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Original Article

Genomic Signatures from Clinical Tumor Sequencing in Patients with Breast Cancer Having Germline *BRCA1/2* Mutation

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Purpose *BRCA1* and *BRCA2* are among the most important genes involved in DNA repair via homologous recombination (HR). Germline *BRCA1/2* (*gBRCA1/2*)-related cancers have specific characteristics and treatment options but conducting *gBRCA1/2* testing and interpreting the genetic imprint are sometimes complicated. Here, we describe the concordance of *gBRCA1/2* derived from a panel of clinical tumor tissues using next-generation sequencing (NGS) and genetic aspects of tumors harboring *gBRCA1/2* pathogenic variants.

Materials and Methods Targeted sequencing was performed using available tumor tissue from patients who underwent *gBRCA1/2* testing. Comparative genomic analysis was performed according to *gBRCA1/2* pathogenicity.

Results A total of 321 patients who underwent *gBRCA1/2* testing were screened, and 26 patients with *gBRCA1/2* pathogenic (*gBRCA1/2p*) variants, eight patients with *gBRCA1/2* variants of uncertain significance (*gBRCA1/2v*), and 43 patients with *gBRCA1/2* wild-type (*gBRCA1/2w*) were included in analysis. Mutations in *TP53* (49.4%) and *PIK3CA* (23.4%) were frequently detected in all samples. The number of single-nucleotide variants per tumor tissue was higher in the *gBRCA1/2w* group than that in the *gBRCA1/2p* group (14.81 vs. 18.86, p=0.278). Tumor mutation burden (TMB) was significantly higher in the *gBRCA1/2w* group than in the *gBRCA1/2p* group (10.21 vs. 13.47, p=0.017). Except for *BRCA1/2*, other HR-related genes were frequently mutated in patients with *gBRCA1/2w*.

Conclusion We demonstrated high sensitivity of *gBRCA1/2* in tumors analyzed by NGS using a panel of tumor tissues. TMB value and aberration of non-*BRCA1/2* HR-related genes differed significantly according to *gBRCA1/2* pathogenicity in patients with breast cancer.

Key words Breast neoplasms, BRCA, High-throughput nucleotide sequencing, Germ-line mutation, Genomic landscape, Tumor mutation burden

Introduction

Advances in precision medicine have maintained pace with the development of genetic profiling technologies. Diseases that were previously classified into only a few types have gradually been subdivided into specific diagnoses, particularly in oncology, leading to an era of personalized treatment. Various therapeutic alternatives to conventional chemotherapy, such as targeted therapy and immunotherapy, have been established and meticulously investigated [1,2].

Next-generation sequencing (NGS) has markedly changed the way breast cancer is identified and treated. In addition to molecular subtyping, physicians should consider numerous genetic factors to combat breast cancer. Testing germline *BRCA1/2* (*gBRCA1/2*) plays a pivotal role in assessing the hereditary risk of breast cancer and in guiding treatment decisions, particularly with the development of poly(ADP-ribose) polymerase (PARP) inhibitors [3,4].

Although *gBRCA1/2* aberration must be confirmed under specific clinical conditions, additional costs and the necessity of blood sampling present practical hurdles to this process. Attempts have been made to infer the result of germline mutations obtained from tissue NGS results, but accuracy remains a concern. In addition, although the clinical and pathological characteristics of *gBRCA*-related breast cancer have been well-acknowledged over several decades [5,6], the genetic aspects of these characteristics have not been fully revealed at the NGS level.

Based on these considerations, this study was conducted to assess the consistency of *gBRCA1/2* aberrations between blood germline testing and tumor tissue sequencing and to characterize the genomic landscape of breast cancer accord-

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ing to *gBRCA1/2* pathogenicity

Materials and Methods

1. Patient selection

After obtaining approval from the Korea University Anam Hospital Institutional Review Board (No. 2017AN0401), we reviewed the charts of patients who were prescribed *gBRCA1/2* testing at Korea University Anam Hospital. The test has been covered by the Korean National Health Insurance since 2012 and was conducted in patients with breast cancer having a family history of breast/ovarian cancer; diagnosed at < 40 years of age; and having bilateral breast cancer, multiple primary cancers, including breast or ovarian malignancy, or male breast cancer. The results of the *gBRCA1/2* test were presented as pathogenic (*gBRCA1/2p*), variants of uncertain significance [VUS; (*gBRCA1/2v*)], and wild-type (*gBRCA1/2w*). Patients who agreed to participate in the K-MASTER project, a Korean National Precision Medicine Cancer Treatment Clinical Trial Platform, were enrolled in the study, and genomic profiles of tumors were collected from all patients in all groups. Detailed information about the K-MASTER project is available in the Supplementary Methods.

2. Germline *BRCA1/2* test

Germline DNA was extracted from peripheral blood using the QIAamp DNA Blood Mini Kit (Qiagen, Chatsworth, CA), according to the manufacturer's instructions. Before April 2018, germline *BRCA1/2* testing based on Sanger sequencing was performed as described previously [7]. In brief, direct sequencing of 73 amplicons covering all exons and flanking introns of *BRCA1* and *BRCA2* was performed on the ABI 3500Dx Genetic Analyser (Applied Biosystems, Foster City, CA) using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). After April 2018, we conducted a multigene NGS panel test that included all exons and flanked intron regions of 31 hereditary cancer susceptibility genes, viz., *ATM, BARD1, BRIP1, CHEK2, NBN, PALB2, RAD50, RAD51C, BRCA1, BRCA2, PTEN, CDH1, TP53, EPCAM, MLH1, MSH2, MSH6, PMS2, MUTYH, APC, MEN1, RET, STK11, RAD51D, TSC1, TSC2, NF1, NF2, SMARCB1, LZTR1*, and *VHL*). NGS was performed on the Illumina MiSeq platform (Illumina Inc., San Diego, CA), generating 2×150-bp paired-end reads. Sequencing reads were aligned using the hg19 reference with BWA (ver. 0.7.10) algorithm. For the post-alignment process, duplicates were removed using Picard (ver. 1.115), and indel realignment and base recalibration were performed using GenomeAnalysisTK-Lite (ver. 2.3.9). Variant calling was performed using GATK HaplotypeCaller ver. 3.8, annotation was performed using ANNOVAR, and conversion to Human Genome Variation Society nomenclature was performed with SnpEff ver. 4.3t. Germline variants were classified according to the five-tier system of the American College of Medical Genetics and Genomics guidelines [8].

3. NGS of tumor tissue

Mutation analysis of solid tumor tissues was performed using targeted NGS. DNA was extracted, purified, and quantified from formalin-fixed, paraffin-embedded breast tumor specimens according to the K-MASTER protocol. Using the K-MASTER panel, which allows the detection of variants of 409 representative genes using the HiSeq sequencing platform, we investigated the mutation profile of the collected tissues. After passing the quality control process, the pipeline demonstrated the results of single-nucleotide variants (SNVs), copy number variants (CNVs), and genomic fusion data from each sample. Detailed laboratory and bioinformatics protocols are available in the Supplementary Methods.

In this study, the average depth of targeted sequencing coverage, duplication rate, on-target rate, pass rate score, and uniformity were 699.16 (141.51-1,202.54), 29.72% (11.8%-84.32%), 94.42% (88.74%-99.14%), 97.97% (25.9%-100%), and 77% (70%-91%), respectively. In terms of quality control, 74 of 77 samples (96.1%) covered a depth of more than 300.

4. Statistical analysis

The Student's t test was used to compare continuous and numerical values between the groups. Normally distributed data are presented as the mean and standard deviation (SD), and deviated data are presented as the median and interquartile range. p-values were calculated using the Student's t test or Mann-Whitney U test, based on Levene's test. The correlation between two factors was assessed using Pearson or Spearman correlation coefficients, according to data distribution. Statistical analyses were performed using IBM SPSS Statistics software ver. 26 (IBM Corp., Armonk, NY) and visualized using R ver. 4.0.0 (R Software, R Foundation for Statistical Computing, Vienna, Austria).

Results

1. Study population

Of the 321 patients who underwent the *gBRCA1/2* testing, 45 patients were categorized as harboring *gBRCA1/2p*, 34 were categorized as harboring *gBRCA1/2v*, and 242 were categorized as harboring *gBRCA1/2w*. After obtaining informed consent from patients and ensuring quality control of the extracted DNA, 26 *gBRCA1/2p* breast cancer tissues, eight

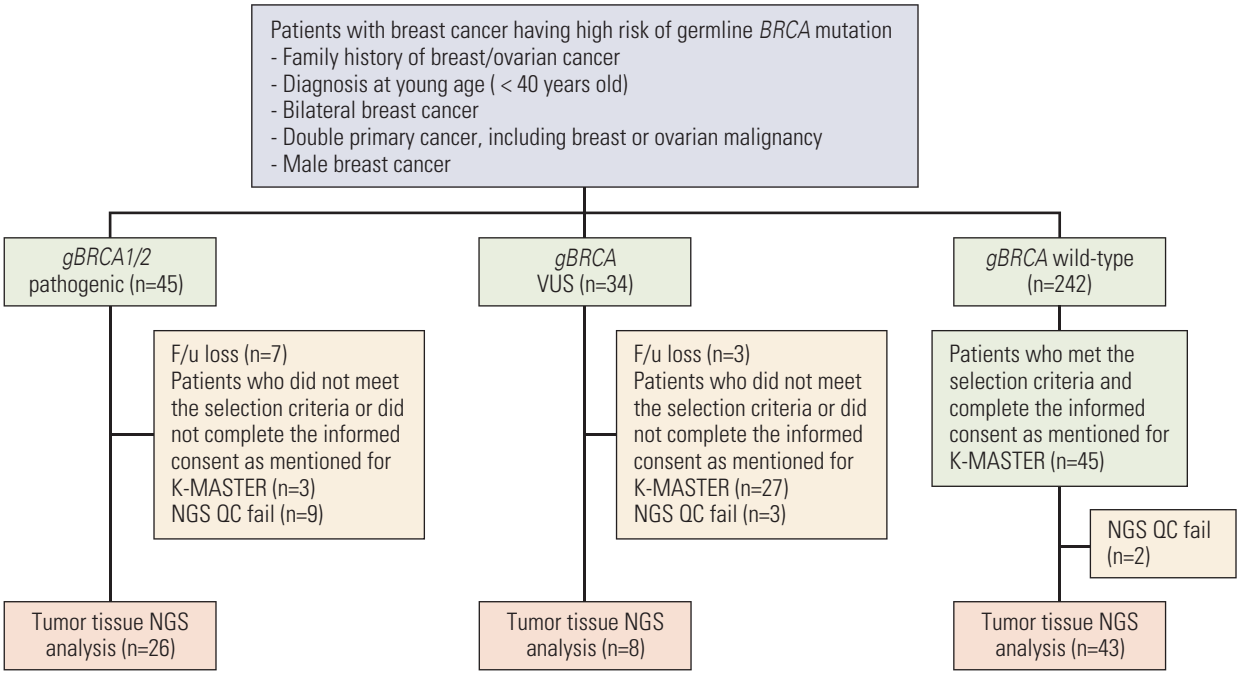

Fig. 1. Schematic flow chart of the study. Among the patients who underwent *gBRCA1/2* testing covered by Korean National Health Insurance, next-generation sequencing (NGS) analysis was conducted with available tumor tissue. After quality control, 26 *gBRCA1/2p* breast cancer tissues, 8 *gBRCA1/2* variants of uncertain significance (VUS) tissues, and 43 *gBRCA1/2w* tissues were analyzed.

Table 1. Sample characteristics

Characteristic	No. of patients (%) (n=77)	*gBRCA* Pathogenic (n=26)	*gBRCA* VUS (n=8)	*gBRCA* Wild-type (n=43)
Age at diagnosis (yr)	42 (27-75)	45.5 (29-74)	34.5 (28-62)	39 (27-75)
Sex				
Female	75 (97.4)	26 (100)	8 (100)	41 (95.3)
Male	2 (2.6)	0	0	2 (4.7)
Stage at diagnosis				
DCIS	2 (2.6)	0	0	2 (4.7)
I	9 (11.7)	6 (23.1)	0	3 (7.0)
II	44 (57.1)	17 (65.4)	5 (62.5)	22 (51.2)
III	12 (15.6)	2 (7.7)	0	10 (23.3)
IV	10 (13.0)	1 (3.8)	3 (37.5)	6 (13.6)
Molecular subtype				
Hormone receptor+	23 (29.9)	10 (38.6)	6 (75.0)	17 (39.5)
HER2+	17 (22.1)	5 (19.2)	2 (25.0)	10 (23.3)
TNBC	27 (35.1)	11 (42.3)	0	16 (37.2)
Family history				
Breast/Ovarian cancer patients in 1st degree relatives	28 (36.4)	14 (53.8)	2 (25.0)	12 (27.9)
Breast/Ovarian cancer in 2nd degree relatives	5 (6.5)	1 (3.8)	1 (12.5)	3 (7.0)
Breast/Ovarian cancer in 3rd degree relatives	2 (2.6)	0	1 (12.5)	1 (2.3)
Any other cancer in 1st, 2nd, or 3rd degree relatives	14 (18.2)	3 (11.5)	0	11 (25.6)
No family history of cancer	28 (36.4)	8 (30.8)	4 (50.0)	16 (37.2)

Values are presented as median (range) or number (%). DCIS, ductal carcinoma *in situ*; HER2, human epidermal growth factor receptor 2; TNBC, triple-negative breast cancer; VUS, variant of unidentified significance.

Table 2. *gBRCA1/2* mutation profile and consistency with tumor NGS assay

	Blood *gBRCA1*				Tissue NGS *BRCA1*					Pathogenicity	Consistency
	REFSEQ	Nucleotide change	AA change	Function	REFSEQ	Nucleotide change	AA change	Function	VAF (%)		
***BRCA1* sample No.**											
S00500	NM_007294	c.154C>T	L52F	Nonsynonymous SNV	NM_007294	c.154C>T	L52F	Nonsynonymous SNV	60.13	VUS	Consistent
S02550	NM_007294	c.154C>T	L52F	Nonsynonymous SNV	NM_007300	c.154C>T	L52F	Nonsynonymous SNV	49.58	VUS	Consistent
S03566	NM_007294	c.154C>T	L52F	Nonsynonymous SNV	NM_007300	c.154C>T	L52F	Nonsynonymous SNV	44.48	VUS	Consistent
S00292	NM_007294	c.390C>A	Y130X	Stopgain	NM_007294	c.390C>A	Y130*	Stopgain SNV	70.83	Pathologic	Consistent
S00564	NM_007294	c.1397G>A	R466Q	Nonsynonymous SNV	NM_007294	c.1397G>A	R466Q	Nonsynonymous SNV	54.43	VUS	Consistent
S04032	NM_007294	c.1716del	E572fs	Frameshift deletion	NM_007294	c.1716delA	E572fs	Frameshift deletion	38.5	Pathologic	Consistent
S04106	NM_007294	c.2048delA	K683fs	Frameshift deletion	NM_007294	c.2048delA	K683fs	Frameshift deletion	40.37	Pathologic	Consistent
S00231	NM_007294	c.2433delC	N810fs	Frameshift deletion	NM_007294	c.2433delC	N810fs	Frameshift deletion	36.73	Pathologic	Consistent
S04059	NM_007294	c.3228_3229delAG	1076_1077del	Frameshift deletion	NM_007294	c.3228_3229del	G1077Afs*8	Frameshift deletion	37.44	Pathologic	Consistent
S04382	NM_007294	c.3448C>T	P1150S	Nonsynonymous SNV	NM_007300	c.3448C>T	P1150S	Nonsynonymous SNV	50.34	VUS	Consistent
S04231	NM_007294	c.4060_4061del	1354_1354del	Frameshift deletion	NM_007294	c.4060_4061del	N1354*	Frameshift deletion	43.99	Pathologic	Consistent
S03086	NM_007294	c.5074+1G>T		Splicing	NM_007300	c.5137+1G>T		Splicing	78.99	Pathologic	Consistent
S01088	NM_007294	c.509G>A	R170Q	Nonsynonymous SNV	NM_007294	c.509G>A	R170Q	Nonsynonymous SNV	36.54	VUS	Consistent
S00275	NM_007294	c.520C>T	Q174*	Stopgain SNV	NM_007294	c.520C>T	Q174*	Stopgain SNV	82.3	Pathologic	Consistent
S04083	NM_007294	c.5339T>C	L1801P	Nonsynonymous SNV	NM_007300	c.5402T>C	L1801P	Nonsynonymous SNV	58.43	Pathologic	Consistent
S03871	NM_007294	c.5444G>A	W1836*	Stopgain SNV	NM_007300	c.5508G>A	W1836*	Stopgain SNV	70.84	Pathologic	Consistent
S04178	NM_007294	c.5445G>A	W1836*	Stopgain SNV	NM_007300	c.5508G>A	W1836*	Stopgain SNV	65.06	Pathologic	Consistent
S03838	NM_007294	c.5467+1G>A		Splicing	NM_007300	c.5530+1G>A		Splicing	61.34	Pathologic	Consistent
S01005	NM_007294	c.5497_5506del	V1833fs	Frameshift deletion	NM_007294	c.5493_5502del	V1832Efs*8	Frameshift deletion	60.31	Pathologic	Inconsistent

(Continued to the next page)

Table 2. Continued

	Blood *gBRCA1*				Tissue NGS *BRCA1*					Pathogenicity	Consistency
	REFSEQ	Nucleotide change	AA change	Function	REFSEQ	Nucleotide change	AA change	Function	VAF (%)		
***BRCA2* sample No.**											
S00601	NM_000059	c.1399A>T	K467*	Stopgain SNV	NM_000059	c.1399A>T	K467*	Stopgain SNV	43.72	Pathologic	Consistent
S03840	NM_000059	c.1399A>T	K467*	Stopgain SNV	NM_000059	c.1399A>T	K467*	Stopgain SNV	73.55	Pathologic	Consistent
S02371	NM_000059	c.1399A>T	K467*	Stopgain SNV	NM_000059	c.1399A>T	K467*	Stopgain SNV	51.11	Pathologic	Consistent
S03566	NM_000059	c.1399A>T	K467*	Stopgain SNV	NM_000059	c.1399A>T	K467*	Stopgain SNV	71.71	Pathologic	Consistent
S00374	NM_000059	c.2435delA	K811fs	Frameshift deletion	NM_000059	c.2431delA	K811fs	Frameshift deletion	30.93	Pathologic	Consistent
S02987	NM_000059	c.2808_2811del	A938fs	Frameshift deletion	NM_000059	c.2808_2811del	A938fs	Frameshift deletion	46.82	Pathologic	Consistent
S03938	NM_000059	c.353G>A	R118H	Nonsynonymous SNV	NM_000059	c.353G>A	R118H	Nonsynonymous SNV	46.99	Pathologic	Consistent
S03896	NM_000059	c.3744_3747del	S1248fs	Frameshift deletion	NM_000059	c.3744_3747del	S1248fs	Frameshift deletion	60.52	Pathologic	Consistent
S03987	NM_000059	c.5656C>T	Q1886*	Stopgain SNV	NM_000059	c.5656C>T	Q1886*	Stopgain SNV	50	Pathologic	Consistent
S00484	NM_000059	c.6029T>G	V2010G	Nonsynonymous SNV	NM_000059	c.6029T>G	V2010G	Nonsynonymous SNV	49.12	VUS	Consistent
S00215	NM_000059	c.6486_6489delACAA	K2162fs	Frameshift deletion	NM_000059	c.6486_6489delACAA	K2162fs	Frameshift deletion	72.18	Pathologic	Consistent
S04259	NM_000059	c.6875A>C	E2292A	Nonsynonymous SNV	NM_000059	c.8633_8648TATTATATT		Frameshift substitution	8.37	VUS	Inconsistent
S04084	NM_000059	c.7258G>T	E2420*	Stopgain SNV	NM_000059	c.7258G>T	E2420*	Stopgain SNV	67.92	Pathologic	Consistent
S04378	NM_000059	c.7480C>T	R2494*	Stopgain SNV	NM_000059	c.7480C>T	R2494*	Stopgain SNV	58.44	Pathologic	Consistent
S04288	NM_000059	c.7706G>T	G2569V	Nonsynonymous SNV	NM_000059	c.7706G>T	G2569V	Nonsynonymous SNV	70.72	VUS	Consistent
S03872	NM_000059	c.8023A>G	I2675V	Nonsynonymous SNV	NM_000059	c.8023A>G	I2675V	Nonsynonymous SNV	47.99	Pathologic	Consistent

AA, amino acid; NGS, next-generation sequencing; REFSEQ, reference sequence; SNV, single-nucleotide variant; VAF, variant allele frequency; VUS, variant of unknown significance.

gBRCA1/2 VUS tissues, and 43 *gBRCA1/2w* tissues were analyzed (Fig. 1). The clinicopathologic characteristics are summarized in Table 1. The median age at diagnosis was 45.5 years in *gBRCA1/2p*, 34.5 years in *gBRCA1/2v*, and 39 years in *gBRCA1/2w*. The difference in age between *gBRCA1/2p* and *gBRCA1/2w* group was not statistically significant (p=0.238 by t test) The familial history of breast/ovarian cancer was more prominent in the *gBRCA1/2p* group than in the other two groups (53.8% vs. 25.0% vs. 27.9%, respectively, having 1st degree relatives, p=0.053).

2. Consistency between Sanger sequencing of *gBRCA1/2* testing and tumor NGS

Twelve nonsynonymous SNVs (34.3%), 11 stopgain SNVs (31.4%), 10 frameshift deletions (28.6%), and two splicing variants (5.7%) were present in all patients harboring any type of *gBRCA1/2* aberration (*gBRCA1/2p* or *gBRCA1/2v*). Of the 35 *gBRCA1/2* variants confirmed in the blood samples of 34 patients by Sanger sequencing, 33 variants (94.3%) were also detected in tumor tissue NGS outcomes. Five *BRCA1* aberrations were inconsistent due to different reference sequences (NM_00294 and NM_007300), but all of them were the same variants after conversion. The complete profiles of *BRCA1/2* aberrations are presented in Table 2.

3. Genomic landscape of breast cancer according to *gBRCA1/2* pathogenicity

Fig. 2 represents the genetic profile of breast cancer tissues and genes showing more than 5% variant allele frequency are listed. Except for *BRCA1/2*, *TP53* was the most frequently mutated gene in all three groups (38/77, 49.4%), followed by *PIK3CA* (18/77, 23.4%). Variants in *PIK3CA* and *PTEN*, which are part of the PIK3CA/AKT/mammalian target of rapamycin pathway, were more prevalent in the *gBRCA1/2w* group than in the *gBRCA1/2p* group (PIK3CA 15.4% vs. 25.6%, p=0.38, PTEN 7.7% vs. 18.6%, p=0.299).

As the number of *gBRCA1/2v* samples was small, we compared genomic aberrancies in tumors from the *gBRCA1/2p* and *gBRCA1/2w* groups. Comparison of the two groups revealed that although the total number of reported SNVs was higher in *gBRCA1/2w* patients (mean, 14.81 vs. 18.86; p=0.278), the difference was not statistically significant. The total number of reported CNVs and fusions was comparable between the two groups (mean CNV, 5.77 vs. 5.47, p=0.343; fusion, 0.27 vs. 0.23, p=0.740) (S1 Table). In addition, the average estimated tumor mutation burden (TMB) was significantly higher in tumor samples from *gBRCA1/2w* patients than in *gBRCA1/2p* patients (10.21% vs. 13.47%, p=0.017). TMB value showed a linear correlation with the number of reported SNVs with marginal significance (Pearson's correlation coefficient, 0.096; p=0.432) (Fig. 3A). However, after removing one outlier (S01192 SNV 130), the Pearson's correlation coefficient was 0.378 and p-value was 0.001 (Fig. 3B).

4. Homologous recombination DNA damage repair gene aberration in tumor NGS according to *gBRCA* pathogenicity

Considering the significant differences in TMB value according to the *gBRCA* mutation status, homologous recombination DNA damage repair (HR-DDR) genetic variants were analyzed further. The following were defined as HR-DDR genes: *ARID1A, ATM, ATRX, BARD1, BLM, BRCA1, BRCA2, BRIP1, CHEK2, FANCA, FANCD2, FANCE, FANCG, MRE11A, NBN, PALB2, RAD50, RAD51*, and *RAD51B*. All these genes are involved in the HR-DDR pathway, as reported by Heeke et al. [9]. These genes are also included in the K-MASTER NGS panel.

Except for *BRCA1/2*, the *gBRCA1/2p* group showed only a few HR-DDR genetic aberrations, whereas tumors from *gBRCA1/2w* patients harbored several aberrations (Fig. 4). Among HR-DDR genes, 15 of 26 *gBRCA1/2p* samples (57.7%) had exclusively *BRCA1/2* aberrations. Less than 50% of the patients (42.3%) harbored HR-DDR gene aberrations apart from *BRCA1/2*, and only four patients (15.4%) harbored multiple non-*BRCA1/2* HR-DDR gene aberrations. Tumor tissues harboring multiple non-*BRCA1/2* HR-DDR gene aberrations were more common in the *gBRCA1/2w* group (13/43, 30.2%). On average, *gBRCA1/2p* patients had significantly lower numbers of non-*BRCA1/2* HR-DDR gene aberrations per person than *gBRCA1/2w* patients (0.54, 1.26, p=0.008) (S2 Table). In the *gBRCA1/2w* group, *BRIP1* was the most frequently identified HR-related gene (23%), followed by *ARID1A* (16%) and *BLM* (12%). All HR-DDR mutation profiles are shown in S3 Table.

5. Correlation between TMB and homologous recombination deficiency gene aberrations

To determine whether HR-DDR gene aberrations affected DNA instability, we performed a matching analysis of TMB values based on the number of non-*BRCA1/2* HR-DDR gene aberrations (S4 Fig.). Non-*BRCA1/2* HR-DDR gene aberrations and TMB values showed a trend of correlation, but it was not statistically significant (Spearman's correlation coefficient, 0.221; p=0.068). Analysis based on grouping with the number of non-*BRCA1/2* HR-DDR gene aberrations is summarized in S5 and S6 Tables.

To examine the correlation between single gene mutations and TMB or SNV, we compared the average of each value between groups with or without specific genetic aberrations (S7 Table). In the analysis of TMB values with 70 tumor samples, tumor tissue with *BRCA1* mutation presented lower TMB than tumors without *BRCA1* mutation

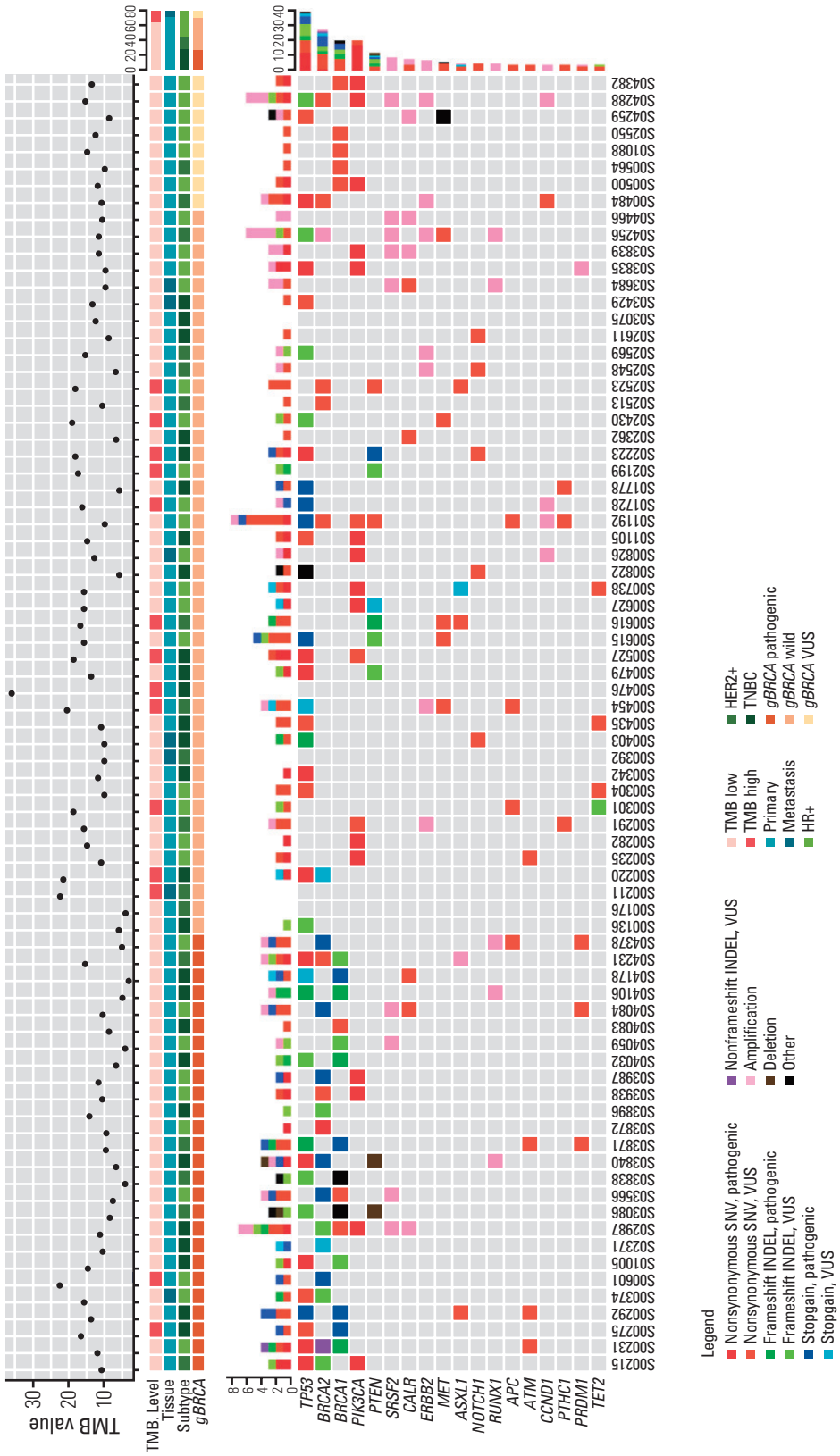

Fig. 2. Genomic landscape of genetic profile according to *gBRCA1/2* pathogenicity. Heatmap visualization of genomic aberration along with tumor mutation burden (TMB) value, breast cancer subtype, and *gBRCA* pathogenicity. The diagram illustrates genes showing more than 5% variant allele frequency (VAF). HER2, human epidermal growth factor receptor 2; HR, hormone receptor; INDEL, insertion and deletion; SNV, single nucleotide variant; TNBC, triple-negative breast cancer; VUS, variant of unidentified significance.

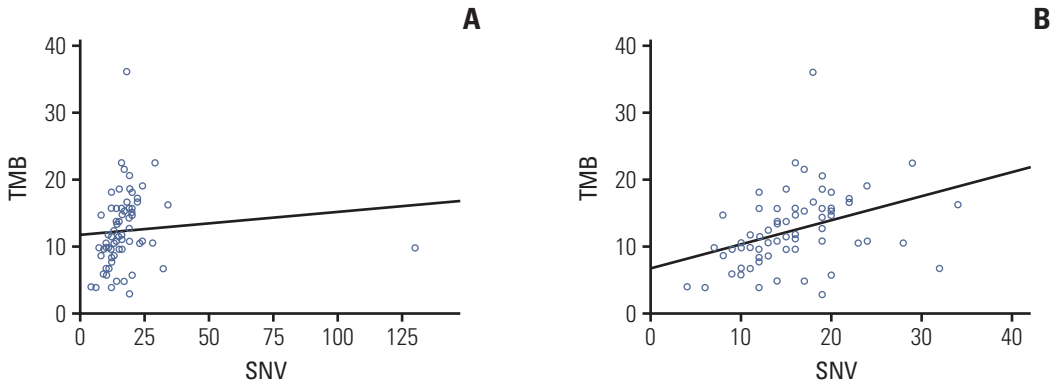

Fig. 3. Tumor mutation burden (TMB) measured by targeted sequencing correlated with total number of single-nucleotide variants (SNVs). (A) Correlation between number of SNV and TMB. (B) Correlation between number of SNV and TMB after removing one outlier.

(p=0.016). Tumor samples with aberrations in *BRIP1* showed a higher mean TMB than samples without *BRIP1* mutations, but this difference was not statistically significant. None of the single gene aberrations correlated with mean SNV, possibly due to the elevated SD caused by one outlier (S01192 SNV 130).

Discussion

In this study, we analyzed breast cancer genomic landscape according to germline *BRCA1/2* pathogenicity. The germline *BRCA1/2* test using DNA extracted from blood samples and tumor *BRCA1/2* test using tumor samples and NGS showed fair consistency (94.3%). Patients harboring the *gBRCA1/2p* mutation showed lower TMB values and fewer SNVs than patients with *gBRCA1/2w*. Although considerable differences were observed for *PIK3CA* and *PTEN* mutations, these were not statistically significant. Higher number of genes related to homologous recombination (HR) repair were mutated more frequently in the *gBRCA1/2w* group than in the *gBRCA1/2p* group, and the differences were remarkable when limited to non-*BRCA1/2* HR-DDR genes. Moreover, the number of non-*BRCA1/2* HR-DDR gene aberrations correlated with the number of reported SNVs.

In the current era of precision medicine, tumor NGS is frequently performed to identify potential therapeutic targets. The major goal of tissue NGS is to detect somatic mutations for actionable targets; however, information beyond somatic SNVs should also be acknowledged. Considerable efforts have been undertaken to determine germline mutations by sequencing clinical tumor samples [10]. However, discrepancies and inaccuracies in identifying germline mutations using tissue NGS have been discussed continuously. A previous study showed that tumor-only sequencing without matched normal samples could not definitively confirm germline aberrations, resulting in high false positivity [11]. Although some discrepancy is inevitable, it is proposed that the number of well-characterized pathogenic genes or their variants should be considered in tumor sequencing. *BRCA1/2*, *MLH1*, *MSH2*, and *MSH6* are often included in cancer panels and are highly associated with inheritance of pathogenicity [12]. Our study revealed a high consistency rate (94.3%) between blood *gBRCA1/2* testing and tumor tissue sequencing, suggesting that it is possible to infer germline *BRCA1/2* aberration from tumor biopsy samples in clinical settings. The two inconsistent cases of our study, S01005 and S04259, presented fair quality of NGS (mean depth 794.59, 442.08 and on-target rate 90.83%, 95.72%). The inconsistency might not be due to low-quality or reference gene diversity. The only possibility is unknown human error in reporting, but clear explanation is not yet determined.

TMB is another key biomarker that can be indirectly inferred by clinical NGS. TMB is defined as the number of base alterations and indels, usually calculated by whole exome sequencing (WES). TMB calculation algorithms used in our study and their clinical efficacy were validated by comparing with TMB calculated by WES, which proved its feasibility with R^2 of 0.71 [13]. Moreover, it is comprehensive with previous studies and demonstrated a fine correlation between TMB by targeted sequencing and WES (R^2=0.74) [14,15]. Similarly, in our study, we assessed the matched relationship between TMB and the number of reported SNVs from a panel of tumor samples (Pearson's correlation coefficient, 0.378; p=0.001).

TMB has emerged as a promising biomarker in the context of immuno-oncology, particularly in melanoma and non–small cell lung cancer [16-18]. High TMB can predict the clinical response to immune checkpoint inhibitors [19,20], but the correlation is not clear in breast cancer [21]. Approxi-

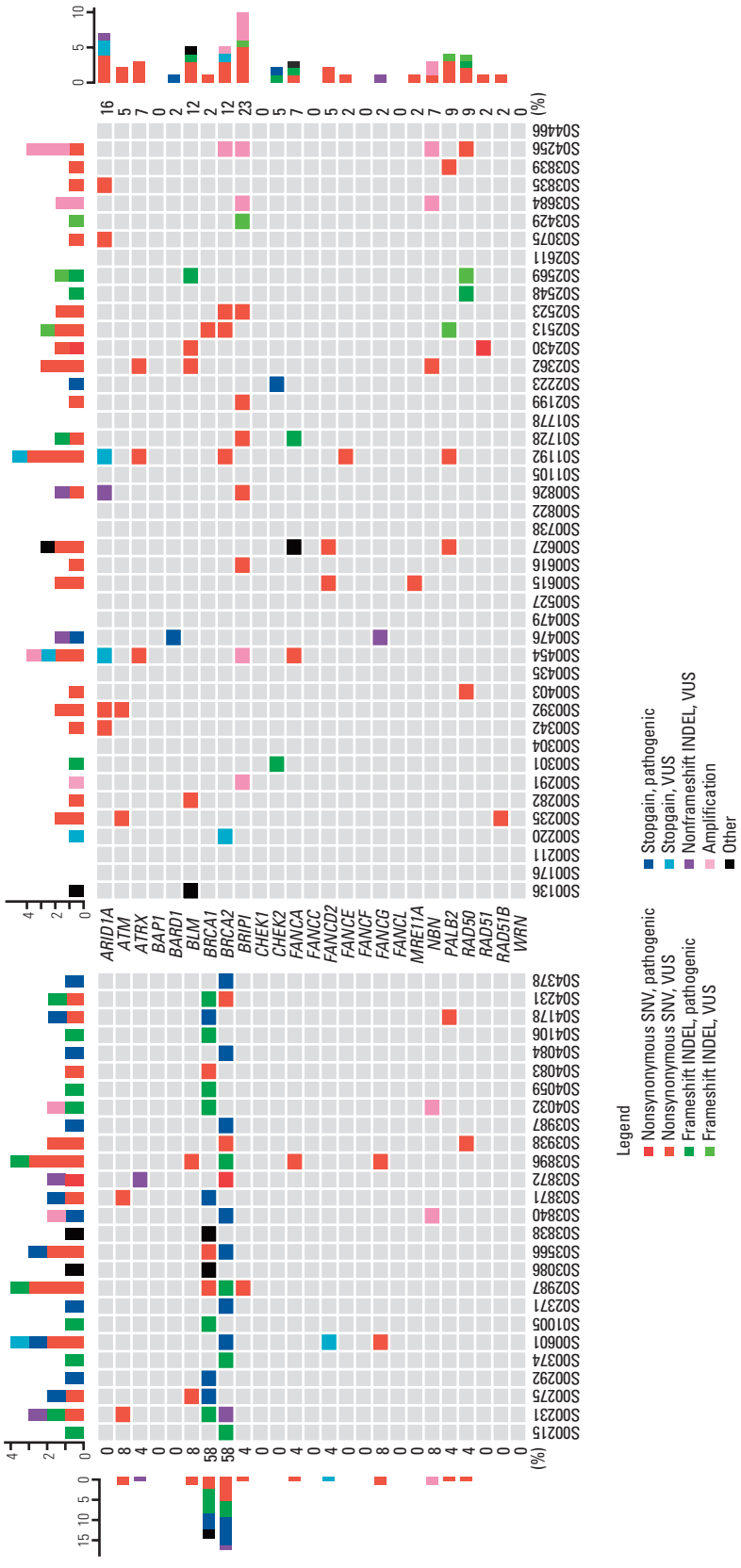

Fig. 4. Genomic landscape of homologous recombination DNA damage repair (HR-DDR) genetic profiles in *gBRCA1/2p* and *gBRCA1/2w* groups. Heatmap visualization of *gBRCA1/2p* and *gBRCA1/2w* tumor tissue confined with HR-DDR genomic aberration. INDEL, insertion and deletion; SNV, single nucleotide variant; VUS, variant of unidentified significance.

mately 20% of metastatic breast cancer cases demonstrate a high TMB [21]. In our study, 14 of 77 patients (18.2%) presented high TMB (> 16 Mb). However, the prevalence sharply increased to 27.9% (12/43) when only the *gBRCA1/2* wild-type group was assessed. Taking these findings into account, further translational studies may reveal whether certain germline pathogenicity is predictive of the therapeutic effect of immunotherapy.

Hypermutated breast cancer can be driven by multiple mechanisms, including homologous recombination deficiency (HRD), and breast cancer with HRD has the highest median TMB among groups with dominant signatures [15]. In our study, the genes were selected for academic purposes and were different from those widely used commercially. With the limited resource and practical barriers with targeted sequencing, we could not clearly present HRD score. Although their important role in HR-DDR were well acknowledged in preclinical studies, genes which had not been included in approved commercial panel, such as *ARID1A* and *NBN*, should be interpreted with caution. Although *BRCA1/2* are two of the essential HR-DDR genes, our data showed frequent HR-DDR aberrations and higher mean TMB in the *gBRCA1/2w* group than in the *gBRCA1/2p* group. Previous studies reported contradictory results about relationship between germline mutation and TMB. Somatic mutation of *BRCA1/2* was associated with higher mutation burden and higher TMB comparing with wild type in The Cancer Genome Atlas–based analysis [22,23]. However, when the patients were classified according to germline *BRCA1/2* mutation, differences of TMB was insignificant or even lower in *gBRCA1/2*-mutated group [24,25]. With these evidences and results of our study, we can infer that germline pathogenic variation in *BRCA1/2* might contribute less to TMB than variations in HRD genes in breast cancer. As with tremendous effort to detect HRDness in solid cancer and applying the results to choosing effective treatment, the role of TMB in breast cancer should be further evaluated. The results of clinical trials evaluating the efficacy of PARP inhibitors in patients with HRDness are anticipated [26].

In our study, we noted more *PIK3CA* and *PTEN* mutations in the *gBRCA1/2w* group than in the *gBRCA1/2m* group. As two of the major cancer driver mutations, our findings provide an evidence for their mutual exclusiveness, consistent with previous studies [24,27]. A recent study also showed that breast cancer with altered phosphoinositide 3-kinase pathway harbors a significantly low rate of homologous recombination co-alterations [28]. In the future, we intend to explore the correlation between germline and somatic mutations using more genomic data from a larger sample size.

In clinical settings, many of the genes known to cause hereditary cancer syndrome are included in most cancer panels. In addition, because of convenience of its application and short turnaround time, NGS panels are increasingly being used to identify germline aberrations. Use of comprehensive genetic analysis based on WES and whole genome sequencing (WGS) is expected to increase as their accessibility has improved. These advances have expanded the possibilities of discovering novel germline mutations. Therefore, further functional genomic studies based on WES and WGS aimed at determining clinical implications of genetic variants and their effects on drug sensitivity are needed.

However, our study has several limitations. First, the sample size was too small to ensure statistical power. There had been number of patients who did their NGS analysis with blood, but they could not be included because the study was performed to investigate concordance rate of germline variants between tumor tissue and peripheral blood mononuclear cell. The statistical results should be interpreted with caution considering selection bias and relatively low prevalence of *gBRCA1/2* mutation in the study cohort. Some patients were enrolled at the early stage of breast cancer, whereas others were enrolled at an advanced stage, making the total genomic profile heterogeneous. In addition, we could not verify the clinical significance of HR-DDR aberration or high TMB in terms of therapeutic targets or predictive markers. As none of the Food and Drug Administration–approved PARP inhibitors are currently reimbursed for patients with metastatic breast cancer under the Korean national health scheme, platinum-based chemotherapy remains the most affordable treatment option.

In conclusion, we herein describe the consistency between *gBRCA1/2* status based on blood testing and tissue sequencing and the differences in genetic landscape according to germline *BRCA1/2* gene variations. The concordance rate of *gBRCA1/2* results by tissue NGS was 94.3%, and a significant difference was observed in TMB value and aberrated non-*BRCA1/2* HR-DDR genes according to germline *BRCA1/2* pathogenicity in patients with breast cancer. In the future, our data should be validated in a larger cohort, and the clinical impact on survival outcomes should be further elucidated.

Electronic Supplementary Material

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

Ethical Statement

This research was conducted under the condition of approval by the Korea University Anam Hospital IRB (No. 2017AN0401). All patients provided written informed consent before sample collection.

Author Contributions

Conceived and designed the analysis: Kim JW, Kim YH, Park KH.
Collected the data: Kim JW, Yun SG, Jung SP, Bae SY, You JY, Choi YJ, Park KH.
Contributed data or analysis tools: Kim JW, Kang HE, Choi J, Yun SG, Park KH.
Performed the analysis: Kim JW, Kang HE, Choi J, Park KH.
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Conflicts of Interest

Conflict of interest relevant to this article was not reported.

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