of 35, carried exons 22–24 deletion (Fig. 2b). The patient had

a first-degree relative with ovarian cancer and a second-degree relative with breast cancer. Estimated mutation probabilities for *BRCA1/2* mutation test were 57.2% by BRCAPRO and 39.2% by Myriad. Genetic testing was performed on her four sisters without breast cancer and of them, two sisters were found to have the deletion.

Patient C, was diagnosed with bilateral stage I breast cancer for the first time at age 33 and for the second time at age 46. She was not a TNBC case, and carried a *BRCA1* gene lacking exons 1–14 (Fig. 2c). The estimated mutation probabilities for *BRCA1/2* mutation test were 51.2% by BRCAPRO and 15.8% by Myriad. After enrollment in this study, she was newly diagnosed with ovarian cancer. Genetic testing was performed on her two sisters and one of her sisters, diagnosed before with bilateral breast cancer, carried a *BRCA1* gene with the same large genomic rearrangements.

Pathogenic variants of *BRCA1/2* genes found in this study

The pathogenic variants of *BRCA1/2* genes found in this study are presented in Table 3. Overall, 24 pathogenic variants in *BRCA1* gene and 19 in *BRCA2* gene were found in 63 index cases mutations. Many of these mutations have been reported in the BIC or previous studies in Korea. The most frequent mutation was c.7480C>T in *BRCA2* and it was found in nine patients (14.3%). The next most frequent mutations were c.1399A>T in *BRCA2* gene and c.390C>A in *BRCA1* gene, which were found in four and three patients, respectively. The large genomic rearrangements found through MLPA were located in *BRCA1* gene, including an exon 1–14 deletion, exon 5–8 deletion, and exon 22–24 deletion.

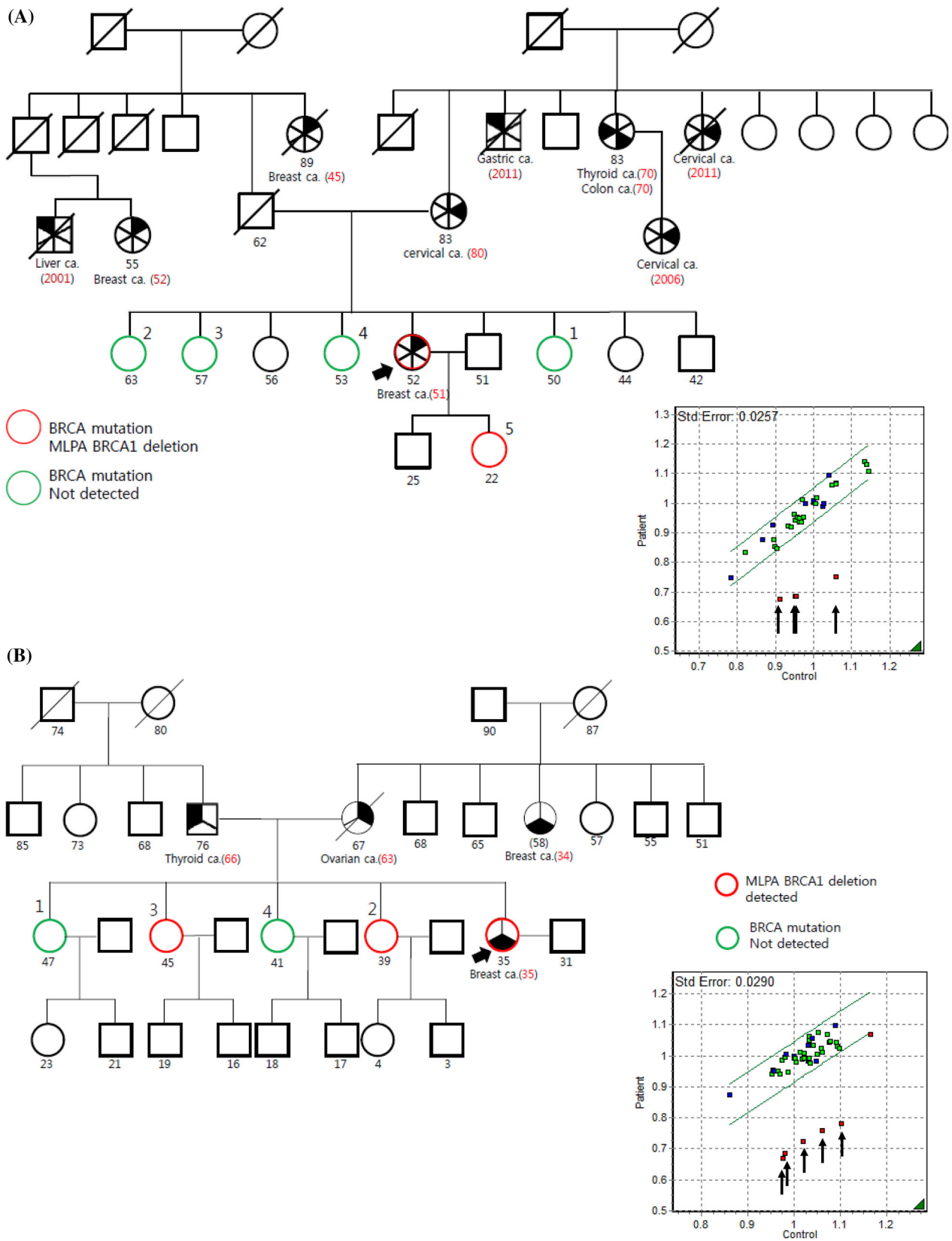


Fig. 2 The 3 *BRCA1* LGRs identified in the study using MLPA screening. The MLPA analysis demonstrates **a** exons 5–8 deletion, **b** exons 22–24 deletion, and **c** exons 1–14 deletion. Exons having a reduced peak ratio are denoted with the *arrows*

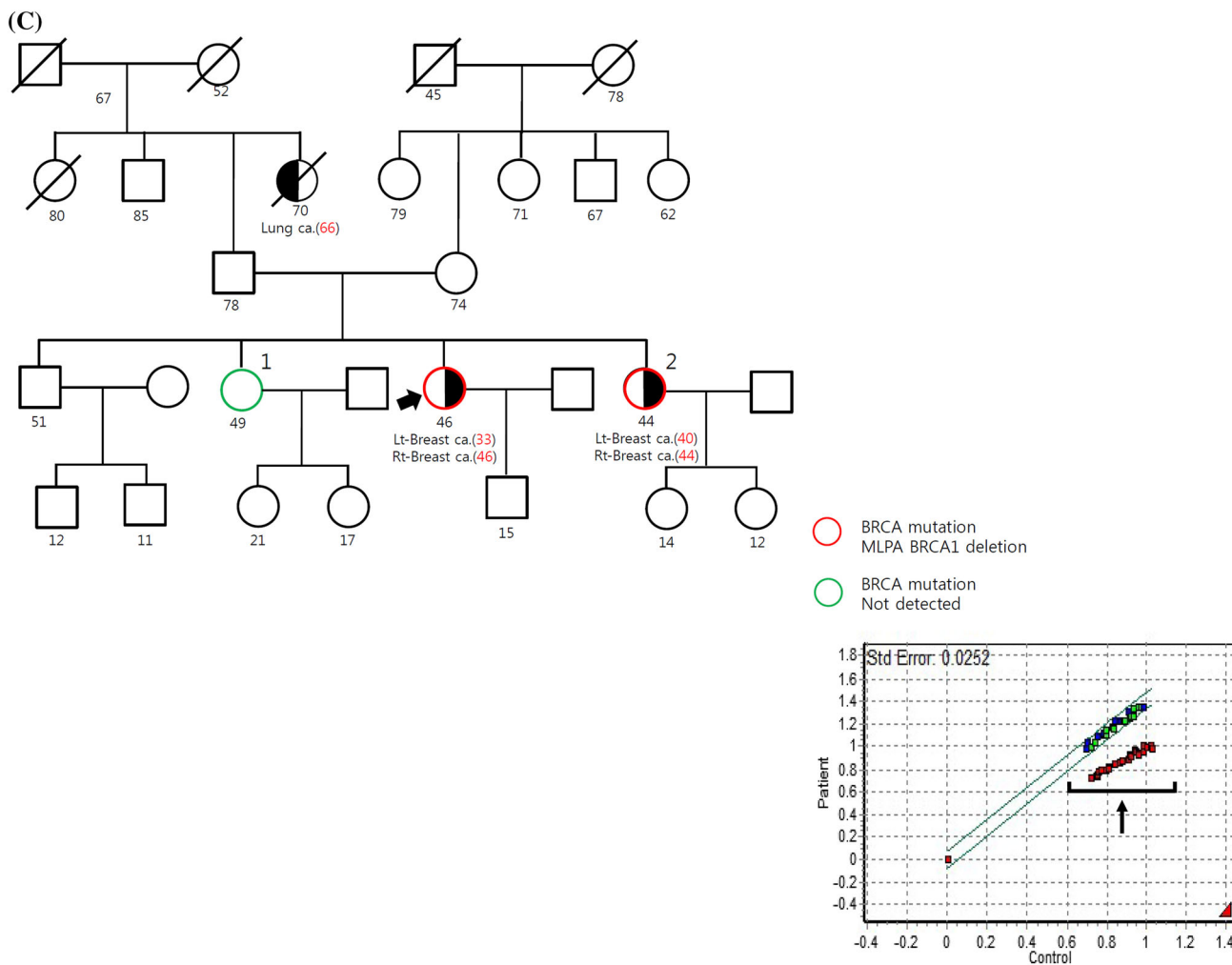


Fig. 2 continued

Discussion

Here, we detected *BRCA1/2* mutations in 13.2% of high-risk breast cancer patients who were referred to a genetic counseling center. Of the *BRCA1/2* carriers, 5% (3 out of 63) were identified by MLPA after negative direct sequencing results. All the large genomic rearrangements were found in *BRCA1* gene. Therefore, 10% of *BRCA1* carriers (3 out of 30) would have not been identified if MLPA had not been conducted. In Korea, *BRCA1/2* mutation screening has been covered by National Health Insurance since 2012 for those who meet certain criteria. Because more than 95% of this study

population was recruited after 2012, our results could provide a more current measure of the prevalence of *BRCA1/2* mutations.

The prevalence in familial breast cancer cases was 15.9% in this study, which was slightly lower than previous results from Korea, which ranged 19.4–30.0% [19–22], and results from Western countries [23]. Mutation prevalence according to each personal factor in this study was comparable with previous Korean studies [19, 21, 22, 24, 25]. TNBC is an important factor used to select breast cancer patients for *BRCA1/2* mutation testing [5] and this was confirmed in our study. Considering that the mutation prevalence varies according to overlapped risk factors

Table 3 Frequency of pathogenic variants of BRCA1 and BRCA2 genes in patients

Gene	Exon/ intron	BIC nomenclature	HGVS cDNA	HGVS protein	N	
<i>BRCA1</i>	IVS5	IVS5+1G>A	c.212+1G>A	–	1	
	7	509C>A	c.390C>A	p.Tyr130*	3	
	11	1041_1042delAG	c.922_923delAG	p.Ser308Glufs*	1	
	11		c.922_924delAGCinsT	p.Ser308*	1	
	11	1137delG	c.1018delG	p.Val340Glyfs*	1	
	11	1599C>T	c.1480C>T	p.Gln494*	1	
	11		c.14923_1494delTC	p.Leu498Hisfs*	1	
	11	1630dupG	c.1511dupG	p.Lys505*	1	
	11		c.1516delA	p.Arg506Glyfs*	2	
	11		c.2354T>A	p.Leu785*	1	
	11	3415delC	c.3296delC	p.Pro1099Leufs*	1	
	11	3746dupA	c.3627dupA	p.Leu1210Glufs*	1	
	11	3819del5	c.3700_3704delGTAAA	p.Val1210Aspfs*	1	
			c.4110C>T	p.Glu1331*	1	
	16	5100G>T	c.4981G>T	p.Glu1661*	2	
	20	5379G>T	c.5260G>T	p.Glu1754*	2	
	IVS21		c.5332+4delA	–	1	
	23		c.5445G>A	p.Trp1815*	2	
	IVS23	IVS23+1G	c.5467+1G>A	–	1	
	24	5602delG	c.5483delG	p.Cys1828Leufs*	1	
	24	5615del11insA	c.5496_5506delGGTGACCCGAGinsA	p.Val1833Serfs*	1	
	1–14	Exon 1–14 deletion			1	
	5–8	Exon 5–8 deletion			1	
	22–24	Exon 22–24 deletion			1	
	<i>BRCA2</i>	7	173G>T	c.518G>T	p.Gly173Val	1
		9	983del4	c.755_758delACAG	p.Asp252Serfs*	2
		10	1222delA	c.994delA	p.Ile332Phefs*	1
		10	1627A>T	c.1399A>T	p.Lys467*	4
		11	3026delCA	c.2798_2799delCA	p.Thr933Argfs*	1
		11		c.3096_3110delAGATATTGAAGAAC	p.Asp1033Ilefs*	1
11		3972del4	c.3744_3747delTGAC	p.Ser1248Glufs*	3	
11		6019C>T	c.5791C>T	p.Gln1931*	1	
11		6781delG	c.6553delG	p.Ala2185Leufs*	1	
14			c.7258G>T	p.Glu2420*	2	
15		7708C>T	c.7480C>T	p.Arg2494*	9	
15			c.7486G>T	p.Glu2420*	1	
18			c.8300_8301insAC	p.Pro2767Hisfs*	1	
22		9179C>G	c.8951C>G	p.Ser2984*	1	
23		9304C>T	c.9076C>T	p.Gln3026*	1	
24			c.9253delA	p.Thr3085Glnfs*	1	
25		9503del2	c.9275_9276delAT	p.Tyr3092Phefs*	1	
25		9641T>G	c.9413T>G	p.Leu3138*	1	

(Appendix Table 4), multiple combinations of familial or personal factors need to be considered for a more detailed risk assessment.

The prevalence of large genomic rearrangement after negative direct sequencing results in previous studies targeting potential hereditary cancer subjects in diverse ethnic groups ranged 0–5%, and was particularly low in Asian countries [12, 26–33] (Appendix Table 5). With the exception of two studies, most large genomic rearrangements have been identified in the *BRCA1* gene [12, 28], and all of the large genomic rearrangements identified in Korea [9, 10], including in this study, were in the *BRCA1* gene. The common characteristics of seven *BRCA1* large genomic rearrangement cases were that they all have a family history of breast and/or ovarian cancer with at least one additional personal factor. These personal factors included bilateral breast cancer, young age at onset (≤ 40 years old), both breast and ovarian cancer, and TNBC. Therefore, at least for *BRCA1*, the MLPA test should be considered for breast cancer patients with a family history of breast and/or ovarian cancer and additional personal factors such as bilateral breast cancer, young age at onset, and TNBC during the genetic counseling process. In our study population with large genomic rearrangements, the compliance with the prophylactic strategies was quite good (Appendix Table 6).

Several limitations of this study should be mentioned. Firstly, this study was performed by the patients of a single institute. Secondly, we could not conduct MLPA test for all *BRCA1/2* small mutation non-carriers due to patient non-participation, suggesting possible participation bias. However, this study was designed to reflect common clinical settings, following the patients in the process of genetic counseling. Thirdly, the detected large genomic rearrangements through MLPA test were not confirmed by different MLPA probes or other platforms. However, the three detected large genomic rearrangements were multi-exon deletions and detected in multiple family members, showing apparent inheritance patterns, suggesting minimum probabilities of false positives. Fourth, the family history was obtained by proband recollection and we did not consider the validity and reliability of the information.

In conclusion, the prevalence of *BRCA1/2* mutations was dependent on familial and personal factors. Subjects with both familial and personal factors had a much higher risk of carrying *BRCA1/2* mutations. The MLPA test for *BRCA1* mutation could be recommended for breast cancer patients with a family member with breast and/or ovarian cancer and additional personal factors, and who tested negative for *BRCA1/2* small mutations in initial testing.

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Compliance with ethical standards

Conflict of interest All of the authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This study protocol was approved by the institutional review board of the National Cancer Center (NCC2015-0177).

Informed consent Among the 418 cases designated as non-carriers by direct sequencing, written informed consent was obtained from 306 patients, and they were screened for the presence of large genomic rearrangements using MLPA assay. From 112 patients from whom the informed consent was not obtained, informed consent was exempted because this study was based on clinical chart review retrospectively. This study was approved by the institutional review board of National Cancer Center (NCC2015-0177).

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Appendix

See Tables 4, 5, and 6.

Table 4 The frequencies of *BRCA1* and *BRCA2* mutations according to combined family and personal characteristics

N of carriers/N of subjects (%)	Relatives with breast cancer				Family history of ovarian cancer	Early-onset breast cancer (≤ 40)	Bi-lateral breast cancer	Both breast and ovarian cancer	Multiple organ cancers	Male breast cancer	None	
	Number		Closest degree									
	1	≥ 2	1st	≥ 2 nd								
Relatives with breast cancer	1		25/186	9/80	6/13	14/78	4/18	2/2	1/15	0/1	11/151	
	Number											
	≥ 2		11/40	4/10	-/0	3/8	2/4	-/0	1/3	-/0	9/36	
	Closest degree											
	1st	13.4	27.5		3/9	10/41	5/13	2/2	2/14	0/1	17/152	
	≥ 2 nd	11.3	40.0		3/4	7/45	1/9	-/0	0/4	-/0	3/35	
Family history of ovarian cancer		46.2	-	33.3	75.0		5/13	1/2	2/2	-/0	-/0	6/27
Early-onset breast cancer (≤ 40)		18.0	37.5	24.4	15.6	38.5		2/15	-/0	0/5	0/2	22/181
Bilateral breast cancer		22.2	50.0	38.5	11.1	50.0	13.3		-/0	1/3	0/2	7/44
Both breast and ovarian cancer		100.0	-	100.0	-	100.0	-	-		-/0	-/0	4/6
Multiple organ cancers ^a		6.7	33.3	14.3	0.0	-	0.0	33.3	-		0/2	2/25
Male breast cancer		0.0	-	0.0	-	-	0.0	0.0	-	0.0		-/0
None		7.3	25.0	11.2	8.6	22.2	12.2	15.9	66.7	8.0		

^a Multiple organ cancer was defined as breast cancer patients with other primary organ cancer except ovarian cancer

Table 5 The prevalence of *BRCA1/2* mutation and large genomic rearrangements in subjects without small mutation from direct sequencing

Country	Number of subject	Total prevalence (%)		Prevalence among subjects with negative result of direct sequencing (%)	
		<i>BRCA1</i>	<i>BRCA2</i>	LRG in <i>BRCA1</i>	LRG in <i>BRCA2</i>
Hong Kong [24]	1236	4.6	5.1	0.4	0.3
Poland [27]	281	28.8	-	1.5	-
Lebanese Arab [22]	250	2.8	2.8	0.0	0.0
Mexico [29]	188	18.0	3.2	2.0	0.0
Slovakia [23]	585	14.5	11.5	5.0	0.0
Malaysia [11]	324	7.4	7.1	0.6 ^a	0.3 ^a
Malaysia [28]	100	-	-	2.0 ^a	0.0
Poland [26]	64	67		4.6	0.0
Singapore [25]	100	-	-	2.0	1.0
Korea [8]	221	35.3		2.1	0.0
Korea [9]	122	-	-	0.8	0.0
Korea—this study	478	6.3	6.9	1.0	0.0

^a Not certain whether the prevalence is from whole subject or from limited subjects with *BRCA1/2* mutation negative

Table 6 Follow-up status of the family members with multiple ligation-dependent probe amplification (MLPA) positive result from family with *BRCA1* large genomic rearrangements

Pt	Relationship (age)	Interval between proband identification and first genetic counseling	Known comorbidities at first genetic counseling	Interval between MLPA (+) to first preventive screening	Result of initial preventive screening for ovarian cancer	Result of initial preventive screening for breast cancer	Conducted prophylactic therapy and family care	Future plan for prophylaxis and family care
A	Daughter (23)	5 months	None	4 days	None	None	Oral contraceptive	Annual breast cancer screening until the age 25 Ovarian cancer screening per 3 months Recommended family screening for brother (age 25)
B	Sister 1 (39)	4 months	Thyroid disease	13 days	None	None	BSO (3 months after identifying carrier)	Recommended family screening for 2 sons in the future (age 19 and 16)
	Sister 2 (45)	4 months	None	13 days	None	None	BSO (3 months after identifying carrier)	
C	Sister (44)	1 months	Left breast cancer (TNBC, age 40) Right breast cancer (HR positive, age 44)	1 day	None	Known breast cancer	–	BSO Recommended family screening for 2 daughters in the future (age 15 and 13)

BSO bilateral salpingo-oophorectomy, *TNBC* triple-negative breast cancer, *HR* hormone receptor

References

- Ford D, Easton DF, Stratton M, Narod S, Goldgar D, Devilee P, Bishop DT, Weber B, Lenoir G, Chang-Claude J et al (1998) Genetic heterogeneity and penetrance analysis of the *BRCA1* and *BRCA2* genes in breast cancer families. The Breast Cancer Linkage Consortium. *Am J Hum Genet* 62(3):676–689
- Antoniou A, Pharoah PD, Narod S, Risch HA, Eyfjord JE, Hopper JL, Loman N, Olsson H, Johannsson O, Borg A et al (2003) Average risks of breast and ovarian cancer associated with *BRCA1* or *BRCA2* mutations detected in case Series unselected for family history: a combined analysis of 22 studies. *Am J Hum Genet* 72(5):1117–1130
- Chen S, Parmigiani G (2007) Meta-analysis of *BRCA1* and *BRCA2* penetrance. *J Clin Oncol* 25(11):1329–1333
- Foulkes WD, Smith IE, Reis-Filho JS (2010) Triple-negative breast cancer. *N Engl J Med* 363(20):1938–1948
- NCCN: Genetic/Familial High-Risk Assessment: Breast and Ovarian. 2015, Version 1
- Moyer VA (2014) Risk assessment, genetic counseling, and genetic testing for *BRCA*-related cancer in women: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med* 160(4):271–281
- Judkins T, Rosenthal E, Arnell C, Burbidge LA, Geary W, Barrus T, Schoenberger J, Trost J, Wenstrup RJ, Roa BB (2012) Clinical significance of large rearrangements in *BRCA1* and *BRCA2*. *Cancer* 118(21):5210–5216
- Sluiter MD, van Rensburg EJ (2011) Large genomic rearrangements of the *BRCA1* and *BRCA2* genes: review of the literature and report of a novel *BRCA1* mutation. *Breast Cancer Res Treat* 125(2):325–349
- Seong MW, Cho SI, Kim KH, Chung IY, Kang E, Lee JW, Park SK, Lee MH, Choi DH, Yom CK et al (2014) A multi-institutional study of the prevalence of *BRCA1* and *BRCA2* large genomic rearrangements in familial breast cancer patients. *BMC Cancer* 14:645
- Seong MW, Cho SI, Noh DY, Han W, Kim SW, Park CM, Park HW, Kim SY, Kim JY, Park SS (2009) Low contribution of *BRCA1/2* genomic rearrangement to high-risk breast cancer in the Korean population. *Fam Cancer* 8(4):505–508
- Ewald IP, Ribeiro PL, Palmero EI, Cossio SL, Giugliani R, Ashton-Prolla P (2009) Genomic rearrangements in *BRCA1* and *BRCA2*: a literature review. *Genet Mol Biol* 32(3):437–446

12. Kang P, Mariapun S, Phuah SY, Lim LS, Liu J, Yoon SY, Thong MK, Mohd Taib NA, Yip CH, Teo SH (2010) Large BRCA1 and BRCA2 genomic rearrangements in Malaysian high risk breast-ovarian cancer families. *Breast Cancer Res Treat* 124(2):579–584
13. Montagna M, Dalla Palma M, Menin C, Agata S, De Nicolo A, Chieco-Bianchi L, D'Andrea E (2003) Genomic rearrangements account for more than one-third of the BRCA1 mutations in northern Italian breast/ovarian cancer families. *Hum Mol Genet* 12(9):1055–1061
14. Palma MD, Domchek SM, Stopfer J, Erlichman J, Siegfried JD, Tigges-Cardwell J, Mason BA, Rebbeck TR, Nathanson KL (2008) The relative contribution of point mutations and genomic rearrangements in BRCA1 and BRCA2 in high-risk breast cancer families. *Cancer Res* 68(17):7006–7014
15. Chen S, Wang W, Broman KW, Katki HA, Parmigiani G (2004) BayesMendel: an R environment for Mendelian risk prediction. *Stat Appl Genet Mol Biol* 3(1):1063
16. Parmigiani G, Berry DA, Aguilar O (1998) Determining carrier probabilities for breast cancer-susceptibility genes BRCA1 and BRCA2. *Am J Hum Genet* 62(1):145–158
17. Frank TS, Deffenbaugh AM, Reid JE, Hulick M, Ward BE, Lingenfelter B, Gumpfer KL, Scholl T, Tavtigian SV, Pruss DR (2002) Clinical characteristics of individuals with germline mutations in BRCA1 and BRCA2: analysis of 10,000 individuals. *J Clin Oncol* 20(6):1480–1490
18. Richards CS, Bale S, Bellissimo DB, Das S, Grody WW, Hegde MR, Lyon E, Ward BE (2008) Committee MSotALQA: ACMG recommendations for standards for interpretation and reporting of sequence variations: revisions 2007. *Genet Med* 10(4):294–300
19. Ahn SH, Son BH, Yoon KS, Noh DY, Han W, Kim SW, Lee ES, Park HL, Hong YJ, Choi JJ et al (2007) BRCA1 and BRCA2 germline mutations in Korean breast cancer patients at high risk of carrying mutations. *Cancer Lett* 245(1–2):90–95
20. Han SA, Kim SW, Kang E, Park SK, Ahn SH, Lee MH, Nam SJ, Han W, Bae YT, Kim HA et al (2013) The prevalence of BRCA mutations among familial breast cancer patients in Korea: results of the Korean Hereditary Breast Cancer study. *Fam Cancer* 12(1):75–81
21. Kang E, Seong MW, Park SK, Lee JW, Lee J, Kim LS, Lee JE, Kim SY, Jeong J, Han SA et al (2015) The prevalence and spectrum of BRCA1 and BRCA2 mutations in Korean population: recent update of the Korean Hereditary Breast Cancer (KOHBRA) study. *Breast Cancer Res Treat* 151(1):157–168
22. Seong MW, Cho S, Noh DY, Han W, Kim SW, Park CM, Park HW, Kim SY, Kim JY, Park SS (2009) Comprehensive mutational analysis of BRCA1/BRCA2 for Korean breast cancer patients: evidence of a founder mutation. *Clin Genet* 76(2):152–160
23. Szabo CI, King M-C (1997) Population genetics of BRCA1 and BRCA2. *Am J Hum Genet* 60(5):1013
24. Son BH, Ahn SH, Kim SW, Kang E, Park SK, Lee MH, Noh WC, Kim LS, Jung Y, Kim KS et al (2012) Prevalence of BRCA1 and BRCA2 mutations in non-familial breast cancer patients with high risks in Korea: the Korean Hereditary Breast Cancer (KOHBRA) Study. *Breast Cancer Res Treat* 133(3):1143–1152
25. 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(9):1638–1645
26. El Saghir NS, Zgheib NK, Assi HA, Khoury KE, Bidet Y, Jaber SM, Charara RN, Farhat RA, Kreidieh FY, Decousus S (2015) BRCA1 and BRCA2 mutations in ethnic Lebanese Arab women with high hereditary risk breast cancer. *Oncologist* 20(4):357–364
27. Konecny M, Milly M, Zavodna K, Weismanova E, Gregorova J, Milkva I, Ilencikova D, Kausitz J, Bartosova Z (2011) Comprehensive genetic characterization of hereditary breast/ovarian cancer families from Slovakia. *Breast Cancer Res Treat* 126(1):119–130
28. Kwong A, Chen J, Shin VY, Ho JC, Law FB, Au CH, Chan T-L, Ma ES, Ford JM (2015) The importance of analysis of long-range rearrangement of BRCA1 and BRCA2 in genetic diagnosis of familial breast cancer. *Cancer Genet* 208(9):448–454
29. Lim Y, Iau P, Ali A, Lee S, Wong JL, Putti T, Sng JH (2007) Identification of novel BRCA large genomic rearrangements in Singapore Asian breast and ovarian patients with cancer. *Clin Genet* 71(4):331–342
30. Ratajska M, Brozek I, Senkus-Konefka E, Jassem J, Stepnowska M, Palomba G, Pisano M, Casula M, Palmieri G, Borg A (2008) BRCA1 and BRCA2 point mutations and large rearrangements in breast and ovarian cancer families in Northern Poland. *Oncol Rep* 19(1):263
31. Rudnicka H, Debnick T, Cybulski C, Huzarski T, Gronwald J, Lubinski J, Gorski B (2013) Large BRCA1 and BRCA2 genomic rearrangements in Polish high-risk breast and ovarian cancer families. *Mol Biol Rep* 40(12):6619–6623
32. Sharifah NA, Nurismah MI, Lee HC, Aisyah AN, Clarence-Ko CH, Naqiyah I, Rohaizak M, Fuad I, Jamal ARA, Zarina AL (2010) Identification of novel large genomic rearrangements at the BRCA1 locus in Malaysian women with breast cancer. *Cancer Epidemiol* 34(4):442–447
33. Villarreal-Garza C, Alvarez-Gómez RM, Pérez-Plasencia C, Herrera LA, Herzog J, Castillo D, Mohar A, Castro C, Gallardo LN, Gallardo D (2015) Significant clinical impact of recurrent BRCA1 and BRCA2 mutations in Mexico. *Cancer* 121(3):372–378