의학박사 학위논문

Detection of germline mutations in breast cancer patients with clinical features of hereditary cancer syndrome using a multi-gene panel test

유전성 암 증후군의 임상적 특징을 갖는 한국인 유방암 환자에서 다중 유전자 패널 검사를 통한 유전성 암 유전자 변이 확인

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지도교수 노 동 영

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신희철의 박사 학위논문을 인준함

2019 년 7 월

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Detection of germline mutations in breast cancer patients with clinical features of hereditary cancer syndrome using a multi-gene panel test

By

Hee-Chul Shin, M.D.

(Directed by Dong-Young Noh, M.D., Ph.D.)

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Approved by Thesis Committee

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Professor _____ Vice Chairman

Professor _____

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Abstract

Background: Hereditary cancer syndrome means that inherited genetic mutations can increase a person's risk of developing cancer. We assessed the frequency of germline mutations using an NGS-based multiple-gene panel containing 64-cancer predisposing genes in Korean breast cancer patients with clinical features of hereditary breast and ovarian cancer syndrome (HBOC).

Materials and Methods: Targeted sequencing using the multi-gene panel was performed to identify germline mutations in 496 breast cancer patients with clinical features of HBOC. Of 496 patients, 95 patients (19.2%) were found to have 48 deleterious germline mutations in 16 cancer susceptibility genes.

Results: The deleterious mutations were found in 39 of 250 patients (15.6%) who had breast cancer and another primary cancer, 38 of 169 patients (22.5%) who had a family history of breast cancer (≥ 2 relatives), 16 of 57 patients (28.1%) who had bilateral breast cancer, and 29 of 84 patients (34.5%) who were diagnosed with breast cancer at younger than 40 years of age. Of the 95 patients with deleterious mutations, 60 patients (63.2%) had *BRCA1/2*mutations and 38 patients (40.0%) had non-*BRCA1/2*mutations. We detected 2 novel deleterious mutations that were not previously reported: NM_000059.3:c.3096_3111del (p.Lys1032Asnfs*6) in *BRCA2* and NM_000249.3:c.849T>A (p.Tyr283*) in *MLH1*.

Conclusions: Using an NGS-based multi-gene panel test, we found that 19.2% of patients with clinical features of HBOC had germline cancer predisposing mutations. Approximately two-thirds of included patients had *BRCA1/2*mutationsandone-thirdhadnon-*BRCA1/2*mutations.NGS-based multiple-

gene panel testing improved the detection rates of deleterious mutations and provided a cost-effective cancer risk assessment.

Keywords: breast cancer, hereditary cancer syndrome, germline cancer predisposing mutation, next-generation sequencing, multi-gene panel testing

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Introduction

Hereditary cancer syndrome means that inherited genetic mutations can increase a person's risk of developing cancer. Specifically, certain genetic mutations can cause changes in the growth control of normal cells and cause them to become cancerous. Genetic mutations that promote cancer can be inherited if the mutations are present in germ cells. It is reported that inherited genetic mutations play a major role in 5% to 10% of all cancers. The most well-known genes associated with hereditary cancer syndrome are the *BRCA1/2* genes for hereditary breast and ovarian cancer syndrome (HBOC) and the *TP53* gene for Li-Fraumeni syndrome. Approximately 7% of breast and 13% of ovarian cancers are estimated to be due primarily to germline mutations in the *BRCA1/2* genes.[1,2] The cumulative risks of breast and ovarian cancers in *BRCA1/2* mutation carriers are reported to be 72% (95% confidence interval [CI]: 65%-79%) and 44% (95% CI: 36-53%), respectively, in *BRCA1* carriers and 69% (95% CI: 61%-77%) and 17% (95% CI: 11%-25%), respectively, in *BRCA2* carriers.[3] The cumulative cancer risk associated with *TP53* mutation may be as high as 90% by the age of 60 years.[4] In addition to mutations in *BRCA1/2* and *TP53*, germline mutations in certain genes were associated with more than 50 hereditary cancer syndromes. Genetic tests for hereditary cancer syndromes can identify individuals and families at increased risk of developing cancer. Once individuals or families are identified for hereditary cancer syndrome, they can be referred for risk assessment and personalized management that may include intensive cancer surveillance, risk-reducing surgery and genetic counseling.

With the rapid progress that has been made in next-generation sequencing (NGS)

technology, simultaneous sequencing of multiple genes has become available through multiple-gene panel testing, which is less expensive and more rapid than single-gene testing.[5] Furthermore, multiple-gene panels using NGS technology have increased the detection rate of mutations compared to conventional gene-by-gene testing.[6]

Currently, several commercial multiple-gene panels provide genetic information for hereditary cancer risk assessment. However, these are differences among ethnicities in cancer-susceptible germline mutations, and the assessment of germline mutations in all ethnic groups with clinical data is mandatory. In Korea and Asia, several studies evaluated the frequency of germline mutations, including *BRCA1/2* and/or other mutations associated with hereditary cancer syndrome. However, the results of most of studies were not representative of the Korean and Asian population because of the relatively small number of patients in included and the limited gene list evaluated.

In this study, we applied multiple-gene panel testing to 64 cancer- susceptibility genes to examine the frequency of mutations and to assess the clinical value of NGS-based multiple-gene panel testing in breast cancer patients with clinical features of HBOC.

Materials and Methods

Patient selection

The study population included 496 breast cancer patients with the following features of HBOC: (1) diagnosed with breast cancer and another primary cancer; (2) a family history that included at least 2 cases of breast cancer in first- or second-degree relatives; (3) bilateral breast cancer; or (4) breast cancer diagnosis before the age of 40 years. Of the patients, 349 patients were admitted to Seoul National University Hospital, Korea, and 147 patients were admitted to National Cancer Center, Korea, between 2002 and 2017. All patients consented to multi-gene panel testing for clinical research. Blood samples of the included patients were collected from each hospital and sent to a central laboratory for sequencing. The medical records were reviewed and personal and family histories and pathologic data of cancer were recorded. This study was approved by the Institutional Review Board of the Seoul National University Hospital (No. 1509-132-689).

NGS assay

Genomic DNA was extracted from the participants' peripheral blood samples. Our panel included 64 hereditary cancer predisposing genes (*ALK, APC, ATM, ATR, BAP1, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, EPCAM, FAM175A, FANCA, FANCB, FANCC, FANCD2, FANCE, FANCF, FANCG, FANCI, FANCL, FH, FLCN, GSTP1, HOXB13, KRAS, LIG4, MEN1, MET, MLH1, MRE11A, MSH2, MSH6, MUTYH, NAT, NBN, NF1, PALB2, PALLD, PMS2,*

PRKAR1A, PRSS1, PTEN, RAD50, RAD51, RAD51C, RAD51D, RB1, RET, SDHB, SDHC, SDHD, SLX4, SMAD4, SPINK1, STK11, TP53, VHL, and XRCC2) (Table1). For mutation analysis, 64 gene-containing DNA fragments were enriched by solution-based hybridization capture and followed by sequencing with an Illumina NextSeq platform (Illumina, San Diego, CA, USA) with the 150-bp paired-end read module. The target region included all coding exons. Capture probes were generated by Celemics, Inc. (Seoul, Korea). The hybridization capture procedure was also performed according to the manufacturer's standard protocol. Genomic DNA was sheared via sonication. Biotynilated RNA oligonucleotide probes were hybridized with sheared DNA. Captured fragments were removed from solution via streptavidin-coated magnetic beads and subsequently eluted. The enriched fragment library was then subjected to polymerase chain reaction (PCR) amplification using primers specific to the linked Illumina adaptors. Resulting libraries were quantified via Agilent 2200 TapeStation before proceeding to Illumina NextSeq platform. All samples were pooled into a single lane on a flow cell and sequenced together.

Raw FASTQ files were filtered using Trimmomatic (Version 0.33) and aligned with the genome of reference (GRCh37/hg19) using Burrows-Wheeler Aligner (Version 0.7.10). PCR duplicates, overrepresented sequences, and low-quality reads were removed. Realignments of insertions and deletions were performed using GATK. Reads with mapping quality of 0 were filtered out. If a read was able to be mapped at 2 different places with an identical percentage, the mapping quality equaled zero. Otherwise, the read was mapped to the most identical region. Variant calling was performed with Samtools (Version 1.1) and Varscan (Version 2.4.0).

Mutation analysis and variant classification

Variants were described according to the nomenclature recommendations of the Human Genome Variation Society (http://www.hgvs.org/mutnomen) and classified according to the following American College of Medical Genetics and Genomics recommendations: pathogenic (P), likely-pathogenic (LP), variants of unknown significance (VUS), likely-benign, and benign/polymorphism.[7] We used online databases, including the Human Gene Mutation Database, the Single Nucleotide Polymorphism Database, the 1000 Genome project, ClinVar, the Sorting Intolerant From Tolerant, Polymorphism Phenotyping-2, and the Korean Reference Genome Database, for *in silico* prediction of identified variants. Variants classified as P or LP were considered deleterious mutations.

Statistical analysis

Participant characteristics and sequencing results were summarized with descriptive statistics, which included medians, means, and standard deviations. The distributions of deleterious mutation according to the inclusion criteria were compared using Pearson's chi-squared analysis and Student's t-test. All *P*-values were 2-sided and a *P* value less than 0.05 was considered significant. All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp, Armonk, NY, USA).

Results

Study population

The clinical characteristics of the patients are shown in Table 1. The median age at diagnosis of cancer was 48 years (range, 19 to 80 years). In these patients, 390 patients (78.6%) had stage I or II disease. More than half of the patients (N=250, 50.4%) had another primary cancer, including ovarian cancer, stomach cancer, colon cancer, lung cancer, or other malignancy. In all, 169 patients (34.1%) reported that they had 2 or more first- or second-degree relatives with breast cancer. Fifty-seven patients (11.5%) had synchronous or metachronous bilateral breast cancer, and 84 patients (16.9%) were diagnosed with breast cancer at an age younger than 40 years. Sixty-four patients had 2 or more risk factors for HBOC (e.g., bilateral breast cancer and breast cancer diagnosis < 40 years old).

Frequency of deleterious mutations

Table 2 summarizes the characteristics of patients with and without deleterious mutations. Of all 496 patients, 95 patients (19.2%) were found to have deleterious germline mutations of cancer-susceptibility genes and 401 patients (80.8%) were not detected to carry deleterious mutations. The breast cancer stage was not different between the 2 groups (P=0.078). The proportions of risk factors, including breast cancer with another primary cancer, family history of breast cancer, and bilateral breast cancer were also not different between the groups. However, the proportion of patients with

deleterious mutations were higher in patients who were diagnosed with breast cancer at younger than 40 years old than

Table 1. Hereditary cancer predisposing genes in the multiple-gene panel test

Gene	Breast	Ovarian	Colorectal	Endometrial	Gastric	Pancreatic	Melanoma	Prostate	Other
ALK									O
APC			O		O	O			O
ATM	O					O			
ATR									O
BAP1									O
BARD1	O								
BLM			O						O
BMPR1A			O		O	O			O
BRCA1	O	O				O		O	
BRCA2	O	O				O	O	O	
BRIP1	O	O							
CDH1	O		O		O				
CDK4							O		
CDKN2A						O	O		
CHEK2	O		O					O	
EPCAM		O	O	O	O	O			O
FAM175A	O	O							
FANCA	O								O
FANCB									O
FANCC	O								O
FANCD2									O
FANCE									O
FANCF									O
FANCG									O
FANCI									O
FANCL									O
FH									O

FLCN									O
GSTP1	O								O
HOXB13								O	
KRAS			O		O	O	O		
LIG4									O
MEN1									O
MET									O
MLH1		O	O	O	O	O			O
MRE11A	O								
MSH2		O	O	O	O	O			O
MSH6		O	O	O	O	O			O
MUTYH			O						O
NAT			O						O
NBN	O							O	
NF1									O
PALB2	O					O			
PALLD									
PMS2		O	O	O	O	O			O
PRKAR1A									O
PRSS1					O	O			
PTEN	O		O	O					O
RAD50	O	O							
RAD51	O	O							
RAD51C	O	O							
RAD51D	O	O							
RB1									O
RET									O
SDHB									O
SDHC									O
SDHD									O

SLX4									O
SMAD4			O		O	O			O
SPINK1									
STK11	O	O	O	O	O	O			O
TP53	O	O	O	O	O	O	O	O	O
VHL									O
XRCC2	O								

Table 2. Characteristics of Patients With and Without Deleterious Mutations

Characteristics	Total (%)	No deleterious mutation (%)	Deleterious mutation (%)	P- value (x^2)
Number of patients	496 (100)	401 (80.8)	95 (19.2)	
Age at diagnosis (years), median (range)	48 (19-80)	49 (19-80)	45 (22-72)	0.027[a]
Breast cancer stage				
0	32 (6.5)	30 (7.5)	2 (2.1)	
I	209 (42.1)	170 (42.4)	39 (41.1)	
II	181 (36.5)	138 (34.4)	43 (45.3)	0.078
III	62 (12.5)	52 (13.0)	10 (10.5)	
IV	10 (2.0)	10 (2.5)	0 (0)	
Unknown	2 (0.4)	1 (0.2)	1 (1.1)	
Risk factors for HBOC[a]				
Breast cancer with another primary cancer	250 (50.4)	211 (52.6)	39 (41.1)	0.052
Family history of breast cancer (≥ 2 relatives)	169 (34.1)	131 (32.7)	38 (40.0)	0.187
Bilateral breast cancer	57 (11.5)	41 (10.2)	16 (16.8)	0.075
Breast cancer diagnosis at < 40 years old	84 (16.9)	60 (15.0)	29 (30.5)	0.022
2 or more risk factors	64 (12.9)	42 (10.5)	22 (23.2)	0.002

HBOC; hereditary breast and ovarian cancer syndrome.

[a]Statistical significance was evaluated by Student's t-test

patients with another risk factors. Breast cancer diagnosis at young age was associated with a higher rate of deleterious mutations (*P*=0.022). Furthermore, having 2 or more risk factors for HBOC was also associated with a higher rate of deleterious mutations (*P* = 0.001).

Breast cancers can be divided into four major subtypes depending on hormone receptor (HR) and human epidermal growth factor receptor 2 (HER2) status that have different clinical outcomes and responses to therapy a: luminal A (HR+ and HER2-), luminal B (HR+ and HER2+), HER2-enriched (HR- and HER2+), and triple-negative (HR- and HER2). Table 3 showed the percentage of subtypes in 410 patients whose immunohistochemistry (IHC) data were available. The percentage of patients with *BRCA1* mutations was different with patients without *BRCA1/2* mutations (P < 0.0001). Seventy-five percent of patients with *BRCA1* mutations were triple-negative breast cancer, whereas 20.8% of patients were HR (+) breast cancer including luminal A and luminal B subtypes. In contrast, the percentage of subtypes in *BRCA2* mutations was not statistically different with patients without *BRCA1/2* mutations (P = 0.825).

Table 4 and Figure 1 summarize 48 deleterious mutations found in 95 patients. Of these patients with deleterious mutations, 60 patients (12.1%) had *BRCA1/2* mutations: 31 in *BRCA1* and 30 in *BRCA2*. Patients HOPE_309 and HOPE_502 had 2 *BRCA1* mutations and patient HOPE_57 carried both *BRCA1* and *BRCA2* mutations. In addition, 38 patients (7.7%) had cancer-susceptibility gene mutations other than *BRCA1/2*: 35 patients had non-*BRCA1/2* mutations and 3 patients had both a *BRCA1/2* mutation and a non-*BRCA1/2* mutation (HOPE_14 had *BRCA2* and *SPINK1* mutations; HOPE_33 had *BRCA2*, *CDH1*, and *TP53* mutations; and HOPE_421 had *BRCA1* and *NBN* mutations.

Table 3. The percentage of breast cancer subtypes according to *BRCA1/2* mutations

Subtypes	Patients without *BRCA1/2* mutations (%)	Patients with *BRCA1* mutations (%)	P- value (x^2)	Patients with *BRCA2* mutations (%)	P- value (x^2)
Luminal A	235 (64.6)	5 (20.8)	<0.0001	15 (65.2)	0.825
Luminal B	32 (8.5)	0 (0.0)		1 (4.3)	
HER2-enriched	23 (6.3)	1 (4.2)		1 (4.3)	
Triple-Negative	75 (20.6)	18 (75.0)		6 (26.1)	

Table 4. List of Deleterious Mutations Identified in Patients

Gene	Mutation	Affected transcript	Affected protein	Case number
BRCA1	Frame shift insertion	NM_007294.3:c.3627dup	p.Glu1210Argfs*9	HOPE_112 HOPE_131 HOPE_191 HOPE_309 HOPE_421 HOPE_454 HOPE_502
	Nonsense mutation	NM_007294.3:c.4981G>T	p.Glu1661*	HOPE_287
	Nonsense mutation	NM_007294.3:c.5080G>T	p.Glu1694*	HOPE_11 HOPE_129 HOPE_429 HOPE_478
	Frame shift deletion	NM_007297.3:c.1575del	p.Glu525Aspfs*16	HOPE_399
	Frame shift deletion	NM_007294.3:c.1961del	p.Lys654Serfs*47	HOPE_118
	Missense mutation	NM_007294.3:c.5339T>C	p.Leu1780Pro	HOPE_226 HOPE_337 HOPE_356
	Nonsense mutation	NM_007294.3:c.3991C>T	p.Gln1331*	HOPE_57
	Nonsense mutation	NM_007294.3:c.928C>T	p.Gln310*	HOPE_10 HOPE_65
	Frame shift insertion	NM_007294.3:c.1511dup	p.Lys505*	HOPE_309 HOPE_502
	Frame shift deletion	NM_007294.3:c.923_924del	p.Ser308Lysfs*11	HOPE_36 HOPE_270
	Frame shift deletion	NM_007294.3:c.3700_3704del	p.Val1234Glnfs*8	HOPE_61 HOPE_351

	Nonsense mutation	NM_007294.3:c.5445G>A	p.Trp1815*	HOPE_280
	Nonsense mutation	NM_007294.3:c.390C>A	p.Tyr130*	HOPE_72 HOPE_168 HOPE_182 HOPE_190 HOPE_269
	Splice donor variant	NG_005905.2:c.5467+1G>A	p.=	HOPE_501
BRCA2	Frame shift deletion	NM_000059.3:c.700del	p.Ser234Profs*7	HOPE_229
	Frame shift deletion	NM_000059.3:c.3096_3111del	p.Lys1032Asnfs*6	HOPE_468 novel
	Frame shift insertion	NM_000059.3:c.9253dup	p.Thr3085Asnfs*26	HOPE_64
	Missense mutation	NM_000059.3:c.8023A>G	p.Ile2675Val	HOPE_407
	Nonsense mutation	NM_000059.3:c.1399A>T	p.Lys467*	HOPE_57 HOPE_91 HOPE_177 HOPE_355
	Frame shift deletion	NM_000059.3:c.4092_4093del	p.Ile1364Metfs*3	HOPE_14
	Nonsense mutation	NM_000059.3:c.8140C>T	p.Gln2714*	HOPE_456
	Nonsense mutation	NM_000059.3:c.9076C>T	p.Gln3026*	HOPE_465
	Frame shift deletion	NM_000059.3:c.5576_5579del	p.Ile1859Lysfs*3	HOPE_133
	Nonsense mutation	NM_000059.3:c.7480C>T	p.Arg2494*	HOPE_5 HOPE_31 HOPE_80 HOPE_114

				HOPE_307 HOPE_345 HOPE_389 HOPE_479
	Frame shift deletion	NM_000059.3:c.2798_2799del	p.Thr933Argfs*2	HOPE_350
	Nonsense mutation	NM_000059.3:c.8951C>G	p.Ser2984*	HOPE_359
	Frame shift deletion	NM_000059.3:c.3195_3198del	p.Asn1066Leufs*10	HOPE_33 HOPE_488
	Frame shift deletion	NM_000059.3:c.3744_3747del	p.Ser1248Argfs*10	HOPE_158 HOPE_233 HOPE_274 HOPE_281 HOPE_352
	Frame shift deletion	NM_000059.3:c.755_758del	p.Asp252Valfs*24	HOPE_372
BRIP1	Nonsense mutation	NM_032043.2:c.2392C>T	p.Arg798*	HOPE_485
CDH1	Missense mutation	NM_004360.4:c.2494G>A	p.Val832Met	HOPE_23 HOPE_28 HOPE_33 HOPE_78 HOPE_192 HOPE_222 HOPE_288 HOPE_319
CHEK2	Nonsense mutation	NM_007194.3:c.409C>T	p.Arg137*	HOPE_162
	Nonsense mutation	NM_001005735.1:c.1684C>T	p.Arg562*	HOPE_310
FANCA	Frame shift deletion	NM_000135.3:c.3720_3724del	p.Glu1240Aspfs*36	HOPE_125

	Frame shift deletion	NM_000135.2:c.2546del	p.Ser849Phefs *40	HOPE_66
MLH1	Frame shift insertion	NM_000249.3:c.1758dup	p.Met587Hisfs *6	HOPE_315
	Nonsense mutation	NM_000249.3:c.849T>A	p.Tyr283*	HOPE_378 novel
MRE11A	Missense mutation	NM_005591.3:c.140C>T	p.Ala47Val	HOPE_285
MSH2	Frame shift deletion	NM_000251.2:c.229_230del	p.Ser77Cysfs*4	HOPE_394
MUTYH	Nonsense mutation	NM_001128425.1:c.55C>T	p.Arg19*	HOPE_225
NBN	Missense mutation	NM_002485.4:c.511A>G	p.Ile171Val	HOPE_264 HOPE_421 HOPE_470
RAD51	Missense mutation	NM_002875.4:c.449G>A	p.Arg150Gln	HOPE_24 HOPE_35 HOPE_231 HOPE_266 HOPE_324 HOPE_335 HOPE_418
SPINK1	Missense mutation	NM_003122.4:c.101A>G	p.Asn34Ser	HOPE_14 HOPE_105 HOPE_144 HOPE_179 HOPE_413 HOPE_497
TP53	Missense mutation	NM_000546.5:c.566C>T	p.Ala189Val	HOPE_33 HOPE_395 HOPE_396
	Missense mutation	NM_000546.5:c.638G>A	p.Arg213Gln	HOPE_290

	Missense mutation	NM_000546.5:c.743G>A	p.Arg248Gln	HOPE_115

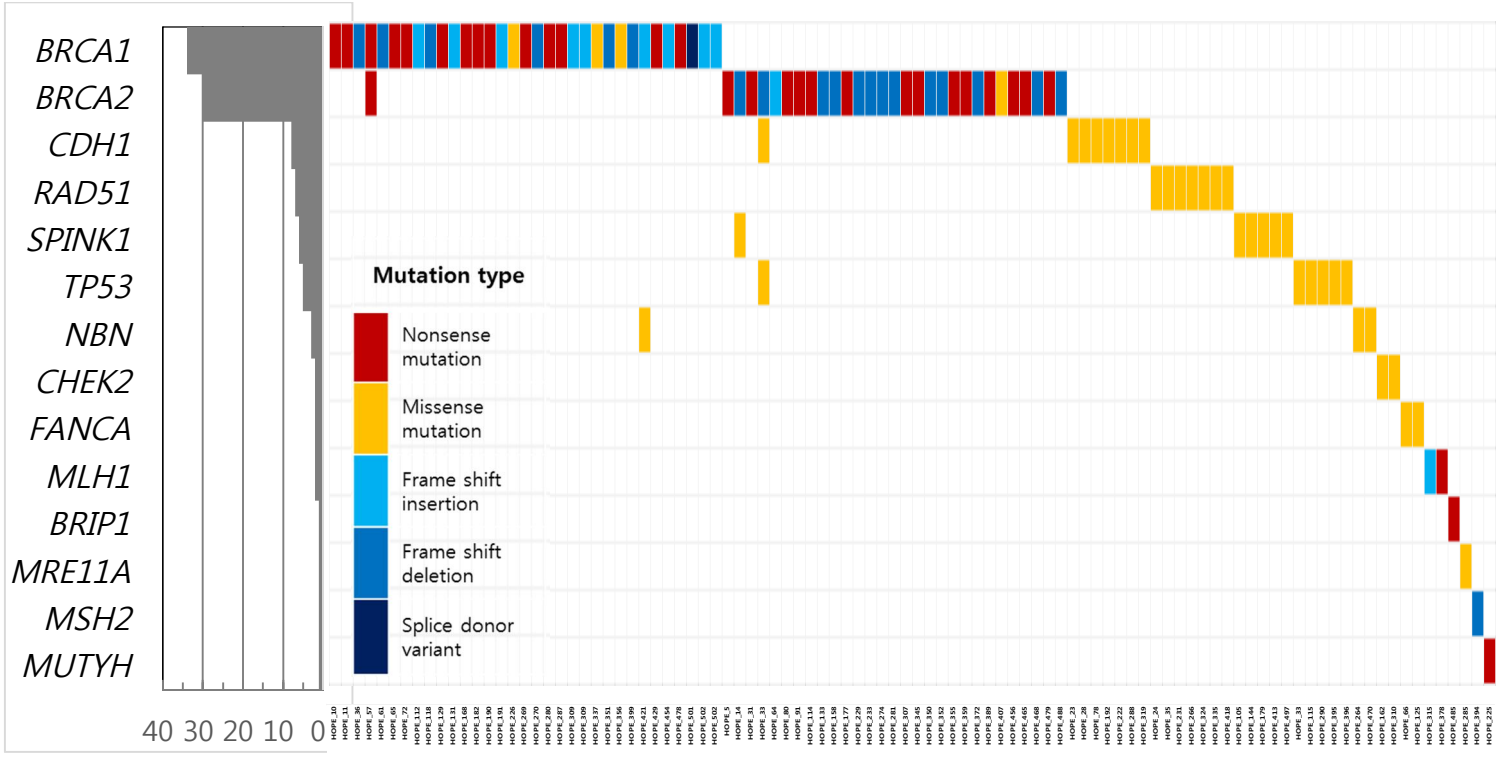


Figure 1. Summary of 48 deleterious mutations in 95 patients. Deleterious *BRCA1* and *BRCA2* mutations were detected in 30 patiensts and 31 patients, respectively. Non-*BRCA1/2* germline mutations were found in 38 patients including *CDH1, RAD51, SPINK1, TP53* and so on.

Most of the deleterious mutations were found in *CDH1* (N=8, 8.4%), *RAD51* (N=7, 7.4%), *SPINK1* (N=6, 6.3%), *TP53* (N=5, 5.3%) and *NBN* (N=3, 3.2%). The remaining patients had deleterious mutations in *CHEK2, FANCA, MLH1* (N=2 of each, 2.1%), *BRIP1, MRE11A, MSH2, and MUTYH* (N=1 of each, 1.1%).

The proportion of deleterious mutations varied according to risk factors. The deleterious mutations were found in 39 of 250 patients (15.6%) who had breast cancer and another primary cancer, 38 of 169 patients (22.5%) who had a family history (≥ 2 relatives) of breast cancer, 16 of 57 patients (28.1%) who had bilateral breast cancer, and 29 of 84 patients (34.5%) who were diagnosed with breast cancer at younger than 40 years old (Figure 2). Furthermore, the distributions of the cancer-susceptibility genes were different according to risk factors (Figure 3). In breast cancer patients with another primary cancer, *BRCA1/2* and non-*BRCA1/2* mutations accounted for 52.3% and 47.7% of mutations, respectively. The non-*BRCA1/2* mutations comprised *CDH1* (11.4%), *SPINK1* (9.1%), *RAD51* (6.8%), and *TP53* (6.8%) mutations. In breast cancer patients with a family history of breast cancer, 65.8% carried a *BRCA1/2* mutation. In 34.2% of non-*BRCA1/2* mutations, 7.9% had *RAD51* and *TP53* mutations, and 5.3% had *CDH1* and *SPINK1* mutations. In bilateral breast cancer patients, 68.4% carried a *BRCA1/2* mutation. Among the 31.6% who had non-*BRCA1/2* mutations, *CHEK2* (10.5%) were found frequently and 5.3% of patients had *CDH1*, *TP53*, *NBN* and *MRE11A* mutations. In patients diagnosed with breast cancer at younger than 40 years old, 62.1% carried *BRCA1/2* mutations and 37.9% carried non-*BRCA1/2* mutations including *RAD51*, *NBN*, *CHEK2*, *CDH1*, *TP53*, *PTEN*, *FANCA* and
MRE11A mutations.

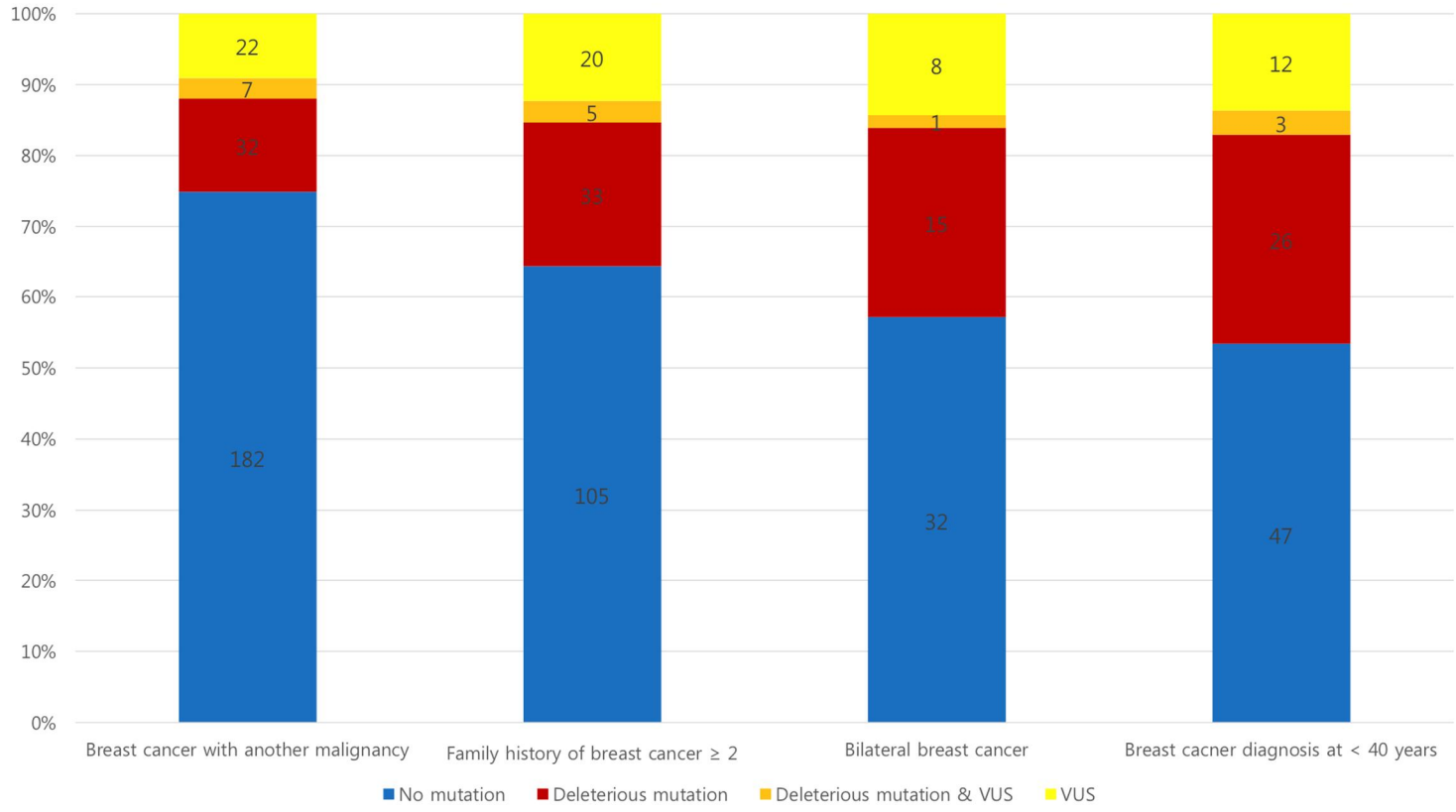


Figure 2. The proportion of deleterious mutations according to risk factors of hereditary cancer syndrome. The highest proportion of deleterious mutations were found in breast cancer patients who were diagnosed at < 40 years old and the lowest were found in breast cancer patient with another primary cancer.

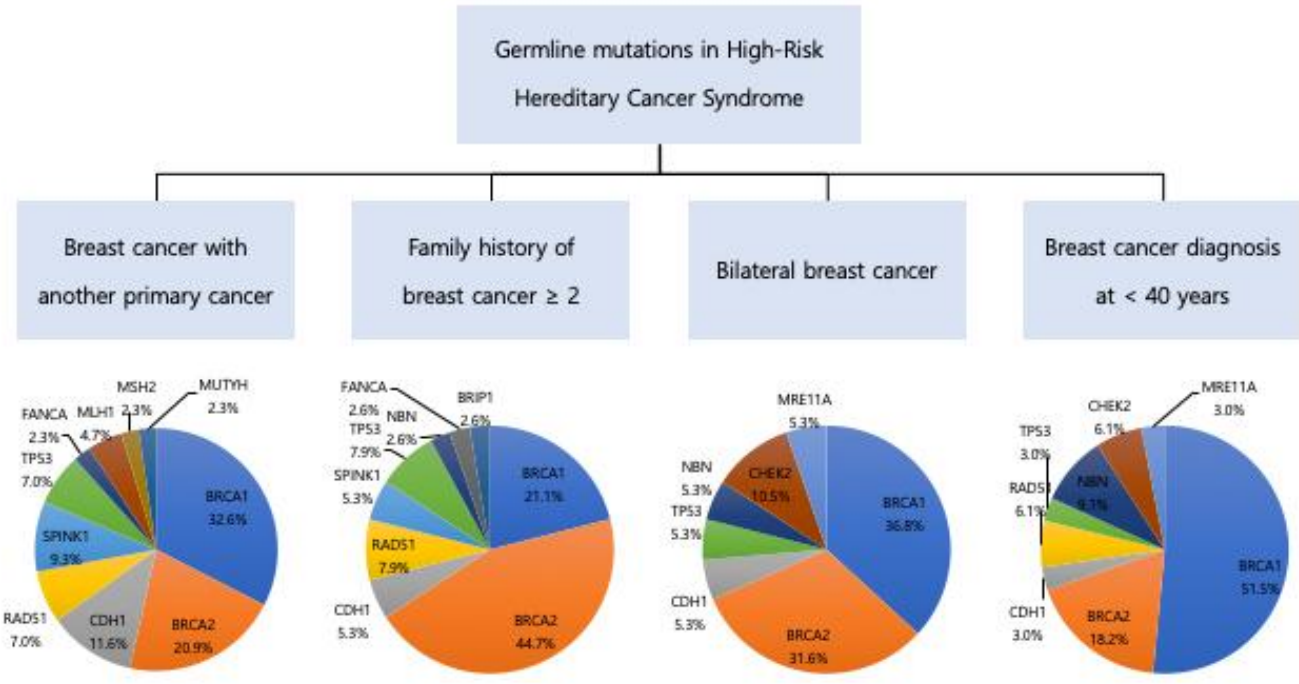


Figure 3. The distributions of the cancer-susceptibility genes according to risk factors hereditary cancer syndrome. The proportion of *BRCA1/2* mutations were relatively small in breast cancer patients with another primary cancer compared with patients with other risk factors.

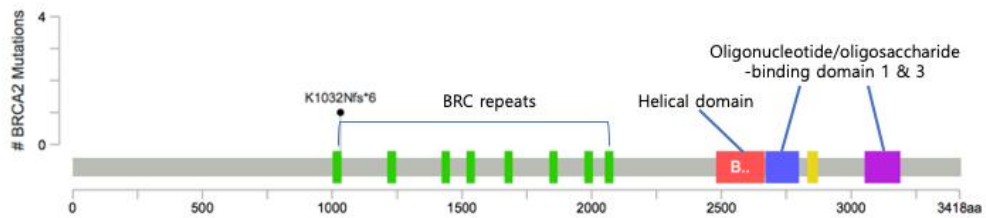
In 64 hereditary cancer predisposing genes, we found deleterious mutations in 16 genes, including *BRCA1/2*. However, we did not find deleterious mutations in the remaining 48 genes.

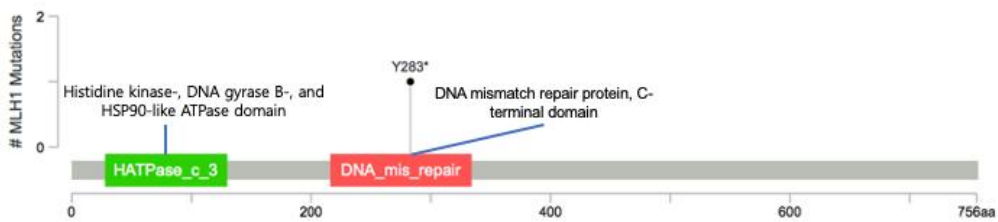
Novel deleterious mutations

We detected 2 novel deleterious mutations that were not previously reported: NM_000059.3:c.3096_3111del (p.Lys1032Asnfs*6) in *BRCA2* and NM_000249.3:c.849T>A (p.Tyr283*) in *MLH1*. The NM_000059.3:c.3096_3111del in *BRCA2* is identified in patient HOPE_468. This mutation encodes a truncated non-functional protein in the domain of the BRC repeats, interfering with cellular response to DNA damage (Fig. 4A). The NM_000249.3:c.849T>A in *MLH1* is identified in patient HOPE_378 and is also predicted to encode a non-functional protein, leading to the disruption of an important functional domain, such as the MutL C-terminal domain (Fig. 4B). The impact of both mutations were predicted deleterious mutations in *in silico* prediction.

Figure 4. Novel deleterious mutations mapped on corresponding protein structures. The impact of mutatinos were predicted in *in silico* analysis

A.





NM_000059.3:c.3096_3111del (p.Lys1032Asnfs*6) in *BRCA2*

B. NM_000249.3:c.849T>A (p.Tyr283*) in *MLH1*

Frequency of VUS

A total of 333 missense mutations were identified in 64 genes. After *in silico* prediction by database and bioinformatics analysis to evaluate pathogenicity, most of the missense mutations were classified as benign or likely-benign. Mutations with conflicting interpretations of pathogenicity but suspicion of being deleterious were classified as VUS. A total of 20 VUS were identified in 67 patients (13.5%) (Table 5). In 15 patients, deleterious mutation and VUS were found concurrently. The proportion of VUS differed among the risk factors for HBOC (Figure 2). VUS was identified in 11.6% of breast cancer patients with another primary cancer, 14.8% of patients with a family history of breast cancer, 15.8% of bilateral breast cancer patients, and 17.0% of patients who were diagnosed with breast cancer younger than 40 years old. Additionally, 13 patients with VUS also had a concurrent deleterious mutation (HOPE_33, 66, 105, 115, 133, 182, 222, 233, 264, 280, 454, 468, and 501).

Table 5. Variants of Uncertain Significance Strongly Suspected of Being Deleterious Mutations

Gene	Mutation	Affected transcript	Affected protein	Case number
ALK	Missense mutation	NM_004304.4:c.3260C>T	p.Thr1087Ile	HOPE_163 HOPE_264
ATR	Missense mutation	NM_001184.3:c.3637A>G	p.Ser1213Gly	HOPE_33 HOPE_204
BLM	Missense mutation	NM_000057.3:c.2371C>T	p.Arg791Cys	HOPE_468 HOPE_387 HOPE_393
BRCA1	Missense mutation	NM_007294.3:c.154C>T	p.Leu52Phe	HOPE_79 HOPE_105 HOPE_187 HOPE_232 HOPE_233
	Missense mutation	NM_007294.3:c.3448C>T	p.Pro1150Ser	HOPE_200
BRCA2	Missense mutation	NM_000059.3:c.7522G>A	p.Gly2508Ser	HOPE_115 HOPE_487 HOPE_306
CDH1	Missense mutation	NM_004360.4:c.1018A>G	p.Thr340Ala	HOPE_124 HOPE_133 HOPE_218 HOPE_436 HOPE_476
CHEK2	Missense mutation	NM_001005735.1:c.1240 C>T	p.His414Tyr	HOPE_164 HOPE_242 HOPE_466
FANCD 2	Missense mutation	NM_001018115.2:c.2480 A>C	p.Glu827Ala	HOPE_34 HOPE_66 HOPE_142 HOPE_214

				HOPE_347 HOPE_415
FANCD 2	Nonsense mutation	NM_001018115.1:c.1318C >T	p.Gln440*	HOPE_172
FANCE	Missense mutation	NM_021922.2:c.991C>G	p.Leu331Val	HOPE_26
FANCI	Missense mutation	NM_001113378.1:c.1111A >G	p.Ser371Gly	HOPE_25 HOPE_86 HOPE_113 HOPE_164 HOPE_202 HOPE_217 HOPE_246 HOPE_280 HOPE_342 HOPE_468 HOPE_501
FH	Missense mutation	NM_000143.3:c.302G>A	p.Arg101Gln	HOPE_145 HOPE_182 HOPE_198 HOPE_439
LIG4	Missense mutation	NM_001098268.1:c.2586T >A	p.His862Gln	HOPE_182 HOPE_291
MSH2	Missense mutation	NM_000251.2:c.14C>A	p.Pro5Gln	HOPE_186 HOPE_209 HOPE_222
	Missense mutation	NM_000251.2:c.1255C>A	p.Gln419Lys	HOPE_35 HOPE_88 HOPE_98 HOPE_232 HOPE_237 HOPE_414 HOPE_435

				HOPE_454 HOPE_462
MSH6	Missense mutation	NM_000179.2:c.3772C>G	p.Gln1258Glu	HOPE_144 HOPE_442 HOPE_490
	Missense mutation	NM_000179.2:c.2503C>G	p.Gln835Glu	HOPE_244
PALB2	Missense mutation	NM_024675.3:c.2509G>A	p.Glu837Lys	HOPE_291 HOPE_293 HOPE_358
PTCH1	Start lost	NM_001083603.2:c.1A>G	p.Met1?	HOPE_89 HOPE_463 HOPE_481
TP53	Missense mutation	NM_001126114.2:c.847C>T	p.Arg283Cys	HOPE_187

Discussion

Patients who carry deleterious mutations are considered to be at high risk for developing cancer, and depending on the target organ, tailored surveillance programs or prophylactic risk reducing surgery are recommended for decreasing cancer-related mortality. Currently, the National Comprehensive Cancer Network (NCCN) guidelines provide principles of genetic risk assessment and surveillance recommendations for various types of cancer.(8) For example, women with *BRCA1/2* mutations are at high risk for breast and ovarian cancers and they are recommended to undergo magnetic resonance imaging (MRI) of the breast for screening and to discuss options for risk-reducing mastectomy or salphingo-oophorectomy.

We investigated the frequency of germline mutations associated with cancer susceptibility using multiple-gene panel testing that included 64 cancer-susceptibility genes in Korean breast cancer patients with clinical features of HBOC. We found that 19.2% of breast cancer patients who had clinical features of HBOC had deleterious mutations of cancer-susceptibility genes. In this study, we found that young breast cancer patients and breast cancer patients with 2 or more risk factors for HBOC carried more deleterious mutations than older patients and patients with single risk factor (Table 2). Although the most detected mutations were *BRCA1/2* mutations, 39.3% and 32.3% of mutations were non-*BRCA1/2* mutations in young breast cancer patients and patients with 2 or more risk factors for HBOC, respectively. Multi-gene panel testing will be useful for patients with features of HBOC, especially, for patients diagnosed with breast cancer at younger than 40 years of age and for patients with 2 or more risk factors for

HBOC.

Table 2 summarizes the characteristics of patients with and without deleterious mutations. Of all 496 patients, 95 patients (19.2%) were found to have germline heterozygous deleterious mutations of cancer susceptibility genes and 401 patients (80.8%) were not found to carry deleterious mutations. The breast cancer stage was not different between the 2 groups (P=0.078), nor were the proportions of risk factors for HBOC (breast cancer with another primary cancer, family history of breast cancer in 2 or more first- or second-degree relatives, and bilateral breast cancer). However, there was a significantly higher rate of patients who were diagnosed with breast cancer at an age younger than 40 years among deleterious mutation-positive patients (P=0.022). Furthermore, having 2 or more risk factors for HBOC was also associated with deleterious mutations (P=0.001).

Among 496 patients who were tested by the multiple-gene panel for cancer-susceptibility genes, 60 patients (12.1%) were *BRCA1/2* positive, which was similar proportion to that reported in Western countries.(9) A previous study that included *BRCA1/2*-negative Korean breast cancer patients with features of hereditary breast cancer found that only 2.5% of non-*BRCA1/2* deleterious mutations were detected: *CHEK2* (0.4%), *PALB2* (0.9%), *MRE11* (0.4%), and *RAD50* (0.9%).(10) Another study including Western patients reported that deleterious mutations were found only in 1.7% of 1994 familial breast cancer patients: *PALB2* (1.3%), *TP53* (0.3%), *CDH1* (0.05%), and *ATM* (0.05%).(11) These studies reported that the frequency of deleterious mutations in each gene was less than 1% and concluded that a small portion of hereditary breast cancer was associated with non-*BRCA1/2* germline mutations. However, Li et al. detected 11.5%

non-*BRCA1/2* mutations, including *ATM, CDH1, CHEK2, PALB2, PTEN, STK11,* and *TP53* in 660 cases of familial breast cancer in a Western population.(12) Ricker et al. reported that multiple-gene panel testing increased the detection rate of deleterious mutations from 8.6% to 15.6% compared with a conventional gene-by-gene approach. Furthermore, they reported that there were no significant differences in the mutation rates according to race or ethnic groups.(6) We identified 35 patients (8.0%) who had non-*BRCA1/2* deleterious mutations. These mutations included *CDH1* in 7 patients (1.6%), *RAD51* in 7 patients (1.6%), *SPINK1* in 5 patients (1.1%), and *TP53* in 4 patients (0.9%). The remaining patients had deleterious mutations in *CHEK2* (0.5%), *FANCA* (0.5%), *MLH1* (0.5%), *NBN* (0.5%), *BRIP1* (0.2%), *MRE11A* (0.2%), *MSH2* (0.2%), and *MUTYH* (0.2%). These results show that multiple-gene panel testing helps to increase the mutation detection rate compared to the conventional *BRCA* test alone. The results of previous studies and of our study are compatible with the suggestion of NCCN guidelines that multiple-gene testing may be more efficient and cost-effective for cancer risk assessment for patients with a high probability of hereditary cancer syndrome.(13)

Germline *CDH1* mutations among the most frequently detected deleterious non-*BRCA1/2* mutations in our study. *CDH1* mutation is known to be associated with invasive lobular carcinoma and diffuse gastric cancer.(14, 15) The NCCN guide lines recommend that women with the *CDH1* mutation receive regular breast examinations with annual mammogram and breast MRI, as well as prophylactic total gastrectomy or regular esophagogastroduodenoscopy with multiple random biopsy. . In this study, we found 8 patients with *CDH1* mutation (NM_004360.4:c.2494G>A), 7 patients with *CDH1* mutation only, and 1 patient (HOPE_33) with *CDH1* and another mutations

(*BRCA2* NM_000059.3:c.3195_3198del and *TP53* NM_000546.5:c.566C>T). Patient HOPE_33 carried *BRCA2*, *CDH1*, and *TP53* mutations; she developed bilateral breast cancer at 33 years of age. Two patients with only the *CDH1* mutation had a family history of breast cancer in 2 or more first- or second-degree relatives. Five patients with only the *CDH1* mutation had breast cancer with another primary cancer including leukemia (HOPE_23), stomach cancer (HOPE_28), colon cancer (HOPE_222), cervical cancer (HOPE_192), and thyroid cancer (HOPE_319). Patients with the *CDH1* mutation should have been recommended to receive close surveillance for contralateral breast cancer and stomach cancer. Further, family members of patient HOPE_28, who already had stomach cancer, should undergo genetic testing and receive close surveillance for breast and stomach cancers.

The *RAD51* gene has a key role in the repair of DNA double-strand breaks through homologous recombination.(16) Germline mutation of *RAD51* is known to cause congenital mirror movement which is characterized by involuntary movements of 1 side of the body that mirror intentional movements on the opposite side.(17) In addition to this congenital neurologic disorder, *RAD51* mutation is associated with the development of malignancy, in including breast cancer, pancreatic cancer, non-small cell lung cancer, and prostate cancer.(18-21) A previous study found that the proteins BRCA2 and PALB2 control the function of RAD51, yielding structural change for cancer susceptibility.(22, 23)). In this study, we found 8 patients with *RAD51* NM_002875.4:c.449G>A. All patients with *RAD51* mutation were *BRCA1/2* negative. Three patients (HOPE_24, 35, and 335) had breast cancer and another primary cancer, including lung cancer, brain tumor, and thyroid cancer. Another 3 patients (HOPE_231, 324, and 418) had a family

history of breast cancer in 2 or more relatives. One patient (HOPE_266) had breast cancer at an age younger than 40 years.

Germline mutation of *SPINK1* has been associated with hereditary pancreatitis by inhibiting the function of SPINK1 protein and causing cellular damage by activated trypsin.(24) Several studies found that the *SPINK1* mutation was associated with pancreatic cancer.(25, 26) In our study, 6 patients carried the deleterious *SPINK1* mutations (NM_003122.4:c.101A>G). One patient (HOPE_14) had both breast cancer and pancreatic cancer and found to carry both *SPINK1* and *BRCA2* mutations (NM_000059.3:c.4092_4093del). Another 3 patients had breast cancer and an additional primary cancer, including stomach cancer, cervical cancer, and common bile duct cancer. The remaining 2 patients had a family history of breast cancer. However, *SPINK1* mutation is not rare despite of deleterious mutation. According to 1000 Genome Project Phase 3, allele frequency of this mutation is 0.003 in American, 0.008 in East Asian and 0.014 in South Asian. Because the allele frequency is relatively high in Asian population, this mutation is thought to have low penetrance in Asian population. Patient HOPE_14 who carried *SPINK1* and *BRCA2* mutations developed breast cancer in 2002 and pancreatic cancer in 2005. Considering that the *BRCA2* mutation is also known for increasing risk of pancreatic cancer and high minor allele frequency of *SPINK1* mutation (NM_003122.4:c.101A>G), the main cause of breast and pancreatic cancer in patients HOPE_14 was *BRCA2* mutation, not *SPINK1* mutation.(27)

Germline mutation of *TP53* is known as Li-Fraumeni syndrome. This mutation is associated with multiple cancers including breast cancer, soft tissue sarcoma, acute leukemia, brain tumor, adrenal carcinoma, and colon cancer. For this reason, Li-

Fraumeni syndrome patients have a poor prognosis. Once this syndrome was revealed to be associated with a germline mutation of *TP53*, it became possible to detect carriers of inherited *TP53* mutations. Currently, individuals with a *TP53* mutation are recommended to undergo targeted surveillance, depending on individual medical history and family history. Villani et al. reported that individuals with *TP53* mutation who received intensive surveillance with colonoscopy, whole body MRI, breast MRI, brain MRI, skin examination, and physical examination showed improved overall survival compared with individuals who did not receive surveillance (P=0.013).(28) This result supports the effectiveness of a tailored surveillance program for increasing survival rates and is beneficial to individuals with deleterious mutations. . In our study, 5 patients were identified to carry *TP53* mutations (NM_000546.5:c.566C>T, NM_000546.5:c.638G>A, and NM_000546.5:c.743G>A). As mentioned, HOPE_33 carried *BRCA2, CDH1* and *TP53* mutations and had bilateral breast cancer at a young age. HOPE_ 395 and 396 carried *TP53* mutation (NM_000546.5:c.566C>T) and suffered from breast cancer and thyroid cancer and had at least 2 relatives with a history of breast cancer. HOPE_290 had *TP53* mutation (NM_000546.5:c.638G>A) and a family history of breast cancer in at least 2 relatives. HOPE_115 had *TP53* mutation (NM_000546.5:c.743G>A) and had breast cancer and lung cancer. Family members of patients with *TP53* mutation need to undergo genetic testing to find out whether they are carriers of the *TP53* mutation or not. Depending on the results of genetic testing, *TP53* mutation carriers, as well as patients with *TP53* mutation, should consider clinical intensive surveillance for early detection of cancer and improved long-term survival.

Surveillance and risk-reducing strategies for patients with germline mutations of

Lynch syndrome (*MLH1, MSH2, MSH6, PMS2,* and *EPCAM2)*, such as colonoscopy, prophylactic hysterectomy, and bilateral salphingo-oophorectomy should be considered. In our study, 3 patients were found to have deleterious mutations in *MLH1* and *MSH2* (NM_000249.3:c.1758dup, NC_000003.11:c.849T>A, and NM_000251.2:c.229_230del). Patient HOPE_315 who carried *MLH1* mutation (NM_000249.3:c.1758dup) had primary breast, colon cancer and lung cancer. Patient HOPE_394 who had *MSH2* mutations (NM_000251.2:c.229_230del) had primary breast and colon cancers. Patient HOPE_378 who carried novel deleterious mutation in *MLH1* (NM_000249.3:c.849T>A) had breast cancer and hepatocellular carcinoma. Because Lynch syndrome is an inherited disorders that increases the risk of various type of cancer, particularly in colorectum, endometrium, ovary, stomach, small bowel, liver, bile duct, upper urinary tract, and brain, these 3 patients' family members should have intensive surveillance for colon cancer and genetic testing for germline mutations of Lynch syndrome.(29)

Among included 496 patients, 6 patients had ≥2 deleterious mutations (HOPE_14, 33, 57, 309, 421, and 502). Patient HOPE_14 who carried *BRCA2* and *SPINK1* mutations (NM_000059.3:c.4092_4093del and NM_003122.4:c.101A>G, respectively) developed primary breast and pancreatic cancer when she was 66 years old and 69 years old. Patient HOPE_33 who carried *BRCA2*, *CDH1*, and *TP53* mutations (NM_000059.3:c.3195_3198del, NM_004360.4:c.2494G>A, and NM_000546.5:c.566C>T, respectively) and patient HOPE_421 who carried *BRCA1* and *NBN* mutations (NM_007294.3:c.3627dup and NM_002485.4:c.511A>G, respectively) developed bilateral breast cancer when she was 33 and 42 years old, respectively. Patient HOPE_57 who carried both *BRCA1* and *BRCA2* (NM_007294.3:c.3991C>T and

NM_000059.3:c.1399A>T, respectively) and patient HOPE_309 who carried two *BRCA1* mutations (NM_007294.3:c.3627dup and NM_007294.3:c.1511dup, respectively) had breast and ovarian cancer in her age of 52 and 47, respectively. Patient HOPE_502 who carried 2 *BRCA1* mutations (NM_007294.3:c.3627dup and NM_007294.3:c.1511dup) developed breast and thyroid cancer at 36 years old. Among 6 patients who carried ≥2 deleterious mutations, 3 patients (50.0%) had 2 or more clinical risk factors for HBOC, which showed higher proportion compared to patients who carried 1 or none deleterious mutation.

We detected 2 novel deleterious mutations that have not been previously reported: NM_000059.3:c.3096_3111del (p.Lys1032Asnfs*6) in *BRCA2* and NM_000249.3:c.849T>A (p.Tyr283*) in *MLH1*. The p.Lys1032Asnfs*6 mutation in *BRCA2* was identified in patient HOPE_468. This mutation encodes a truncated non-functional protein in the domain of the BRC repeats (Figure 4A). The human tumor suppressor protein BRCA2 plays a key role in recombinant DNA repair. BRCA2 recruits RAD51 to sites of DNA damage through interaction with 8 conserved motifs of approximately 35 amino acids, the BRC repeats, although the specific function of each repeat remains unclear (30). The mutation of *BRCA2* p.Lys1032Asnfs*6 is thought to interfere with cellular response to DNA damage, resulting in malignant transformations. The p.Tyr283* mutation in *MLH1* is found in patient HOPE_378 and is also predicted to encode a non-functional protein, leading to the disruption of important functional domain like MutL C-terminal domain (Figure 4B). The subunits of MLH1 and PMS2 make the MutLα complex, which plays an essential role in mismatch repair.(31) A defect in *MLH1* is associated with mismatch repair and results in microsatellite instability and

spontaneous mutation rate. The family history of patients with novel deleterious mutations and genetic tests of family members are required to determine the clinical impact of these newly identified mutations.

It is well known that *BRCA1* and *BRCA2* mutation carriers showed differences in tumor histopathology. A large proportion of breast cancer in women who carry a *BRCA1* mutation exhibited a triple-negative breast cancer. Previous study including Korean familial breast cancer patients reported that triple-negative breast cancer was diagnosed in 57.1% of *BRCA1* mutation carriers.(32) In contrast with *BRCA1* mutations, luminal A and luminal B subtype of breast cancer was found in 83.0% in breast cancer patients who carried *BRCA2* mutations.(33) Our results showed that 75.0% of *BRCA1* mutation carriers were triple-negative breast cancer and 69.5% of *BRCA2* mutation carriers were HR-positive breast cancer including luminal A and B subtype, which are concordant with previous studies.

The proportions and distributions of deleterious mutations in *BRCA1/2* negative patients were quite different in this Asian population than in a previously reported Western population. Maxwell et al. reported that Caucasian and African American breast cancer patients who were *BRCA1/2* negative and had early-onset breast cancer (< 40 years old at diagnosis) carried 11% of non-*BRCA1/2* deleterious mutations.(34) The deleterious mutations were *ATM* (25.8%), *CHEK2* (32.3%), *TP53* (12.9%), and *MRE11A* (6.5%). The remaining mutations were *MSH6, CDKN2A, MUTYH, BARD1, BRIP1, NBN*, and *RAD50* (3.2%). The majority of deleterious mutations in our study in *BRCA1/2*-negative and early-onset breast cancer patients were *NBN* (30.0%), *RAD51* (20.0%), and *CHEK2* (20.0%). Recently, Li et al. reported the results of germline mutations among

Chinese patients with features of hereditary breast cancer.(35) They found that 16.9% of included patients carried *BRCA1/2* mutations and 6.8% of patients had non-*BRCA1/2* mutations including *TP53*, *PALB2*, *CHEK2*, *ATM*, *BARD1*, *BRIP*, *CDH1* and *RAD50*. Recent studies reported that mutations in *PALB2* and *RAD51C* were found to be an important cause of HBOC.(36, 37) Additionally, *CDH1* mutations were not found in the Western study but detected in the Chinese study. Although we did not find the *ATM, PALB2* and *RAD51C* mutations in any of our study patients, we should have caution to interpret sequencing results of these important genes for HBOC.

In this study, 67 patients (13.5%) were shown to have 20 VUS in 18 genes (Table 5). Compared with other studies, the rate of VUS in this study was relatively low. This could be because we excluded most of the missense mutations with conflicting interpretations and considered benign or likely-benign. We only considered mutations as VUS when mutations had conflicting interpretation of pathogenicity but a suspicion of being deleterious. Most of the VUS will be re-categorized as benign or deleterious. Until the significance is fully understood, VUS should not be used for making clinical decisions. It is also important to reduce the number of VUS in clinical practice. Potential deleterious mutations can be selected by mutation frequency analysis and *in silico* analysis. Lin et al. performed structural analysis to view whether the mutation affected the protein structure.(38) Recently, Findlay et al. used saturation genome editing to assay single-nucleotide variants in exons that encode functional domains of *BRCA1*.(39) They found that functional effects of saturation genome editing were almost perfectly concordant with established assessments of pathogenicity. The saturation genome editing will be useful for accurate classification of VUS in clinically actionable genes.

The application of multi-gene panel testing has been rapidly increasing in clinical practice, especially in the evaluation of germline mutations which are associated with cancer susceptibility. The identification of deleterious mutations in cancer susceptibility genes in individuals with a high risk for hereditary cancer can improve the effectiveness of personalized surveillance, leading to early detection or prophylactic treatment of hereditary cancer in both individuals and their family members. Intensive surveillance for early detection and prophylactic treatment are directly linked with better survival in patients with deleterious mutations.

However, there are limitations to multi-gene panel testing. The prevalence of pathogenic mutations and VUS vary across races and ethnicities. Furthermore, the penetrance and phenotype of mutations are different among individuals. Detection of a deleterious mutation does not always mean an individual will develop cancer, and conversely, a negative result from a multi-gene panel test does not mean an individual has no risk of getting cancer. Another limitation is the attitude and knowledge gaps of physicians who provide care for individuals who undergo genetic testing for a disease. One survey reported that, although most physicians received formal genetic education and agreed that genetic tests are clinically useful for assessing disease risk, they were not confident about interpreting test results and were not prepared for managing individuals at high risk for genetic disease.(40) For these reasons, genetic education and genetic counseling, as well as the appropriate and accurate interpretation of results, are important for the effective clinical application of risk management strategies. Stadler et al. proposed that the results of germline genetic testing using multi-gene panels, including cancer-related findings and other incidental findings, should be integrated with

traditional risk assessments, such as personal and family histories, to establish cancer and non-cancer risk management and follow-up plans.(41) The paradigm shift toward personalized and precision medicine requires the incorporation of NGS technologies into clinical practice.

To the best of our knowledge, this is the largest study to include Korean breast cancer patients with clinical features of HBOC and examine the frequency and characteristics of germline mutations in *BRCA1/2* and non-*BRCA1/2* cancer-susceptibility genes.

We analyzed germline mutations from 496 breast cancer patients of Asian ethnicity with clinical features of HBOC using NGS-based multi-gene panel testing. Overall, 95 patients (19.2%) were found to carry 48 deleterious germline mutations in 16 cancer-susceptibility genes. Of these 95 patients, 60 patients (63.2%) had *BRCA1/2* mutations, 38 patients (40.0%) had non-*BRCA1/2* mutations and 3 patients (3.2%) had both *BRCA1/2* and non-*BRCA1/2* mutations. The NGS-based multi-gene panel test improved the detection rates of deleterious mutations and provided a cost-effective cancer risk assessment compared with a gene-by-gene approach.

Authors Contribution

All the authors have made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data. Hee-Chul Shin wrote the manuscript. Hee-Chul Shin, Eun-Shin Lee and Ryong Nam Kim carried out analysis of the variants of each patient. Han-Byoel Lee, Tae-Kyoung Yoo, Boyoung Park, Kyong-Ah Yoon, and Charny Park participated in the collecting patients' samples and medical records for this research. Eun Sook Lee, Hyeong-Gon Moon, and Dong-Young Noh participated in the study design and helped to draft the manuscript. Dong-Young Noh advised to interpret variants and find clinical meaning of detected variants. Wonshik Han performed *in silico* analysis for novel deleterious mutations and helped to make Figure 4A and 4B. Sun-Young Kong, Dong-Young Noh and Wonshik Han designed and coordinated the research. The authors have been involved in drafting the manuscript or revising it for intellectual content.

References

1. Pal T, Permuth-Wey J, Betts JA, Krischer JP, Fiorica J, Arango H, et al. BRCA1 and BRCA2 mutations account for a large proportion of ovarian carcinoma cases. Cancer. 2005;104(12):2807-16.

2. Zhang S, Royer R, Li S, McLaughlin JR, Rosen B, Risch HA, et al. Frequencies of BRCA1 and BRCA2 mutations among 1,342 unselected patients with invasive ovarian cancer. Gynecol Oncol. 2011;121(2):353-7.

3. Kuchenbaecker KB, Hopper JL, Barnes DR, Phillips KA, Mooij TM, Roos-Blom MJ, et al. Risks of Breast, Ovarian, and Contralateral Breast Cancer for BRCA1 and BRCA2 Mutation Carriers. JAMA. 2017;317(23):2402-16.

4. Mai PL, Khincha PP, Loud JT, DeCastro RM, Bremer RC, Peters JA, et al. Prevalence of Cancer at Baseline Screening in the National Cancer Institute Li-Fraumeni Syndrome Cohort. JAMA Oncol. 2017;3(12):1640-5.

5. Castera L, Krieger S, Rousselin A, Legros A, Baumann JJ, Bruet O, et al. Next-generation sequencing for the diagnosis of hereditary breast and ovarian cancer using genomic capture targeting multiple candidate genes. Eur J Hum Genet. 2014;22(11):1305-13.

6. Ricker C, Culver JO, Lowstuter K, Sturgeon D, Sturgeon JD, Chanock CR, et al. Increased yield of actionable mutations using multi-gene panels to assess hereditary cancer susceptibility in an ethnically diverse clinical cohort. Cancer Genet. 2016;209(4):130-7.

7. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and

Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-24.

8. NCCN Clinical Practice Guidelines in Oncology: NCCN; 2018 [Available from: https://www.nccn.org/].

9. Hall MJ, Reid JE, Burbidge LA, Pruss D, Deffenbaugh AM, Frye C, et al. BRCA1 and BRCA2 mutations in women of different ethnicities undergoing testing for hereditary breast-ovarian cancer. Cancer. 2009;115(10):2222-33.

10. Kim H, Cho DY, Choi DH, Oh M, Shin I, Park W, et al. Frequency of pathogenic germline mutation in CHEK2, PALB2, MRE11, and RAD50 in patients at high risk for hereditary breast cancer. Breast Cancer Res Treat. 2017;161(1):95-102.

11. Thompson ER, Rowley SM, Li N, McInerny S, Devereux L, Wong-Brown MW, et al. Panel Testing for Familial Breast Cancer: Calibrating the Tension Between Research and Clinical Care. J Clin Oncol. 2016;34(13):1455-9.

12. Li J, Meeks H, Feng BJ, Healey S, Thorne H, Makunin I, et al. Targeted massively parallel sequencing of a panel of putative breast cancer susceptibility genes in a large cohort of multiple-case breast and ovarian cancer families. J Med Genet. 2016;53(1):34-42.

13. Hall MJ, Forman AD, Pilarski R, Wiesner G, Giri VN. Gene panel testing for inherited cancer risk. J Natl Compr Canc Netw. 2014;12(9):1339-46.

14. Fitzgerald RC, Hardwick R, Huntsman D, Carneiro F, Guilford P, Blair V, et al. Hereditary diffuse gastric cancer: updated consensus guidelines for clinical management and directions for future research. J Med Genet.

2010;47(7):436-44.

15. Masciari S, Larsson N, Senz J, Boyd N, Kaurah P, Kandel MJ, et al. Germline E-cadherin mutations in familial lobular breast cancer. J Med Genet. 2007;44(11):726-31.

16. Cousineau I, Abaji C, Belmaaza A. BRCA1 regulates RAD51 function in response to DNA damage and suppresses spontaneous sister chromatid replication slippage: implications for sister chromatid cohesion, genome stability, and carcinogenesis. Cancer Res. 2005;65(24):11384-91.

17. Depienne C, Bouteiller D, Meneret A, Billot S, Groppa S, Klebe S, et al. RAD51 haploinsufficiency causes congenital mirror movements in humans. Am J Hum Genet. 2012;90(2):301-7.

18. Martin RW, Orelli BJ, Yamazoe M, Minn AJ, Takeda S, Bishop DK. RAD51 up-regulation bypasses BRCA1 function and is a common feature of BRCA1-deficient breast tumors. Cancer Res. 2007;67(20):9658-65.

19. Maacke H, Jost K, Opitz S, Miska S, Yuan Y, Hasselbach L, et al. DNA repair and recombination factor Rad51 is over-expressed in human pancreatic adenocarcinoma. Oncogene. 2000;19(23):2791-5.

20. Mitra A, Jameson C, Barbachano Y, Sanchez L, Kote-Jarai Z, Peock S, et al. Overexpression of RAD51 occurs in aggressive prostatic cancer. Histopathology. 2009;55(6):696-704.

21. Qiao GB, Wu YL, Yang XN, Zhong WZ, Xie D, Guan XY, et al. High-level expression of Rad51 is an independent prognostic marker of survival in non-small-cell lung cancer patients. Br J Cancer. 2005;93(1):137-43.

22. Pellegrini L, Yu DS, Lo T, Anand S, Lee M, Blundell TL, et al. Insights into DNA recombination from the structure of a RAD51-BRCA2 complex.

Nature. 2002;420(6913):287-93.

23. Buisson R, Dion-Cote AM, Coulombe Y, Launay H, Cai H, Stasiak AZ, et al. Cooperation of breast cancer proteins PALB2 and piccolo BRCA2 in stimulating homologous recombination. Nat Struct Mol Biol. 2010;17(10):1247-54.

24. Witt H, Luck W, Hennies HC, Classen M, Kage A, Lass U, et al. Mutations in the gene encoding the serine protease inhibitor, Kazal type 1 are associated with chronic pancreatitis. Nat Genet. 2000;25(2):213-6.

25. Midha S, Sreenivas V, Kabra M, Chattopadhyay TK, Joshi YK, Garg PK. Genetically Determined Chronic Pancreatitis but not Alcoholic Pancreatitis Is a Strong Risk Factor for Pancreatic Cancer. Pancreas. 2016;45(10):1478-84.

26. Masamune A, Mizutamari H, Kume K, Asakura T, Satoh K, Shimosegawa T. Hereditary pancreatitis as the premalignant disease: a Japanese case of pancreatic cancer involving the SPINK1 gene mutation N34S. Pancreas. 2004;28(3):305-10.

27. Zhen DB, Rabe KG, Gallinger S, Syngal S, Schwartz AG, Goggins MG, et al. BRCA1, BRCA2, PALB2, and CDKN2A mutations in familial pancreatic cancer: a PACGENE study. Genet Med. 2015;17(7):569-77.

28. Villani A, Shore A, Wasserman JD, Stephens D, Kim RH, Druker H, et al. Biochemical and imaging surveillance in germline TP53 mutation carriers with Li-Fraumeni syndrome: 11 year follow-up of a prospective observational study. Lancet Oncol. 2016;17(9):1295-305.

29. Lynch HT, Lynch JF. What the physician needs to know about Lynch syndrome: an update. Oncology (Williston Park). 2005;19(4):455-63;

discussion 63-4, 66, 69.

30. Carreira A, Kowalczykowski SC. Two classes of BRC repeats in BRCA2 promote RAD51 nucleoprotein filament function by distinct mechanisms. Proc Natl Acad Sci U S A. 2011;108(26):10448-53.

31. Kadyrov FA, Dzantiev L, Constantin N, Modrich P. Endonucleolytic function of MutLalpha in human mismatch repair. Cell. 2006;126(2):297-308.

32. Seong MW, Kim KH, Chung IY, Kang E, Lee JW, Park SK, et al. A multi-institutional study on the association between BRCA1/BRCA2 mutational status and triple-negative breast cancer in familial breast cancer patients. Breast Cancer Res Treat. 2014;146(1):63-9.

33. Krammer J, Pinker-Domenig K, Robson ME, Gonen M, Bernard-Davila B, Morris EA, et al. Breast cancer detection and tumor characteristics in BRCA1 and BRCA2 mutation carriers. Breast Cancer Res Treat. 2017;163(3):565-71.

34. Maxwell KN, Wubbenhorst B, D'Andrea K, Garman B, Long JM, Powers J, et al. Prevalence of mutations in a panel of breast cancer susceptibility genes in BRCA1/2-negative patients with early-onset breast cancer. Genet Med. 2015;17(8):630-8.

35. Li JY, Jing R, Wei H, Wang M, Xiaowei Q, Liu H, et al. Germline mutations in 40 cancer susceptibility genes among Chinese patients with high hereditary risk breast cancer. Int J Cancer. 2019;144(2):281-9.

36. Antoniou AC, Casadei S, Heikkinen T, Barrowdale D, Pylkas K, Roberts J, et al. Breast-cancer risk in families with mutations in PALB2. N Engl J Med. 2014;371(6):497-506.

37. Li N, McInerny S, Zethoven M, Cheasley D, Lim BWX, Rowley SM, et al. Combined tumor sequencing and case/control analyses of RAD51C in breast cancer. J Natl Cancer Inst. 2019.

38. Lin PH, Kuo WH, Huang AC, Lu YS, Lin CH, Kuo SH, et al. Multiple gene sequencing for risk assessment in patients with early-onset or familial breast cancer. Oncotarget. 2016;7(7):8310-20.

39. Findlay GM, Daza RM, Martin B, Zhang MD, Leith AP, Gasperini M, et al. Accurate classification of BRCA1 variants with saturation genome editing. Nature. 2018;562(7726):217-22.

40. Hauser D, Obeng AO, Fei K, Ramos MA, Horowitz CR. Views Of Primary Care Providers On Testing Patients For Genetic Risks For Common Chronic Diseases. Health Aff (Millwood). 2018;37(5):793-800.

41. Stadler ZK, Schrader KA, Vijai J, Robson ME, Offit K. Cancer genomics and inherited risk. J Clin Oncol. 2014;32(7):687-98.

국문초록

유전성 암 증후군의 임상적 특징을 갖는 한국인 유방암 환자에서 다중 유전자 패널 검사를 통한 유전성 암 유전자 변이 확인

서울대학교 대학원

의학과 외과학 전공

신희철

연구목적: 유전성 암 증후군은 생식세포의 병적인 돌연변이를 통해 개인의 특정 암 발생의 위험도가 높아지는 것을 의미한다. 현재 유방암에서의 *BRCA1/2* 유전자 돌연변이가 가장 많이 알려진 유전성 암 유전자 중 하나이다. 우리는 64개의 유전성 암 유전자를 포함한 다중 유전자 패널을 차세대 염기 서열 분석 방식을 통해 개발하였다. 이 다중 유전자 패널 검사를 통해 임상적으로 유전성 암 증후군의 특징을 가지고 있는 한국인 유방암 환자를 대상으로 하여 유전성 암과 관련된 유전자의 돌연변이 빈도를 분석하여, 다중 유전자 패널의 유용성을 알아보고자 한다.

대상 및 방법: 64개의 유전성 암과 관련된 유전자 목록을 문헌 검색을 통해 선택 후 차세대 염기 서열 분석 방식을 기반으로 한 다중 유전자 패널을 개발하였다. 2002년부터 2017년까지 서울대학교병원과 국립암센터에서 진단된 유방암 환자 중 임상적인 유전성 암 증후군의 특징을 가진 496명의 환자를 대상으로 유전자 분석을 시행하여

생식세포 돌연변이 여부를 확인하였다. 임상적인 유전성 암 증후군은 1) 유방암 이외 다른 원발성 암이 발생한 경우, 2) 2명이상의 유방암 가족력이 있는 경우, 3) 양측성 유방암이 발생하나 경우, 4) 40세 이하에서 유방암이 발생한 경우 중 하나 이상 포함되는 경우로 정의하였다.

결과: 총 496 명의 환자 중 95 명(19.2%)의 환자에서 48 개의 암의 발생 가능성이 매우 높은 유전자 돌연변이(deleterious mutation)가 발견되었다. 유방암과 또다른 원발성암이 발생한 250 명 중 39 명(15.6%), 2 명이상의 유방암 가족력이 있는 169 명 중 38 명(22.5%), 양측성 유방암이 진단된 57 명 중 16 명(28.1%), 40 세 이하에서 유방암이 진단된 84 명 중 29 명(34.5%)에서 deleterious mutation 이 발견되었다. Deleterious mutation 이 발견된 95 명 중 60 명(63.2%)는 *BRCA1/2* 유전자 돌연변이가 있었다. 38 명(40.0%)은 *BRCA1/2* 유전자 이외의 유전성 암 유전자의 돌연변이가 있는 것으로 나타났으며 *CDH1* (8.4%), *RAD51* (7.4%), *SPINK1* (6.3%), *TP53* (5.3%), *NBN* (3.2%), *CHEK2* (2.1%), *FANCA* (2.1%), *MLH1* (2.1%), *BRIP1* (1.1%), *MRE11A* (1.1%), *MSH2* (1.1%), *MUTYH* (1.1%)등의 유전자 돌연변이가 관찰되었다. 3 명에서는 *BRCA1/2* 유전자 변이와 *BRCA1/2* 이외의 유전자 변이가 동시에 있었다. 이 외에 67 명(13.5%)의 환자에서 deleterious mutation 의 가능성이 있을 것으로 보이는 Variant of Unknown Significance(VUS)가 발견되었다. 또한 이번 연구를 통해 현재까지 보고되지 않은 새로운 2 개의 deleterious mutation 을 발견하였으며, *BRCA2* 유전자에서 발생한 NM_000059.3:c.3096_3111del

(p.Lys1032Asnfs*6) 변이, *MLH1* 유전자에서 발생한 NM_000249.3:c.849T>A (p.Tyr283*) 변이이다.

결론: 차세대 염기 서열 분석 방식을 이용한 다중 유전자 패널 검사를 통해 유전성 암 증후군의 임상적 특징을 보이는 유방암 환자에서 생식세포 돌연변이 중 deleterious mutation이 19.2%에서 있음을 확인하였다. Deleterious mutation 중 약 2/3 가량은 *BRCA1/2* 변이였으며 1/3 가량은 *BRCA1/2* 이외의 다른 유전자 변이가 있었다. 이를 통해 다중 유전자 패널 검사를 시행할 경우 단일 유전자 검사에 비해 임상적으로 의미가 있는 deleterious mutation을 보다 많이 효율적으로 찾아낼 수 있음을 확인하였다. 이를 통해 유전성 암 증후군의 맞춤 진단 및 치료를 시행하는데 도움이 될 것으로 보인다.

주요어 : 유방암, 유전성 암 증후군, 유전성 암 유전자, 차세대 염기 서열 분석, 다중 유전자 패널 검사

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