



Original Article

Implication and Influence of Multigene Panel Testing with Genetic Counseling in Korean Patients with *BRCA1/2* Mutation–Negative Breast Cancer

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Purpose The aim of the study was to evaluate the clinical implication of multigene panel testing of beyond *BRCA* genes in Korean patients with *BRCA1/2* mutation-negative breast cancer.

Materials and Methods Between 2016 and 2019, a total of 700 *BRCA1/2* mutation-negative breast cancer patients received comprehensive multigene panel testing and genetic counseling. Among them, 347 patients completed a questionnaire about cancer worry, genetic knowledge, and preference for the method of genetic tests during pre- and post-genetic test counseling. The frequency of pathogenic and likely pathogenic variants (PV/LPV) were analyzed.

Results At least one PV/LPV of 26 genes was found in 76 out of 700 patients (10.9%). The rate for PV/LPV was 3.4% for high-risk genes (17 *PALB2*, 6 *TP53*, and 1 *PTEN*). PV/LPVs of clinical actionable genes for breast cancer management, high-risk genes and other moderate-risk genes such as *ATM*, *BARD1*, *BRIP1*, *CHEK2*, *NF1*, and *RAD51D*, were observed in 7.4%. Patients who completed the questionnaire showed decreased concerns about the risk of additional cancer development (average score, 4.21 to 3.94; $p < 0.001$), influence on mood (3.27 to 3.13; $p < 0.001$), influence on daily functioning (3.03 to 2.94; $p = 0.006$); and increased knowledge about hereditary cancer syndrome (66.9 to 68.8; $p = 0.025$) in post-test genetic counseling. High cancer worry scales (CWSS) were associated with age ≤ 40 years and the identification of PV/LPV. Low CWSSs were related to the satisfaction of the counselee.

Conclusion Comprehensive multigene panel test with genetic counseling is clinically applicable. It should be based on interpretable genetic information, consideration of potential psychological consequences, and proper preventive strategies.

Key words Breast neoplasms, Genetic testing, Multigene panels, Beyond *BRCA*, Cancer worry

Introduction

With the discovery of breast cancer susceptibility genes and recognizing the significantly increased breast cancer risks in the carriers with pathogenic variant (PV) or likely pathogenic variant (LPV), clinical practice regarding hereditary or familial breast cancers has undergone considerable changes for several decades. The most well-known hereditary breast cancer susceptibility genes, *BRCA1* and *BRCA2* mutations result in cumulative risk of female breast cancer by the age of 80 of 57%-72% and 45%-69%, respectively [1-3]. Many current clinical guidelines recommend preventive strategies for carriers of *BRCA* mutations [4-7].

In addition, with the commercialization of multigene panel tests using next-generation sequencing, it has become more common to test germline mutations of other breast cancer susceptibility genes beyond *BRCA* using multigene

panels. Despite the cost effectiveness and shortened turnaround time to test multiple genes, comprehensive multigene panel tests still have several limitations, including a high likelihood for detection of variants of unknown significance (VUS) or secondary findings, as well as limited information and preventive strategies especially for the carriers with low to moderate-penetrance cancer susceptibility genetic variants.

A recent study reported that multigene panel tests did not increase cancer worry in the patients with breast cancer, compared to those who underwent *BRCA1/2*-only testing [8]. However, because the results of multigene panel tests can provoke negative emotional effects exceeding potential preventive benefit for some patients, there is still the opinion that multigene panel tests should be carefully applied in accordance with phenotypical features of multiple hereditary cancer syndrome or limited to individuals without a known

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Received August 30, 2021 Accepted November 15, 2021 Published Online November 17, 2021

genetic mutation of a single syndrome [7,9]. Therefore, the use of comprehensive multigene panel testing requires discussion of clinical actionability and consideration of possible negative emotional effects.

In this study, we prospectively tested the germline genetic variants beyond *BRCA* in Korean *BRCA1/2* mutation-negative breast cancer patients with high risk of hereditary cancer syndrome using a comprehensive multigene panel. Subsequently, we evaluated the frequency of PV/LPV in clinically actionable genes for breast cancer, cancer worry, genetic knowledge, and preference for the sequence and methods of multigene panel testing among the patients. In this manner, we considered clinical actionability and emotional effect of comprehensive multigene panel testing.

Materials and Methods

1. Study population

We enrolled Korean *BRCA1/2* mutation-negative breast cancer patients with at least one high-risk factor for hereditary breast cancer syndrome. Risk factors of hereditary breast cancer were defined as follows: (1) at least one case of breast or ovarian cancer in first- or second-degree relatives; (2) a first diagnosis of breast cancer before age 40; (3) bilateral breast cancer; (4) male breast cancer and (5) co-diagnosis with breast and other cancers in the same patient. Between March 2016 and December 2019, 1,866 breast cancer patients with high-risk factors were tested for *BRCA1/2* germline mutations, and 76 carriers with *BRCA1* mutations and 119 carriers with *BRCA2* mutations (one patient had both *BRCA1* and *BRCA2* mutations) were identified in Yonsei Cancer Center, Yonsei University College of Medicine, Seoul, Republic of Korea. Out of the patients without *BRCA1/2* mutations, we conducted comprehensive multigene panel tests for 700 participants, and additionally evaluated cancer worry, genetic knowledge, and attitude toward the multigene panel tests for 374 participants who agreed to answer questionnaires before and after the genetic tests. A flowchart including study design and process is shown in S1 Fig.

2. Comprehensive multigene panel-based variant analysis

Genomic DNA was extracted from the patients' peripheral blood samples. We used a customized targeted capture sequencing panel which included all coding sequences and intron-exon boundaries of the coding exon from 65 cancer predisposition genes (*APC*, *ALK*, *ATM*, *AXIN1*, *AXIN2*, *BARD1*, *BLM*, *BMPR1A*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CDK4*, *CDKN2A*, *CHEK2*, *CTNNB1*, *EPCAM*, *EXO1*, *FANCM*, *FLCN*, *GALNT12*, *GPC3*, *GREM1*, *KIF1B*, *KRAS*, *LMO1*, *MEN1*, *MLH1*, *MLH3*, *MRE11A*, *MSH2*, *MSH6*, *MUTYH*, *NBN*, *NF1*,

NF2, *NRAS*, *NTRK1*, *PALB2*, *PAX6*, *PHOX2B*, *PMS1*, *PMS2*, *POLD1*, *POLE*, *PPM1D*, *PRSS1*, *PTCH1*, *PTEN*, *RAD50*, *RAD51*, *RAD51C*, *RAD51D*, *RB1*, *RET*, *RUNX1*, *SDHA*, *SDHAF2*, *SDHB*, *SLX4*, *SMAD4*, *STK11*, *TP53*, *VHL*, and *WT1*). Products with each capture reaction were sequenced by 151 base pair paired-end reads on a NextSeq 550Dx instrument (Illumina, San Diego, CA). High-quality sequencing data with an average depth of 500-1,000 fold was obtained.

We identified all single base pair substitutions, insertion-deletions, and copy number variants (CNVs) in each gene. All likely deleterious variants were validated by Sanger sequencing. Split-read-based detection of large insertions and deletions was conducted using the Pindel and Manta algorithms. CNVs detected by ExomeDepth software [10] were further crosschecked with a base-level read depth normalization algorithm implemented in the DxSeq Analyzer (Dxome, Seoul, Korea). All possible large rearrangements were confirmed by the multiplex ligation-dependent probe amplification method. Genetic variants were classified using a five-tier system following guidelines from the American College of Medical Genetics and Genomics [11], and PV/LPV was considered to be a mutation in the current study [12].

3. Clinical data collection

Sociodemographic factors (sex, current age, age at first diagnosis of breast cancer, education level, marital status, and the number of children) were obtained during the baseline interview prior to pre-test counseling. The family history of cancer within the third-degree relatives was assessed by drawing a pedigree for each family during the pre-test counseling. The characteristics of breast cancer (pathological diagnosis, laterality, and subtype) and presence of other primary malignancy were obtained by review of medical records with permission from each participant.

4. Definition of the genes of interest

Among the genes tested using the comprehensive multigene panel, 14 genes were defined as clinically actionable genes [13] for risk-reduction of breast cancer using recommended strategies according to the NCCN, ASCO, or ESMO guidelines [4,6,7]: *ATM*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CHEK2*, *NF1*, *PALB2*, *PTEN*, *RAD51C*, *RAD51D*, *STK11*, and *TP53*. Considering the penetrance for hereditary breast cancer in the previous reports and guidelines, we defined *BRCA1*, *BRCA2*, *CDH1*, *PALB2*, *PTEN*, *STK11*, and *TP53* as high-risk genes; *ATM*, *BARD1*, *BRIP1*, *CHEK2*, *NF1*, *RAD51C*, and *RAD51D* as moderate-risk genes; and other genes as unknown-risk genes for breast cancer [7,14-16].

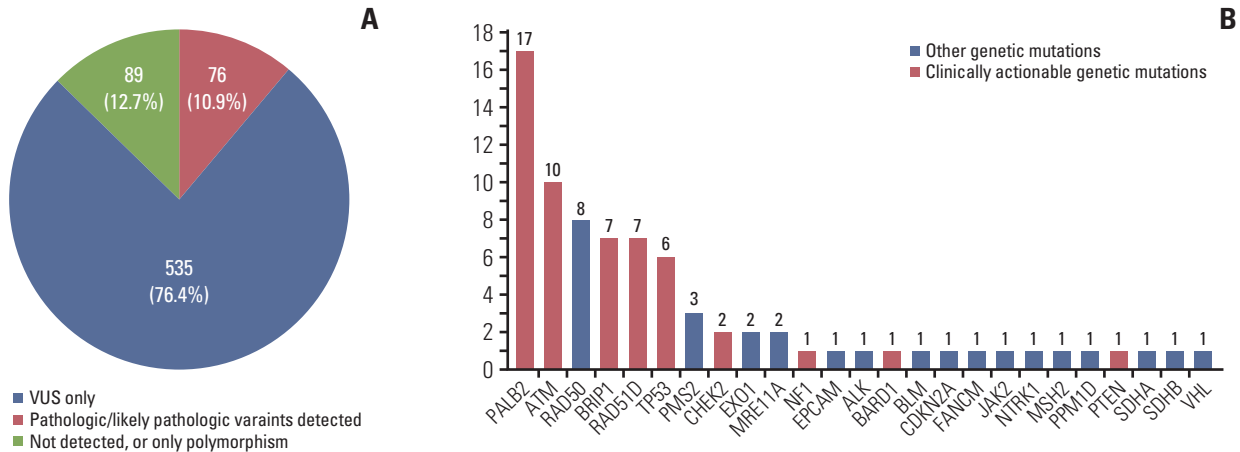


Fig. 1. Result of germline multigene panel tests in *BRCA* mutation-negative patients with high risk for hereditary breast cancer (n=700). (A) Proportion of the patients according to the results. (B) Frequency of likely pathogenic/pathogenic genetic variants (genes, n=26; patients, n=76)^a. VUS, variants of unknown significance. ^aThree patients had two likely pathogenic/pathogenic variants simultaneously (*RAD50* and *PMS2*, *EPCAM* and *SDHA*, and *JAK2* and *NTRK1*).

5. Genetic counseling

For all the patients enrolled in this study, the researchers provided pre- and post-test counseling. Genetic counseling was conducted by a trained medical oncologist and two registered nurses. The three researchers had completed a genetic counseling program certified by the Korean Breast Cancer Society. The pre-test counseling included the significance and utility of genetic variants with information, possible discrimination in insurance and employment, and alternatives to genetic testing. Post-test counseling was regarding interpretation of the genetic tests results and recommendations based on the results. For the mutation carriers, we provided preventive strategies via a multidisciplinary clinic consisting of various cancer specialists. We also recommended familial disclosure of genetic test result, and provided familial genetic testing with counseling for the family members.

6. Questionnaires about cancer worry, genetic knowledge, and attitude to genetic tests

In this study, cancer worry and its influence on mood and daily functioning were measured using a five-point Likert scale from Lerman’s Cancer Worry Scale (CWS) [17], which was modified under Korean translation [18,19], with a Cronbach’s alpha of 0.853. Genetic knowledge was measured using a 12-item true-false scale test adapted from Erlich’s Breast Cancer Genetic Counseling Knowledge Questionnaire (BGKQ) [20], which was translated and applied to previous studies [21,22], with a Cronbach’s alpha of 0.817 in this study. Total score of the test was calculated on a scale of 100 points. After a genetic test and post-test counseling, we assessed the patient’s satisfaction about the comprehensive multigene

panel tests with counseling using the question, “How much were your questions regarding the possibility of hereditary breast cancer answered after the multigene panel test?” with answer choices using the five ordinal variables of “very satisfied,” “satisfied,” “neutral,” “dissatisfied,” and “very dissatisfied.” The second question was asked to assess the patient preference for the sequence of genetic tests, “You did multigene panel tests after confirmation of negative for *BRCA1/2* mutation. If you can select the sequence for testing *BRCA1/2* genes and other genetic variants beyond *BRCA*, which of the method would you prefer?” with four choices, “concurrent tests using multigene panel,” “multigene panel test only for *BRCA1/2* negative patients,” “*BRCA1/2* mutation tests only,” or “not sure.”

7. Statistical analysis

Correlation between each risk factor and identified PV/LPV was analyzed using a chi-square test or Fisher’s exact test if indicated. Differences between pre-test and post-test values of CWS and BGKQ were compared using paired t tests. Clinico-genetic factors associated with genetic test results and post-test cancer worry were analyzed using single and multiple linear logistic regression modeling. Multiple linear logistic regression modeling was conducted using the variables with a p-value < 0.2 in the simple linear logistic regression model. A p-value < 0.05 was designated as statistically significant and all tests were two-sided. All statistical analyses were performed using IBM SPSS ver. 25.0 (IBM Corp., Armonk, NY).

Table 1. Baseline characteristics of the patients according to the genetic results (n=700)

Clinicopathological variable	PV/LPV (n=76)	VUS or ND (n=624)	p-value
Age at first diagnosis of breast cancer (yr)	44 (25-82)	43 (17-83)	0.628
Sex			
Male	2 (2.6)	5 (0.8)	0.171 ^{a)}
Female	74 (97.4)	619 (99.2)	
Breast cancer, laterality			
Unilateral	64 (84.2)	554 (88.8)	0.250 ^{a)}
Bilateral (metachronous)	6 (7.9)	24 (3.8)	
Bilateral (synchronous)	6 (7.9)	46 (7.4)	
Pathology			
IDC	55 (72.4)	435 (69.7)	0.545 ^{a)}
ILC	5 (6.6)	18 (2.9)	
DCIS	11 (14.5)	119 (19.1)	
LCIS	1 (1.3)	10 (1.6)	
Others	4 (5.2)	39 (6.3)	
Unknown	0	3 (0.5)	
Hormone receptor			
Positive	51 (67.1)	156 (25.0)	0.255 ^{a)}
Negative	23 (30.3)	460 (73.7)	
Unknown	2 (2.6)	8 (1.3)	
TNBC			
TNBC	15 (19.7)	98 (15.7)	0.367
Others	61 (80.3)	526 (84.3)	
Education			
University/College graduate	43 (56.6)	375 (60.1)	0.301 ^{a)}
High school graduate	24 (31.6)	161 (25.8)	
Middle graduate	1 (1.3)	26 (4.2)	
No/Elementary school graduate	3 (3.9)	9 (1.5)	
Unknown	5 (6.6)	53 (8.5)	
Family history of breast cancer			
Yes	30 (39.5)	288 (46.2)	0.269
No	46 (60.5)	336 (53.8)	
Family history of ovarian cancer			
Yes	7 (9.2)	26 (4.2)	0.076 ^{a)}
No	69 (90.8)	598 (95.8)	

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Results

1. Overview of clinical characteristics of the patients according to the results of genetic tests

This study included a total of 700 *BRCA1/2* mutation-negative breast cancer patients aged 18-83 years who had at least one high-risk factor for hereditary breast cancer syndrome. Among the patients, we identified at least one PV/LPV of 26 genes in 76 patients (10.9%). The frequency and spectrum of genetic variants are shown in Fig. 1. Another 535 patients (76.4%) had at least one VUS of 63 genes. No mutation nor

VUS was found in 89 patients (12.7%) in this study. The baseline characteristics of the patients according to the presence of PV/LPV are presented in Table 1.

Among the 76 patients with any PV/LPV, 24 patients (31.6% of the PV/LPV carriers, and 3.4% of the total participants) had PV/LPV in one of three high-risk genes: 17 in *PALB2*, six in *TP53*, and one in *PTEN*. Information on the genetic variants is shown in Table 2 with detailed clinicopathologic characteristics of the patients. PV/LPV in moderate-risk genes were identified in 28 patients (36.8% of the PV/LPV carriers, and 4% of the total participants) for six

Table 1. Continued

Clinicopathological variable	PV/LPV (n=76)	VUS or ND (n=624)	p-value
Second cancer history (multi-selection)			
Yes	19 (25.0)	86 (13.8)	0.010
Thyroid	9 (11.8)	40 (6.4)	0.080
Colorectal	4 (5.3)	10 (1.6)	0.055 ^{a)}
Lung	2 (2.6)	4 (0.6)	0.131 ^{a)}
Endometrium	1 (1.3)	6 (1.0)	0.554 ^{a)}
Ovary	2 (2.6)	3 (0.6)	0.131 ^{a)}
Pancreas	0	2 (0.3)	> 0.99 ^{a)}
Sarcoma	0	2 (0.3)	> 0.99 ^{a)}
Lymphoma	0	2 (0.3)	> 0.99 ^{a)}
Leukemia	0	1 (0.2)	> 0.99 ^{a)}
Kidney	0	4 (0.6)	> 0.99 ^{a)}
Urothelial	0	1 (0.2)	> 0.99 ^{a)}
Stomach	0	12 (1.9)	0.630 ^{a)}
Small bowel	1 (1.3)	1 (0.2)	0.205 ^{a)}
Paranglioma	1 (1.3)	0	0.109 ^{a)}
Liver	0	3 (0.5)	> 0.99 ^{a)}
Uterine cervix	4 (5.3)	2 (0.3)	0.002 ^{a)}
No	57 (75.0)	538 (86.2)	
Experience of full-term delivery			
Yes	63 (82.9)	461 (73.9)	0.087
No	13 (17.1)	163 (26.1)	

Values are presented as median (range) or number (%). DCIS, ductal carcinoma *in situ*; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; LCIS, lobular carcinoma *in situ*; LPV, likely pathogenic variant; ND, not detected; PV, pathogenic variant; TNBC, triple negative breast cancer; VUS, variant of unknown significance. ^{a)}These values were analyzed using Fisher exact tests.

genes: 10 in *ATM*, seven in *BRIP1*, seven in *RAD51D*, two in *CHEK2*, one in *BARD1*, and one in *NF1* (S2 Table). PV/LPV in unknown-risk genes were found in 24 patients (31.6% of the PV/LPV carriers, and 3.4% of the total participants) for 16 genes: seven in *RAD50*, two in *PMS2*, two in *EXO1*, two in *MRE11A*, one in *ALK*, one in *BLM*, one in *CDKN2A*, one in *FANCM*, one in *MSH2*, one in *PPM1D*, one in *SDHB*, one in *VHL*, one in both *EPCAM* and *SDHA*, one in both *JAK2* and *NTRK1*, and one in both *PMS2* and *RAD50* (S3 Table).

Fifty-two patients with PV/LPV in clinically actionable genes beyond *BRCA* (68.4% of the PV/LPV carriers and 7.4% of the total participants) were more likely to have bilateral breast cancer compared to those without any PV/LPV and VUS (odds ratio, 5.619; 95% confidence interval, 1.623 to 19.455; p=0.006) (Table 3).

2. Cancer worry and genetic knowledge before and after multigene panel testing with genetic counseling

A total of 374 patients completed the questionnaires regarding cancer worry, its influence on mood and daily functioning, and genetic knowledge before and after genetic

tests with counseling with a median time interval of 21 days between questionnaires (range, 14 to 85). After genetic tests with counseling about multigene panel, the patients showed decreased concern about the possibility of cancer in the future (average score of pre-test, 4.21±0.883 to post-test, 3.94±1.048; p < 0.001), decreased influence of cancer worry on mood (average score of pre-test, 3.27±0.645 to post-test, 3.13±0.694; p < 0.001), and decreased influence of cancer worry on daily functioning (average score of pre-test, 3.03±0.758 to post-test, 2.94±0.729; p=0.006). In addition, there was a slight but significant increase in the average score of knowledge about hereditary cancer (pre-test, 66.9±21.7 to post-test, 68.8±21.8; p=0.025) (Table 4).

3. Satisfaction and preference about comprehensive multigene panel tests beyond BRCA

Among the 374 patients who answered the survey about satisfaction after the comprehensive multigene panel tests with counseling, the answer about hereditary cancer risks were “very satisfied” for 173 patients (46.3%) and “satisfied” for 182 patients (48.7%). Another 11 patients (2.9%) were dis-

Table 2. Characteristics of patients with pathogenic or likely pathogenic variants in high-risk genes for breast cancer beyond BRCA (n=24)

Case	Sex	Age at first diagnosis of breast cancer (yr)	Breast cancer Side/Path (subtype)	Family history kind (degree*n)	Second cancer	Affected genes	Nucleotide change	Amino acid change	Effect	Mode of inheritance	Zygoty	dbSNP
P027	F	34	Rt/IDC (TNBC)	CRC (2*1)	-	PALB2	c.1381C>G	p.Gln461Glu	MS	AD	Hetero	-
P030	F	38	Lt/ILC (ER+/HER2-)	Breast (1*1), Lung (1*1)	-	PALB2	c.1426delA	p.Arg476Glufs	FS	AD	Hetero	-
P041	F	50	Lt/DCIS (ER+/HER2-)	Breast (1*1), Lung (1*1)	-	PALB2	c.1426delA	p.Arg476Glufs	FS	AD	Hetero	-
P032	F	38	Lt/LCIS (ER+/HER2-)	Breast (2*2)	-	PALB2	c.1516C>T	p.Gln506Ter	NS	AD	Hetero	-
P036	F	47	Rt/IDC (ER+/HER2-)	Breast (1*1), Stomach (1*2)	AoV	PALB2	c.2257C>T	p.Arg753Ter	NS	AD	Hetero	-
P042	F	54	Lt/IDC (ER+/HER2-)	Breast (1*1), Kidney (1*1), Esophagus (1*1)	-	PALB2	c.228_229delAT	p.Ile76Metfs	FS	AD	Hetero	-
P048	F	44	Rt/DCIS (ER+/HER2-)	Stomach (2*1)	Ovary	PALB2	c.355C>T	p.Gln119Ter	NS	AD	Hetero	-
P005	F	28	Rt/DCIS (ER+/HER2-)	Stomach (2*1), Pros (2*1), Liver (2*1)	-	PALB2	c.2406_2407delTG	p.Cys802Ter	NS	AD	Hetero	-
P040	F	52	Lt/IDC (ER+/HER2-)	Stomach (1*1), Skin (1*1)	Cervix	PALB2	c.2485C>T	p.Gln829Ter	NS	AD	Hetero	-
P014	F	25	Lt/Medullary (TNBC)	Breast (2*2), CRC (2*2)	-	PALB2	c.2748+1G>A	-	Splicing	AD	Hetero	rs753153576
P049	F	62	Lt/IDC (TNBC)	Breast (1*1), Stomach (1*2)	-	PALB2	c.2748+1G>A	-	Splicing	AD	Hetero	rs753153576
P063	F	45	Rt/ILC (ER+/HER2-)	Breast (2*1, 3*1)	-	PALB2	c.3256C>T	p.Arg1086Ter	NS	AD	Hetero	rs587776527
P101	F	60	Lt/IDC (ER+/HER2-)	Ovary (1*1)	-	PALB2	c.3350+5G>A	-	Splicing	AD	Hetero	rs587782566
P102	F	38	BiI(syn)/IDC (ER+/HER2+)	Ovary (1*1)	-	PALB2	c.3350+5G>A	-	Splicing	AD	Hetero	rs587782566
P010	F	50	Rt/IDC (ER+/HER2-)	Breast (1*1), Stomach (1*1), Panc (2*1), Sarcoma (1*1)	-	PALB2	Exon 11 deletion	-	Exon deletion	AD	Hetero	-
P011	F	47	BiI(met)/IDC (TNBC)	Breast (1*2)	-	PALB2	Exon 11 deletion	-	Exon deletion	AD	Hetero	-

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Table 2. Continued

Case	Sex	Age at first diagnosis of breast cancer (yr)	Breast cancer Side/path (subtype)	Family history kind (degree*n)	Second cancer	Affected genes	Nucleotide change	Amino acid change	Effect	Mode of inheritance	Zygoty	dbSNP
P059	F	47	Rt/IDC (TNBC)	Breast (2*2), CRC (1*1, 2*1), cervix	-	PALB2	Exon 8 deletion	-	Exon deletion	AD	Hetero	-
P070	F	47	Lt/mucinous (ER+/HER2-)	Breast (2*1), Lung (2*1), Leukemia (2*1)	-	TP53	c.542G>A	p.Arg181His	MS	AD	Hetero	rs397514495
P023	F	51	Lt/IDC (ER+/HER2-)	Breast (1*2), Ovary (1*1)	-	TP53	c.542G>A	p.Arg181His	MS	AD	Hetero	rs397514495
P006	F	41	Bil(met)/DCIS (ER-/HER2+)	0	Lung, melanoma, sarcoma	TP53	c.646G>A	p.Val216Met	MS	AD	Hetero	rs730882025
P034	F	32	Bil(syn)/IDC (ER-/HER2+)	Stomach (1*1), Panc (1*1)	-	TP53	c.733G>A	p.Gly245Ser	MS	AD	Hetero	rs28934575
P025	F	38	Rt/DCIS (unknown)	0	-	TP53	c.743G>A	p.Arg248Gln	MS	AD	Hetero	rs11540652
P021	F	30	Rt/IDC (ER+/HER2+)	Stomach (2*2), Lung (2*1), Liver (2*1), Lymphoma (2*1)	-	TP53	c.824G>A	p.Cys275Iyr	MS	AD	Hetero	-
P062	F	37	Bil(syn)/IDC (ER+/HER2-)	Thyroid (1*1)	-	PTEN	c.813_815delinsCC	p.His272Profs	FS	AD	Hetero	-

AD, autosomal dominant; AoV, ampulla of Vater; Bil(met), bilateral breast cancer, metachronous; Bil(syn), bilateral breast cancer, synchronous; CRC, colorectal cancer; DCIS, ductal carcinoma *in situ*; ER, estrogen receptor; F, female; FS, frameshift; HER2, human epidermal growth factor receptor 2; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; Lt, left; MS, missense; NS, nonsense; Panc, pancreas; Pros, prostate; Rt, right; TNBC, triple negative breast cancer.

Table 3. Risk factors with odds ratio related to the identification of clinically actionable^{a)} genetic mutations for breast cancer beyond *BRCA* (n=52)

Clinicopathological risk factor	Mutation (n=52)	Vs. not detected (n=89)					
		Simple regression			Multiple regression		
		OR	95% CI	p-value	OR	95% CI	p-value
Age at first diagnosis of breast cancer ≤ 40 yr	21	0.758	0.379-1.516	0.433	-	-	-
Male breast cancer	0	N/A	-	-	N/A	-	-
Bilateral breast cancer	10	5.060	1.498-17.087	0.009	5.619	1.623-19.455	0.006
TNBC	10	1.175	0.485-2.847	0.722	-	-	-
Family history of breast cancer	23	0.741	0.373-1.474	0.393	-	-	-
Family history of ovarian cancer	5	4.628	0.864-24.775	0.073	5.470	0.980-30.545	0.053
Presence of other primary cancer	10	2.116	0.798-5.610	0.132	2.665	0.977-7.267	0.055

CI, confidence interval; N/A, not applicable; OR, odds ratio; TNBC, triple negative breast cancer. ^{a)}Genes with inheritance of increased breast cancer risk according to the National Comprehensive Cancer Network, American Society of Clinical Oncology, or European Society of Medical Oncology guidelines: number of patients with mutation in each gene; *ATM* (10), *BARD1* (1), *BRIP1* (7), *CHEK2* (2), *NF1* (1), *PALB2* (17), *PTEN* (1), *RAD51D* (7), *TP53* (6).

Table 4. Difference in cancer worry scores (5-point Likert scale) and genetic knowledge between pre- and post-test (n=374)

	Pre-test	Post-test	p-value
Concern about the possibility of cancer in the future	4.21±0.883	3.94±1.048	< 0.001
Influence on mood	3.27±0.645	3.13±0.694	< 0.001
Influence on daily functioning	3.03±0.758	2.94±0.729	0.006
Knowledge about hereditary cancer (max. point 100)	66.9±21.7	68.8±21.8	0.025

Values are presented as average±standard deviation.

satisfied, and eight patients (2.1%) were neutral about the genetic tests with counseling (Fig. 2A). When the answers were converted to a five-point Likert score (from “very dissatisfied” as point 1, to “very satisfied” as point 5), the median point for satisfaction was 4 (range 2 to 5). Meanwhile, in the simple regression and multiple regression models, a high CWSs were associated with young patients (aged ≤ 40 years) and the identification of PV/LPV, and low CWSs were related to higher satisfaction regarding genetic test with counseling (Tables 5 and 6).

For the sequence of genetic tests, 176 patients (47.1%) preferred to simultaneously test *BRCA1/2* and the genes beyond *BRCA* using comprehensive multigene panel, and 164 patients (43.9%) selected a sequential test including *BRCA1/2* mutation tests followed by multigene panel testing beyond *BRCA* for the *BRCA1/2* mutation-negative patients. Another three patients (0.8%) wanted to test *BRCA1/2* mutations only, and 31 patients (8.3%) had no preference for the sequence or method of genetic tests (Fig. 2B).

Discussion

The current study demonstrated that one out of ten patients with germline *BRCA1/2* mutation-negative breast cancer and risk factors for hereditary breast cancer had PV or LPV of cancer predisposition genes. Considering the general rule of 10 for threshold of certain testing, multigene panel tests can be justified and applicable in clinical practice for patients with germline *BRCA1/2* mutation-negative breast cancer and risk factors for hereditary breast cancer. For those with PV/LPV, clinical actionability and psychological influence should be considered in genetic counseling.

In this study, among the germline *BRCA1/2* mutation-negative breast cancer patients, PV/LPV were identified in 3.4% of the subjects with high-risk genes, and a total of 7.4% of the subjects with clinically actionable genes with recommendations in the current clinical guidelines for hereditary breast cancer, which was consistent with 4.9%-11.4% frequency of PV/LPV beyond *BRCA* in the previous results of multigene panel tests [23-27]. We provided intensive screening using mammography and breast magnetic resonance imaging to all of 52 patients with clinically actionable genetic mutations. No contralateral prophylactic mastectomy was conducted.

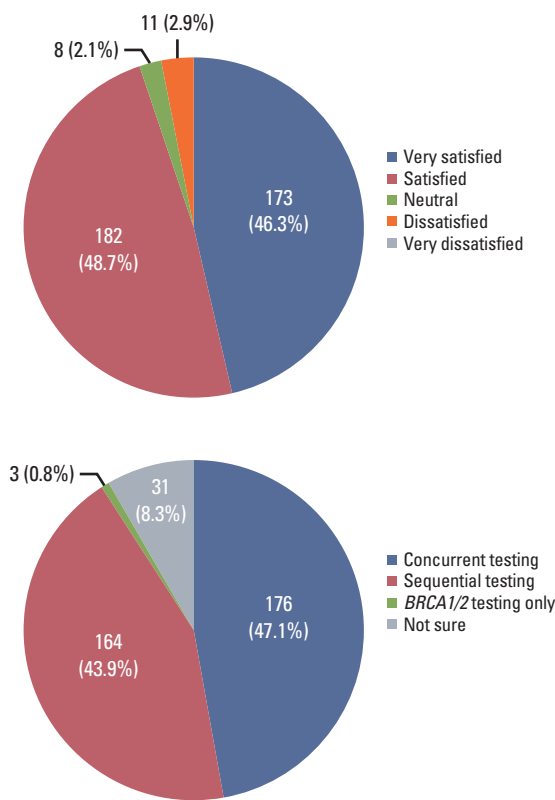


Fig. 2. Result of the survey about genetic counseling on multi-gene panel testing (n=374). (A) Satisfaction about the information gained by genetic tests with counseling. (B) Preference of the sequence and method of genetic testing for *BRCA1/2* mutation test and multigene test beyond *BRCA*.

A

B

Among the high-risk genes, PV/LPV were most frequently identified in *PALB2* gene (n=17). The carriers of *PALB2* PV/LPV were diagnosed with the primary breast cancer at median 47.1 years of age (range, 28.2 to 62.7), and 11 of 17 (54.7%) carriers had family history of breast cancer (Table 2). Zhou et al. [28] reported that *PALB2*-related breast cancer showed clinical characteristics including a family history of cancer, larger tumor, triple-negative breast cancer (TNBC), lymph nodal positivity, and bilateral breast cancer. Although proportion of TNBC (29.4%), frequency of family history of breast and/or cancer (76.5%), and proportion of bilateral breast cancer (11.8%) in this study were slightly higher than those in the report, clinical significance could not be shown due to small number of the participants and control group. The second most frequent high-risk PV/LPV was found in *TP53* (n=6). Among six carriers, five did not meet the criteria for the classic Li-Fraumeni syndrome (LFS) [29], or those of Birch et al. [30], Eeles [31], and Bougeard et al. [32]. All PV/LPVs found in this study were missense variants (Table 2). Bougeard et al. [32] previously suggested early-onset breast cancer diagnosed before age 31 years as a novel criterion for *TP53* genetic testing, based on the clinical findings of the carriers with missense variants in *TP53*, and tumor spectrum of the adult *TP53* PV/LPV carriers. However, most of the carriers in our study had neither personal /family history of LFS tumors nor early-onset breast cancer. In addition, eight kinds of missense VUS were also identified (S4 Table). It is necessary to further investigate the clinical penetrance and tumor spectrum of the carriers with *TP53* missense variants.

The present study additionally focused on the effect of

Table 5. Correlation between clinic-social factors and the cancer worry after multigene panel testing with counseling (simple regression analysis, n=374)

	Concern about the possibility of breast cancer in the future			Influence on mood			Influence on daily functioning		
	B	95% CI	p-value	B	95% CI	p-value	B	95% CI	p-value
Age ≤ 40 yr	0.255	0.042 to 0.467	0.019	0.150	0.009 to 0.291	0.037	0.173	0.025 to 0.321	0.022
Bilateral breast cancer	-0.391	-0.716 to -0.065	0.019	-0.146	-0.363 to 0.071	0.187	-0.079	-0.307 to 0.150	0.498
TNBC	0.283	-0.005 to 0.570	0.054	0.140	-0.050 to 0.331	0.149	0.214	0.014 to 0.414	0.036
Family history of breast or ovarian cancer	-0.052	-0.266 to 0.161	0.630	0.031	-0.110 to 0.172	0.666	-0.016	-0.164 to 0.133	0.837
Highly educated (above college graduates)	0.002	-0.002 to 0.006	0.388	0.0005	-0.002 to 0.003	0.724	0.0003	-0.002 to 0.003	0.816
PV /LPV detected	0.503	0.140 to 0.866	0.007	0.142	-0.100 to 0.384	0.250	0.102	-0.152 to 0.357	0.477
Counselor's satisfaction to genetic test with counseling	-0.176	-0.333 to -0.019	0.028	-0.125	-0.229 to -0.021	0.018	-0.134	-0.243 to -0.025	0.016
Stage IV breast cancer	0.396	-0.453 to 1.244	0.360	0.547	-0.013 to 1.107	0.055	0.235	-0.356 to 0.825	0.435
Genetic knowledge (post-test)	0.339	-0.150 to 0.829	0.174	0.049	-0.276 to 0.375	0.765	0.136	-0.206 to 0.477	0.435

B, beta regression coefficient value; CI, confidence interval; LPV, likely pathogenic variant; PV, pathogenic variant; TNBC, triple negative breast cancer.

Table 6. Correlation between clinic-social factors and the cancer worry after multigene panel testing with counseling (multiple regression analysis, n=374)

	Concern about the possibility of breast cancer in the future			Influence on mood			Influence on daily functioning		
	B	95% CI	p-value	B	95% CI	p-value	B	95% CI	p-value
Age ≤ 40 yr	0.201	-0.015 to 0.417	0.068	0.149	0.009 to 0.288	0.037	0.173	0.018 to 0.312	0.027
Bilateral breast cancer	-0.292	-0.624 to -0.040	0.085	-	-	-	-	-	-
TNBC	0.196	-0.090 to 0.481	0.179	-	-	-	0.181	-0.018 to 0.379	0.075
PV/LPV detected	0.427	0.057 to 0.797	0.024	-	-	-	-	-	-
Counselor's satisfaction to genetic test with counseling	-0.117	-0.276 to 0.043	0.152	-0.125	-0.228 to -0.022	0.018	-0.125	-0.234 to -0.017	0.024
Stage IV breast cancer	-	-	-	0.535	-0.019 to 1.089	0.058	-	-	-

B, beta regression coefficient value; CI, confidence interval; LPV, likely pathogenic variant; PV, pathogenic variant; TNBC, triple negative breast cancer.

genetic counseling after multigene panel tests. Among 374 patients who answered the questionnaire, 35 patients (9.4%) had PV/LPV in the genes beyond *BRCA* (S5 Table). Our results demonstrated that comprehensive multigene panel tests with genetic counseling can decrease the patients' cancer worry and increase the patients' knowledge about hereditary cancer syndrome. However, cancer worry of the PV/LPV carriers did not change after genetic tests with counseling (S6 Table), which was consistent with the previous study [33]. Decreased cancer worry was probably related to the psychological relief of the patients with VUS or negative results. In a previous study regarding *BRCA1/2* mutation tests, Richter et al. [34] reported that 36% of the VUS carriers failed to recall the clinical significance of their result, and their cancer worry and cancer preventive strategies were similar to those for patients without mutation. Otherwise, in a meta-analysis study including the results of 13 multigene panel tests and two exome sequencing tests of hereditary syndromes, the patients with VUS had higher genetic test-specific concerns compared to those with negative results, and lower concerns compared to those with positive results [35]. Katz et al. [8] suggested that the impact of cancer worry was not different by genetic test type or test results, but is rather influenced by ethnic and educational factors. In addition to the debate about the correlation between genetic testing result and cancer worry, the impact of the multigene panel testing result and clinical factors on cancer worry of Asian breast cancer patients has not been fully evaluated, since most previous studies were conducted in Western countries [8,34,35].

Genetic counseling is defined as a communication process which deals with human problems associated with the occurrence, or risk of occurrence, of a genetic disorder in a family [36]. Considering that one of the goals of genetic counseling is to facilitate the ability to use genetic information under

the cognitive interpretation [37], we assessed the satisfaction level using the counselees' subjective degree of interpretation of the genetic information to the possibility of hereditary breast cancer. Although the satisfaction of the counselee was distributed at lower scores in the carriers with PV/LPV (median, 4; range, 2 to 5) than in those with VUS (median, 4; range, 2 to 5; $p < 0.001$), or than in those with negative result (median, 5; range, 2 to 5; $p = 0.001$), 85.7% of the patients answered that they were satisfied with the information gained by genetic testing with counseling, even among the carriers with PV/LPV (S7 Fig.).

Based on the results that clinically actionable PV/LPV were commonly identified in multigene panel tests and that cancer worry was decreased after multigene panel tests with genetic counseling, the authors suggest that multigene panel tests can be usefully applied in clinical practice. However, we are needed to embrace the potential discomfort of the patients who still prefer *BRCA1/2* mutation tests prior to multigene panel tests beyond *BRCA*. In this study, the patients who preferred concurrent multigene panel tests were younger (median years of age, 39.8 vs. 44.6; $p = 0.004$) and more highly educated (proportion of college or university graduated, 74.2% vs. 62.7%; $p = 0.029$) than the patients who preferred sequential tests. Given that comprehensive multigene panel includes complex genetic information about multiple disease penetrance and diverse kinds of malignancy, well-structured genetic counseling will help to support comprehension and clinical decisions of the patients who have difficulties in getting multigene tests.

There are several limitations in this study. First, considering the frequency of PV/LPV in moderate- or low-penetrance genes beyond *BRCA*, a larger number of patients is needed to analyze an accurate incidence rate of each variant and clinical features of the carriers. Second, clinical action-

ability was assessed only based on the detection of genetic variant described in current clinical guidelines. Whether the identification of genetic mutation with counseling can actually improve a long-term preventive strategy and the survival outcome of the carriers is still controversial. Third, cancer worry and satisfaction of the patients with VUS and negative results could be influenced by miscomprehension about VUS and uninformative results, respectively. Despite the limitations, to the best of our knowledge, this is the first study simultaneously analyzed the potential actionability and psychological influence of comprehensive multigene panel tests in hereditary breast cancer.

Despite several debates, multigene panel tests are rapidly replacing the traditional single-gene direct sequencing methods. It is important for clinicians to improve the comprehensive multigene panel tests with genetic counseling programs based on the interpretable genetic information, consideration of potential psychological consequences, and proper preventive strategies for the carrier.

Electronic Supplementary Material

Supplementary materials are available at Cancer Research and Treatment website (<https://www.e-ert.org>).

Ethical Statement

The ethical principles for medical research established by the World Medical Association Declaration of Helsinki were followed throughout the study. The institutional review board at Severance Hospital, Seoul, Korea reviewed and approved this study (IRB approval number: 4-2015-0819 and 4-2018-0259). We obtained informed consent from all patients who participated in this study.

Author Contributions

Conceived and designed the analysis: Park JS, Park HS.

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Contributed data or analysis tools: Park JS, Shin S, Lee YJ, Lee ST, Nam EJ, Han JW, Lee SH, Kim TI, Park HS.


Performed the analysis: Park JS, Shin S, Lee YJ, Lee ST, Park HS.

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Conflicts of Interest

Conflict of interest relevant to this article was not reported.

Acknowledgments

Some of the results of this study were presented as a poster presentation at the 2019 ESMO ASIA held on November 22-24, 2019, in Singapore, and as an E-poster presentation at the 13th Annual Meeting of Korean Society of Medical Oncology & 2020 International Conference held on September 3-4, 2020, in Republic of Korea.

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (NRF-2018R1C1B6009449).

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