# Clinical Characteristics and Exploratory Genomic Analyses of Germline BRCA1 or BRCA2 Mutations in Breast Cancer

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## ABSTRACT

gBRCA1/2 mutations increase the incidence of breast cancer by interrupting the homologous recombination repair (HRR) pathway. Although gBRCA1 and gBRCA2 breast cancer have similar clinical profiles, different molecular characteristics have been observed. In this study, we conducted comprehensive genomic analyses and compared gBRCA1/2 breast cancer. Sanger sequencing to identify gBRCA1/2 mutations was conducted in 2,720 patients, and gBRCA1 ($n = 128$) and gBRCA2 ($n = 126$) mutations were analyzed. Within this population, deep target sequencing and matched whole-transcriptome sequencing (WTS) results were available for 46 and 34 patients, respectively. An internal database of patients with breast cancer with wild-type gBRCA was used to compile a target sequencing ($n = 195$) and WTS ($n = 137$) reference dataset. Three specific mutation sites, p.Y130X ($n = 14$) and p.1210Afs ($n = 13$) in gBRCA1 and p.R294X ($n = 22$) in gBRCA2, were comparably frequent. IHC subtyping determined that the incidence of triple-negative breast cancer was higher among those with a gBRCA1 mutation (71.9%), and estrogen receptor–positive breast cancer was dominant in those with a gBRCA2 mutation (76.2%). gBRCA1/2 mutations were mutually exclusive with *PIK3CA* somatic mutations ($P < 0.05$), and gBRCA1 frequently cooccurred with *TP53* somatic mutations ($P < 0.05$). The median tumor mutation burden was 6.53 per megabase (MB) in gBRCA1 and 6.44 per MB in gBRCA2. The expression of *AR, ESR1*, and *PGR* was significantly upregulated with gBRCA2 mutation compared with gBRCA1 mutation. gBRCA1 and gBRCA2 breast cancer have similar clinical characteristics, but they have different molecular subtypes, coaltered somatic mutations, and gene expression patterns.

**Implications:** Even though gBRCA1 and gBRCA2 mutations both alter HRR pathways, our results suggest that they generate different molecular characteristics and different mechanisms of carcinogenesis.

## Introduction

Among Asian patients with breast cancer, 2% to 3% are reported to harbor mutations in germline BRCA1 or BRCA2 (1–4). Compared with the general population, the incidence of breast cancer in these patients is increased by 69% to 72% until the age of 80 years. Germline mutations in both BRCA1 and BRCA2 also increase the incidence of breast and many other cancers, such as ovarian, peritoneal, pancreatic, and prostate (5, 6). These risks are understood to be determined by the position and type of mutation in BRCA1/2, as assessed by the Consortium of Investigators of Modifiers of BRCA1/2 (7).

Both BRCA1 and BRCA2 function in DNA damage repair (DDR) by playing a major role in the homologous recombination repair process. The BRCA1 protein induces a 5′ to 3′ resection of double-strand breaks to generate an overhanging 3′ ssDNA and localizes damaged DNA by loading DNA recombinase RAD51 onto ssDNA (8).

gBRCA1- and gBRCA2-mutant breast cancer have similar clinical profiles, but different molecular characteristics. The onset of the disease differs between them in that the incidence increases until age 30 to 40 years for gBRCA1 and age 40 to 50 years for gBRCA2, although it then remains constant until age 80 in both cases (9). The proportion of molecular subtypes also differs in that gBRCA1-mutated breast cancer is predominantly triple-negative breast cancer (TNBC), whereas gBRCA2-mutated breast cancer is predominantly estrogen receptor (ER)-positive (10, 11). Nevertheless, both gBRCA1- and gBRCA2-mutant breast cancer shows superior progression-free survival (PFS) and a higher overall response rate with a poly adenosine diphosphate-ribose polymerase inhibitor than with standard treatment, which indicates that gBRCA1/2-mutant breast cancer is highly dependent on other DDR pathways regardless of its underlying molecular subtype (12, 13).

On the basis of the unique characteristics of gBRCA1/2 mutations in breast cancer, we performed comprehensive clinical and genomic

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analyses of samples from operable patients with breast cancer with gBRCA1/2 mutations.

## Materials and Methods

### Study population and data collection

The medical records of patients who received a germline BRCA1/2 test at Samsung Medical Center (SMC, Seoul, Republic of South Korea) between January 2007 and October 2018 were retrospectively reviewed ($n$ = 2,720). The study population was selected using the following criteria: patients with pathologic gBRCA1 or gBRCA2 variants who received surgery with curative intent, were diagnosed at least 1 year before data acquisition, and had adequate clinical information available for analysis (**Fig. 1A**). Pathology and IHC data were collected from surgical and biopsy reports. Genomic analyses were performed on samples with available genomic outcomes using a deep target sequencing ($n$ = 45) protocol called CancerSCAN and whole-transcriptome sequencing (WTS, $n$ = 34). The patients with WTS data were a subset of the population with deep target sequencing outcomes. Genomic data from an internal database ($n$ = 195) were used as a gBRCA wild-type (WT) reference (**Fig. 1B**). This study was approved, and written informed consent was waived by the SMC Institutional Review Board (Seoul, Republic of South Korea, IRB#2019-03-067). The data that support the findings of this study are available from the corresponding author upon reasonable request.

### Germline BRCA mutation test

Genomic DNA was isolated from peripheral blood leukocytes. Germline BRCA1/2 mutation status was tested by direct Sanger sequencing. Annotation used a predefined internal calling algorithm based on previous reports. Only patients with a pathogenic variant in the reports were considered to have a gBRCA mutation. Patients with equivocal variants and variants of unknown significance were excluded from the analyses.

### Target sequencing (CancerSCAN) and whole-transcriptome sequencing

Target sequencing results were extracted from previously calculated CancerSCAN data. CancerSCAN is designed to enrich the exons of 381 genes curated from the literature (Supplementary Table S1). DNA (250 ng) from cancer tissue was sheared in a Covaris S220 Ultrasonicator (Covaris) and used to construct a library with CancerSCAN probes and a SureSelect XT Reagent Kit, HSQ (Agilent Technologies) according to the manufacturer's protocol (14, 15). After being enriched, exome libraries were multiplexed and sequenced using the 100-bp paired-end mode in a TruSeq Rapid PE cluster kit and TruSeq Rapid SBS kit on the Illumina HiSeq 2500 Sequencing Platform (Illumina Inc.). The DNA sequence data were aligned to the human genome reference (hg19) using the MEM algorithm in BWA 0.7.5 (16). Duplicate read removal was performed using Picard v.193 and SAMTOOLS v0.1.18 (samtools.sourceforge.net). Local alignment was optimized using the Genome Analysis Toolkit (GATK) v3.1-1 (https://software.broadinstitute.org/gatk/). BaseRecalibrator from GATK was used for the recalibration based on known SNPs and insertion/deletions (indels) from Mills, dbSNP138, 1000G gold standard, 1000G phase1, and Omni 2.5. Single-nucleotide variations (SNV) were detected using MuTect and LoFreq (17, 18). Falsely detected variants were filtered out using a script developed in-house. ANNOVAR was used to annotate the detected variants with dbSNP138, the Catalogue of Somatic Mutations in Cancer (COSMIC), The Cancer Genome Atlas, and an in-house Korean SNP database. Indels were detected by Pindel (19). Germline variants were filtered out by removing the variants with an allele frequency greater than or equal to 97%, and suspected germline variants were removed on the basis of an allele frequency greater than or equal to normal Korean samples. LOH status was calculated using PureCN (R-package; Supplementary Fig. S1A; Supplementary Table S11).

For WTS, sequencing libraries were prepared using the TruSeq RNA Sample Preparation Kit v2 (Illumina). Sequencing of the RNA libraries was performed on an Illumina HiSeq2500 in the 100-bp paired-end mode of a TruSeq Rapid PE cluster kit and TruSeq Rapid SBS kit. After trimming poor quality bases from the FASTQ files obtained from the sequencing, we aligned the reads to the human reference genome (hg19) with TopHat (v 2.0.6; ref. 20) and performed a reference-guided assembly of transcripts with Cufflinks (v 2.1.1; ref. 21). Alignment quality was verified with SAMTOOLS (v 0.1.19; ref. 22). Gene expression was estimated from the RNA-sequencing data of the patients using a count-based method with HTSeq (22). We selected 20,345 protein-coding genes, and genes expressed in at least three samples were retained. A total of 16,971 genes were thus considered for analysis. Gene counts were used as the input for trimmed mean of M value normalization by the R package, edgeR (23), and normalized counts were transformed to $\log_2$-counts per million by applying voom from the R package limma (ref. 24; Supplementary Fig. S1B). Raw data are available at NCBI SRA (PRJNA625821) and Gene Expression Omnibus (GSE149276).

### Tumor mutation burden, 50-gene prediction analysis of microarray calculation, and gene set analyses

Tumor mutation burden (TMB) was defined as the number of somatic variants per megabase (MB) of the genome. We assessed TMB on the basis of variants detected by the targeted sequencing in CancerSCAN. Among the variants in the coding region, nonsynonymous SNVs and frameshift indels were counted. Germline variants with a population frequency >0.001 in the Exome Aggregation Consortium (25) database and the Korean population were excluded. Variants listed in COSMIC (26) were filtered. To calculate the TMB per MB, the total number of mutations counted was divided by the size of the coding region in the targeted region.

We performed intrinsic subtyping with log-scaled normalized expression values using the 50-gene prediction analysis of microarray (PAM50) subtype predictor, as described by Parker and colleagues (27). The PAM50 subtype predictor classified tumors into the following groups: Luminal A, Luminal B, HER2-enriched, basal-like, and normal-like.

Gene set analyses were performed using R package gene set variation analysis (GSVA) (28), which calculates the gene set enrichment score for each sample based on gene expression data. For the gene set enrichment analysis, we used the Molecular Signatures Database (MSigDB v6.2, http://software.broadinstitute.org/gsea/msigdb/index.jsp), which is a collection of 17,810 annotated gene sets, including 50 hallmark gene sets (29).

### Statistical analysis

Descriptive statistics were used to describe the characteristics of the study population. Overall survival (OS) and PFS in patients with gBRCA1-mutated versus gBRCA2-mutated status and gBRCA1/2-mutated versus WT status were analyzed using the Kaplan–Meier method.

The Maftools R package (30) was used to present the somatic mutation profile and detect a mutually exclusive set of genes, which was identified by Fisher exact test. To compare the TMB between

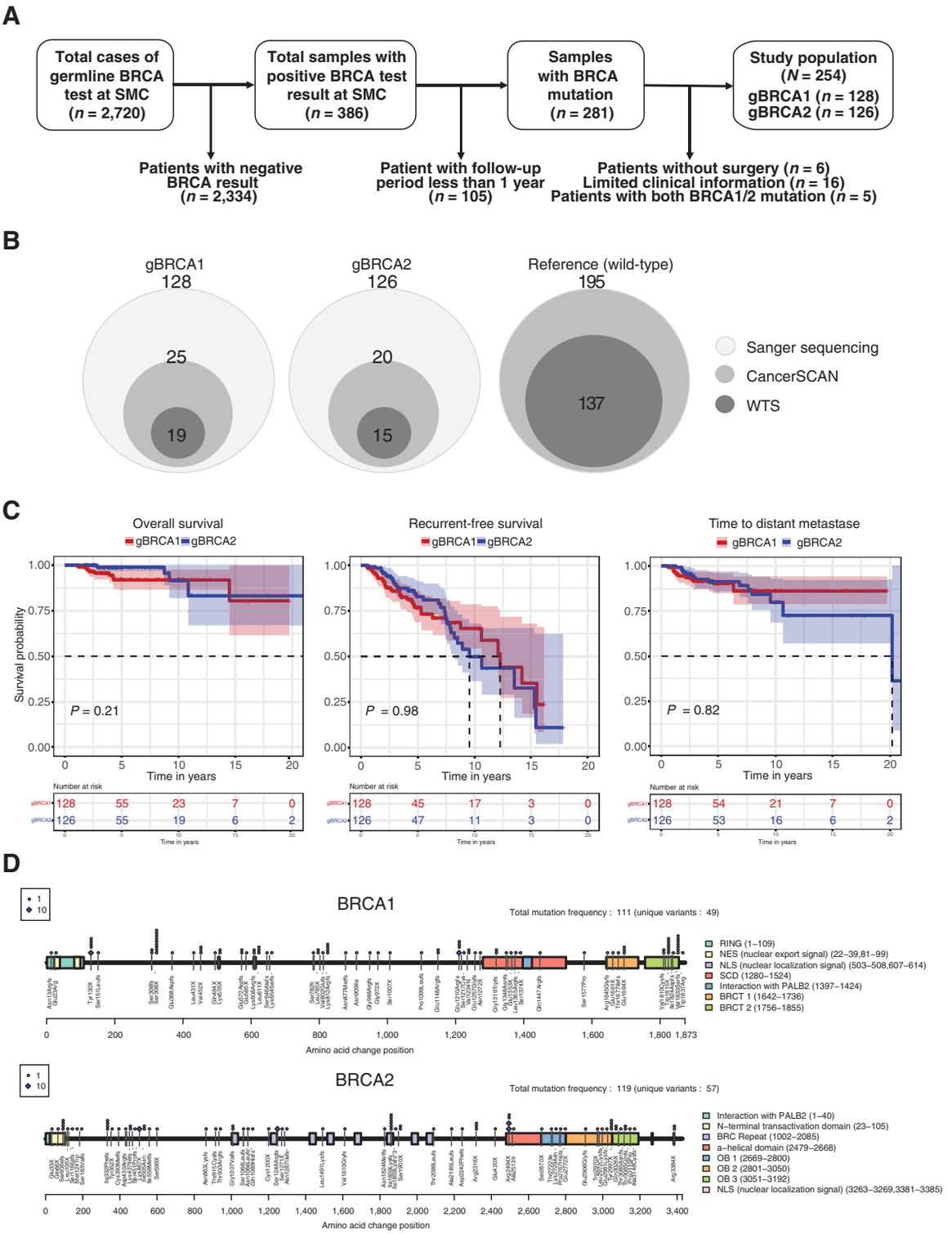



**Figure 1.**

**A,** Consort flow of study population. **B,** Sample status of the study population and reference subjects. **C,** Kaplan–Meier curve of OS, RFS, and time to distant metastases. **D,** Nucleotide position of germline BRCA1 and BRCA2 in the study population.

gBRCA1/2 and WT, ANOVA and *t* test were used. Fisher exact test was used to identify genes whose mutations were associated with the triple negative or ER-positive subtypes.

Differentially expressed gene (DEG) analyses for gBRCA1/2 status used a two-group *t* test. The raw *P* value was adjusted to an FDR. Genes within the cut-off criteria of FDR $P < 0.01$ and an absolute fold change >2 were designated as DEGs for gBRCA1/2. The thresholds of $P < 0.05$ and absolute fold change >1.5 were used for significant DEGs between gBRCA1/2 and WT. The thresholds for gene set enrichment analyses between gBRCA1 and gBRCA2 were $P < 0.01$ and an absolute difference in GSVA score >0.3. For GSVA between gBRCA1/2 and WT, both $P < 0.01$ and an absolute difference in GSVA score >0.2 were required.

A significance level of $P < 0.05$ was generally held to be significant in the analyses. All statistical tests were performed using R software v.3.4.2 (https://www.r-project.org/).

# Results

### Baseline demographics of the study population

Descriptions of the baseline demographics are given by gBRCA1 ($n = 128$) and gBRCA2 ($n = 126$) mutation status (**Fig. 1A**). The median age was 39 years old (range, 25–67) for patients with gBRCA1 mutations and 40 years old (range, 23–68) for patients with gBRCA2 mutations. Most patients were tested for the gBRCA mutation due to a family history of either breast or ovarian cancer (65.7%), followed by bilateral breast cancer (21.3%), and disease diagnosed at younger than 40 years old (18.0%). Most patients were in a premenopausal state (81.2%), and this ratio was similar in patients with gBRCA1 (89.1%) and gBRCA2 (73.0%) mutations. Patients received either modified radical mastectomy and total mastectomy (37.5%) or breast-conserving surgery and partial mastectomy (62.6%). Clinical stage at diagnosis was stage III in 24.5%, stage II in 40.6%, and stage I in 30.3%, according to the American Joint Committee on Cancer, Breast Cancer Staging, 7th edition. The main histopathology type was invasive ductal carcinoma (86.2%) for both gBRCA1 mutations (86.7%) and gBRCA2 mutations (85.7%). Details of the baseline demographics are provided in **Table 1**.

### Clinical outcomes of the study population

Median follow-up duration of the study population was 55.3 months (range, 13.7–261.7). Neoadjuvant chemotherapy was applied in 24.8% of patients, and 77.6% of patients received adjuvant treatment. Disease recurrence after surgery was observed in 26.4% of the study population, and the median time to recurrence was 147.3 months (range, 127.4–186.2) for patients with a gBRCA1 mutation and 114.8 months (range, 95.2–183.6) for those with a gBRCA2 mutation. Palliative chemotherapy was applied in 9.5% of patients, and 5.5% of patients had died by the time of data cutoff (**Table 2**). Survival analyses between gBRCA1 and gBRCA2 showed no significant difference in OS ($P = 0.21$), recurrence-free survival (RFS, $P = 0.98$), or time to distant metastases ($P = 0.82$; **Fig. 1C**). *Post hoc* analyses conducted with individual loci showed that patients with mutations in gBRCA2 p.Lys467X had comparably shorter RFS ($P = 0.05$) than those with other gBRCA2 mutations. In a similar manner, RFS compared in a range of 200 amino acids showed that patients with BRCA2 mutations in 400–600 ($P < 0.01$) and 800–1,000 ($P = 0.02$) amino acids had shorter RFS than the rest of the patients (Supplementary Fig. S2). No specific locus in BRCA1 correlated with RFS. In addition, survival analyses conducted on the basis of ER status dose did not show difference between gBRCA1 and gBRCA2 mutation. As an exploratory analysis, we looked in to the pathologic complete response (pCR) rate based on gBRCA mutation. In gBRCA1 mutant, 14 patients among 35 patients (40.0%) who received neoadjuvant chemotherapy achieved pCR. However, gBRCA2 mutant only showed one pCR among 28 patients (3.6%). Similarly, in WT patients, we observed pCR in 8% of the patients.

### Germline BRCA1/2 mutation profiles and coaltered somatic mutations identified by CancerSCAN

The mutations that occur in gBRCA are mostly frameshift mutations (45.2%), followed by nonsense mutations (43.3%), splicing mutations (7.1%), and missense mutations (2.0%; Supplementary Table S2). Among the mutations, Tyr130X (10.9%) and Glu1210Argfs (10.2%) in gBRCA1 and Arg2494X (17.5%) in a helical domain of gBRCA2 were observed frequently. Other mutations were observed in a broad range of loci not limited to a specific domain (**Fig. 1D**). We assessed coaltered somatic mutation profiles in samples available for target sequencing ($n = 45$; gBRCA1 $n = 25$ and gBRCA2 $n = 20$) and identified coaltered somatic mutations in 44 (97.8%) of the samples. Mutations in *TP53* (60%), *FAT3* (33%), and *FANCI* (27%) were commonly observed. The mutations identified in BRCA1/2 were identical to previously detected germline mutations (**Fig. 2A**). A test for cooccurrence and exclusivity shows that gBRCA2 mutations were mutually exclusive with somatic mutations in *TP53* (35.0%; $P < 0.05$) and *MET* (0%; $P < 0.05$). In contrast, gBRCA1 mutations highly cooccurred with somatic mutations in *TP53* (84.0%; $P < 0.05$) and *MET* (24.0%; $P < 0.05$; **Fig. 2B**).

To identify the overall difference in somatic mutations between gBRCA mutants and the WT, target sequencing results from the merged gBRCA1/2 samples ($n = 45$) were compared with the results from the gBRCA WT reference samples ($n = 195$). Interestingly, the *PIK3CA* mutation was mutually exclusive with gBRCA mutation ($P < 0.05$). Specifically, only 13.3% of patients with a gBRCA mutation had a somatic alteration in PIK3CA. However, the somatic PIK3CA mutation rate was 27.7% in patients with WT gBRCA (**Fig. 2C**; Supplementary Fig. S3).

Median TMB was 6.525 per MB in gBRCA1, 6.437 per MB in gBRCA2, and 6.439 per MB in the WT reference patients. There were no significant differences between the groups (**Fig. 2D**).

### IHC subtypes and PAM50 molecular subtypes

In the IHC subtyping, patients with gBRCA1 mutations were more frequently diagnosed with TNBC (71.9%), and those with gBRCA2 mutations were more frequently diagnosed with ER-positive breast cancer (76.2%). This result was compared with the PAM50 results from those available for WTS ($n = 34$; **Fig. 3A**). Among the patients with TNBC diagnosed by IHC, 87% were still predicted as a basal type by PAM50. A significant discrepancy was also observed in the ER-positive subjects analyzed by IHC: 31% of them ($n = 5$) were predicted as a basal subtype by PAM50. These results were compared with the expression profile of *ESR1*, which was arbitrarily classified into high ($n = 13$), intermediate ($n = 8$), and low ($n = 13$). As expected, in most of the patients with a gBRCA2 mutation (73%), *ESR1* was upregulated, but *ESR1* expression was either intermediate or low (90%) in those with a gBRCA1 mutation (**Fig. 3B**). In terms of the gene expression pattern of representative genes, the samples rated ER-positive by IHC and basal type by PAM50 had relatively upregulated *BRCA2* expression (Supplementary Fig. S4).

On the basis of the IHC molecular subtype, we again compared the somatic mutation profiles with those of patients with WT gBRCA. A comparison between patients with TNBC with a gBRCA1 mutation or WT found a higher *ARID1B* somatic mutation rate in patients with

**Table 1.** Baseline demographics of the study population: patients with single BRCA1 and BRCA2 mutations.

| | | Study population ($N = 254$) | BRCA1 ($n = 128$) | BRCA2 ($n = 126$) |
|---|---|---|---|---|
| Age at diagnosis BRCA mutation[a] | Median (range) | 40 (23–68) | 39 (25–67) | 40 (23–68) |
| | Frameshift | 115 (45.2%) | 54 (42.2%) | 61 (48.4%) |
| | Missense | 5 (2.0%) | 4 (3.1%) | 1 (0.8%) |
| | Nonsense | 110 (43.3%) | 53 (41.4%) | 57 (45.2%) |
| | Splicing | 18 (7.1%) | 12 (9.4%) | 6 (4.8%) |
| Reason for BRCA test[b] | | | | |
| | FHx breast or ovary cancer | 167 (65.7%) | 87 (68.0%) | 80 (63.0%) |
| | FHx other cancer | 5 (2.0%) | 4 (3.1%) | 1 (0.8%) |
| | Bilateral cancer | 54 (21.3%) | 27 (21.1%) | 27 (21.4%) |
| | Age under 40 | 46 (18.0%) | 19 (14.8%) | 27 (21.4%) |
| | Breast and ovarian cancer | 8 (3.1%) | 5 (3.9%) | 3 (2.4%) |
| | Male breast cancer | 2 (0.8%) | 0 (0.0%) | 2 (1.6%) |
| | Unidentified | 4 (1.6%) | 3 (2.3%) | 1 (0.8%) |
| Menopausal status | | | | |
| | Premenopausal | 207 (81.2%) | 114 (89.1%) | 92 (73.0%) |
| | Postmenopausal | 42 (16.5%) | 10 (7.8%) | 32 (25.4%) |
| | Other[c] | 6 (2.4%) | 4 (3.1%) | 2 (1.6%) |
| Prophylactic mastectomy | | 17 (6.7%) | 11 (8.6%) | 6 (4.8%) |
| Prophylactic bilateral oophorectomy | | 95 (37.4%) | 46 (35.9%) | 49 (38.9%) |
| Surgical record | | | | |
| | MRM or TM | 95 (37.4%) | 43 (33.6%) | 52 (41.3%) |
| | BCS or PM | 159 (62.6%) | 85 (66.4%) | 74 (58.7%) |
| Clinical stage at diagnosis | | | | |
| | 0 | 12 (4.7%) | 2 (1.6%) | 10 (7.9%) |
| | 1A | 73 (28.7%) | 34 (26.6%) | 39 (31.0%) |
| | 1B | 4 (1.6%) | 1 (0.8%) | 3 (2.4%) |
| | 2A | 72 (28.4%) | 45 (35.2%) | 27 (21.4%) |
| | 2B | 31 (12.2%) | 17 (13.3%) | 14 (11.1%) |
| | 3A | 37 (14.6%) | 20 (15.6%) | 17 (13.5%) |
| | 3B | 3 (1.2%) | 2 (1.6%) | 1 (0.8%) |
| | 3C | 22 (8.7%) | 7 (5.5%) | 15 (11.2%) |
| Pathology | | | | |
| | Invasive ductal carcinoma | 219 (86.2%) | 111 (86.7%) | 108 (85.7%) |
| | Other[d] | 35 (13.8%) | 17 (13.3%) | 18 (14.3%) |
| $HR^+$ | | 125 (49.2%) | 29 (22.7%) | 96 (76.2%) |
| $HR/HER2^+$ | | 10 (3.9%) | 4 (3.1%) | 6 (4.8%) |
| $HER2^+$ | | 3 (1.2%) | 3 (2.3%) | 0 (0.0%) |
| Triple negative | | 116 (46.7%) | 92 (71.9%) | 24 (19.1%) |

Abbreviations: BCS, breast-conserving surgery; FHx, family history; HR, hormone receptor; MRM, modified radical mastectomy; PM, partial mastectomy; TM, total mastectomy.

[a]Some patients are omitted because of limited information about the locus of mutation.

[b]Some patients are counted several times for multiple reasons.

[c]Other reasons include previous oophorectomy, total hysterectomy, and unknown menstrual history.

[d]Other types include ductal carcinoma *in situ*, invasive lobular carcinoma, micropapillary carcinoma, and metaplastic carcinoma.

WT gBRCA, whereas the patients with a gBRCA1 mutation had a higher *RET* and *PIK3CA* somatic mutation rate (**Fig. 3C**). Similarly, the comparison between ER-positive patients with a gBRCA2 mutation and WT gBRCA showed a higher *PIK3CA* somatic mutation rate in patients with WT gBRCA, whereas patients with a gBRCA2 mutation had a higher *NOTCH1* and *ZNF217* somatic mutation rate (**Fig. 3D**).

### Gene expression comparison between gBRCA1 and gBRCA2

Initial analyses were conducted by comparing the gene expression patterns of patients with a gBRCA1 mutation ($n = 19$) with those with a gBRCA2 mutation ($n = 15$; **Fig. 4A**). Notably, the gBRCA2-mutated samples showed upregulation in hormone receptor or hormone-induced genes, such as *AR* (FDR = 0.001), *KIAA1324* (FDR = 0.003), and *HSPB8* (FDR = 0.004); membrane tyrosine kinases, such as *ERBB4* (FDR = 0.002); and other breast cancer–related genes, such as *BCAS1* (FDR = 0.005) and *GATA3* (FDR = 0.006). On the other hand, the gBRCA1 samples showed upregulation in cytokines, such as *CXCL5* (FDR = 0.008; **Fig. 4B**; Supplementary Fig. S5; Supplementary Table S3). Although, it did not satisfy the predefined threshold, well-known breast cancer genes such as *ERBB3, ESR1, PGR*, and *BRCA1* were upregulated in gBRCA2 samples, and *EGFR* and *PIK3CA* were upregulated in gBRCA1 samples (**Fig. 4C**).

The top-ranked gene set identified by GSVA (FDR < 0.002) in the gBRCA1 mutants was consistent with a molecular subtype in which the ESR1-related gene set was downregulated and the basal type gene set was upregulated, whereas the reverse was true in the gBRCA2 mutants (Supplementary Fig. S6; Supplementary Table S4). Among the

**Table 2.** Treatment patterns and survival outcomes based on BRCA mutation profile.

| | | Study population ($N = 254$) | BRCA1 ($n = 128$) | BRCA2 ($n = 126$) |
|---|---|---|---|---|
| Median duration of follow-up (months) | | 55.3 (13.7–261.7) | 55.7 (13.7–237.4) | 54.8 (14.9–161.7) |
| Neoadjuvant chemotherapy | | 63 (24.8%) | 35 (27.3%) | 28 (22.2%) |
| | AC followed by docetaxel | 49 (19.3%) | 25 (19.5%) | 24 (19.0%) |
| Adjuvant therapy | | 197 (77.6%) | 92 (71.9%) | 105 (83.3%) |
| Adjuvant CTx (percent calculated among CTx) | | 171 (67.3%) | 95 (74.2%) | 76 (60.3%) |
| | CMF | 8 (4.7%) | 5 (5.3%) | 3 (4.0%) |
| | FAC | 49 (28.7%) | 32 (33.7%) | 17 (22.4%) |
| | AC | 17 (9.9%) | 8 (8.4%) | 9 (11.8%) |
| | AC and taxane | 58 (33.9%) | 27 (28.4%) | 31 (40.8%) |
| | Other | 39 (22.8%) | 23 (24.2%) | 16 (20.1%) |
| Adjuvant hormone therapy | | 132 (52.0%) | 33 (25.8%) | 99 (78.6%) |
| Post-op RT | | 198 (78.0%) | 94 (73.4%) | 104 (82.5%) |
| Recurrence event | | 67 (26.4%) | 34 (26.6%) | 33 (26.2%) |
| | Local recurrence | 47 (18.5%) | 26 (20.3%) | 21 (16.7%) |
| | Contralateral recurrence | 41 (16.1%) | 23 (18.0%) | 18 (14.3%) |
| | Systemic recurrence | 28 (11.0%) | 13 (10.2%) | 15 (11.9%) |
| | Median time to recurrence (months)[a] | 145.0 (107.3–170.2) | 147.3 (127.4–186.2) | 114.8 (95.2–183.6) |
| Palliative chemotherapy | | 24 (9.5%) | 12 (9.4%) | 12 (9.5%) |
| Survival status | | | | |
| | Deceased | 14 (5.5%) | 9 (7.0%) | 5 (4.0%) |

Abbreviations: AC, adriamycin and cyclophosphamide; CMF, cyclophosphamide, methotrexate, and fluorouracil; CTx, chemotherapy; FAC, fluorouracil, adriamycin, and cyclophosphamide; Op, operation; RT, radiotherapy.
[a]Median (range).

hallmark gene sets, E2F targets, $G_2$–M-phase checkpoints, hypoxia, MYC targets, UV response, mitotic spindle, TNFA signaling via NF-κB, and glycolysis were upregulated ($P < 0.01$) in the gBRCA1 mutants, and early estrogen response, bile acid metabolism, pancreas beta cells, and late estrogen response were upregulated in the gBRCA2 mutants (Supplementary Fig. S7).

### Gene expression comparison between gBRCA mutation and WT under same IHC subtype

In an extension of the WTS analysis, we conducted comparative analyses to identify the unique gene expression patterns shown with gBRCA mutations under the same IHC subtypes.

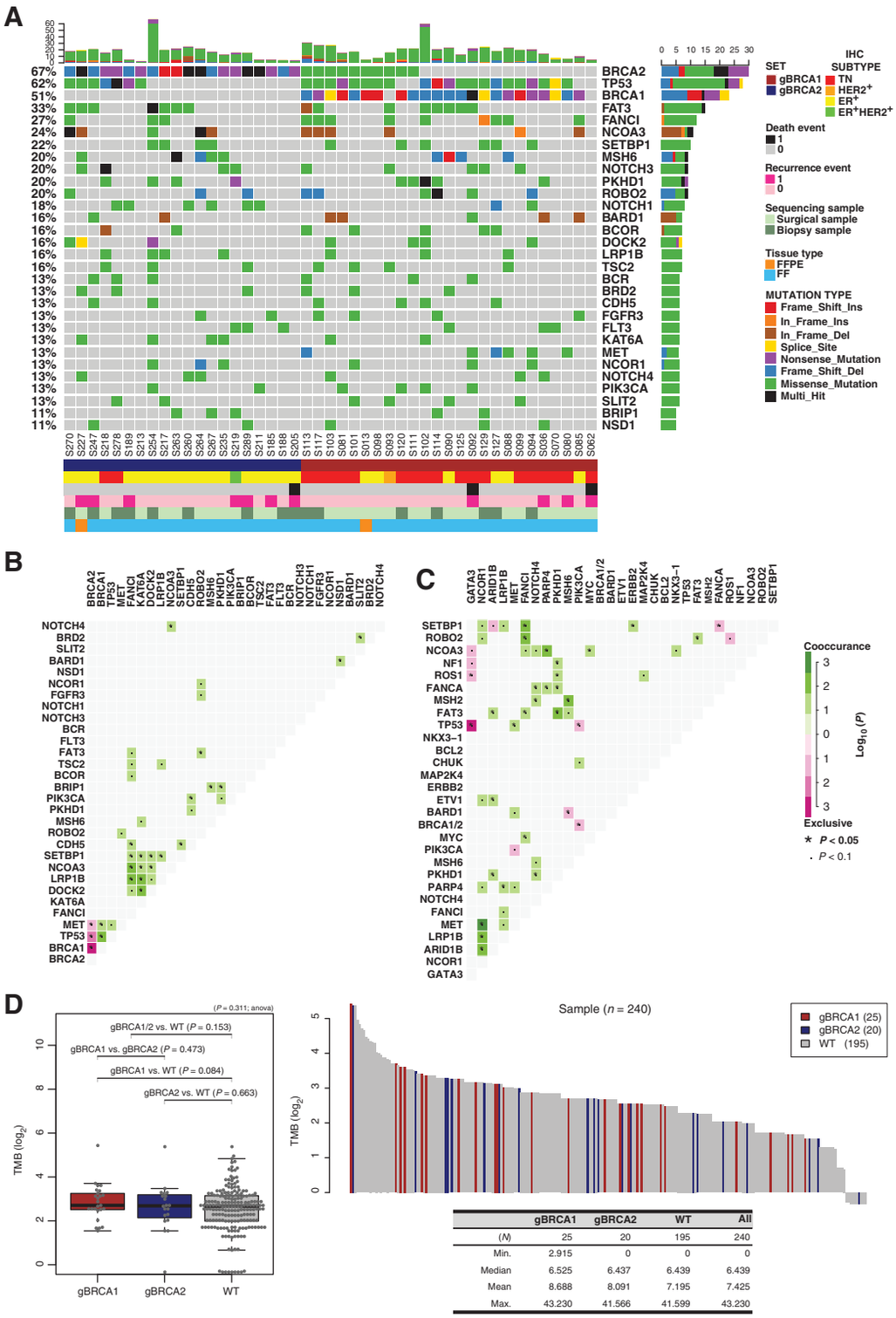
In our initial analyses, we compared a merged study population with a WT reference group and identified 27 genes with significant differences. Representatively, gBRCA1/2 mutations showed upregulation of *CXCL5* and *FGFBP1* and downregulation of genes normally expressed in breast tissue, such as *TPSAB1, TPSB2*, and *ADIPOQ* (**Fig. 4D**; Supplementary Table S5). Additional functional annotation using gene ontology indicated that the 27 DEGs were clustered in serine-type endopeptidase activity and positive regulation of cell proliferation (Supplementary Table S6). Subgroup analyses were conducted by comparing gBRCA1 versus WT in patients with TNBC and gBRCA2 versus WT in ER-positive patients, and we found that genes and gene sets were expressed differently in each group (Supplementary Fig. S8; Supplementary Tables S7–S10).

## Discussion

Since the 1990s, when BRCA1 and BRCA2 were first reported to be associated with hereditary breast cancer (31, 32), their function and clinical utility have been researched extensively. Over the decades, this research has led to the approval of BRCA1/2-mutant–specific clinical treatments (12, 13). With easy access to genomic testing and the development of consensus guidelines for high-risk candidates, an increasing number of patients are being found to have germline BRCA mutations. Consequently, we have questioned the underlying carcinogenesis mechanism triggered by BRCA mutations.

As an initial step, we searched for the underlying coaltered somatic mutation profiles of breast cancer with gBRCA mutations. We emphasize that our results showed a significant cooccurrence of somatic *TP53* mutation only with gBRCA1 mutation, not with gBRCA2 mutation. The TP53 pathway plays a pivotal role in maintaining genomic stability through the $G_1$–S-phase checkpoint, which is activated by phosphorylated *BRCA1* (33, 34). Because a high incidence rate of somatic *TP53* mutation occurs only with gBRCA1 mutation, a second-hit alteration in *TP53* might trigger the carcinogenesis mechanism in gBRCA1-mutated breast cancer by dysregulating the cell cycles maintained by *TP53*. In addition, we noticed that the overall somatic *PIK3CA* mutation status among patients with gBRCA mutations was inconsistent with our knowledge of the general breast cancer population. The mutation rate was significantly lower in patients with breast cancer with gBRCA mutations (13.3%) than in those with WT gBRCA (27.7%). This finding is especially evident in ER-positive gBRCA2 patients (11.8%), even though the *PIK3CA* mutation is associated with hormone receptor–positive breast cancer (35).

Next, we questioned what underlying genomic profile might eventually generate the different molecular subtypes found with gBRCA1 and gBRCA2 mutation (36). As in previous reports, our data demonstrate a high incidence of TNBC with a gBRCA1 mutation and ER-positive breast cancer with a gBRCA2 mutation. As mentioned previously, we thought that the coalteration of gBRCA1 and *TP53*, which is enriched in the basal-like subtype (37), might be related to the unique carcinogenesis mechanism of TNBC with a gBRCA1 mutation. In a similar manner, we focused on the genomic instability caused by hormones as a potential mechanism of carcinogenesis with gBRCA2 mutations. In this study, we identified upregulated hormone-related genes, *ESR1, AR*, and *PGR*, only with a gBRCA2 mutation. At the same time, it has been reported that the baseline estrogen level is higher than




| | gBRCA1 | gBRCA2 | WT | All |
|---|---|---|---|---|
| (N) | 25 | 20 | 195 | 240 |
| Min. | 2.915 | 0 | 0 | 0 |
| Median | 6.525 | 6.437 | 6.439 | 6.439 |
| Mean | 8.688 | 8.091 | 7.195 | 7.425 |
| Max. | 43.230 | 41.566 | 41.599 | 43.230 |

**Figure 2.**

**A,** Coaltered somatic mutations in patients with gBRCA1 mutation ($n = 25$) and gBRCA2 mutation ($n = 20$) with data for additional deep target sequencing (CancerSCAN). Association between identified somatic mutation and gBRCA1 or gBRCA2 mutations (**B**) and merged gBRCA1/2 mutation (**C**). **D,** Estimated TMB using CancerSCAN. $ER^+$, ER positive; FFPE, formalin-fixed, paraffin-embedded; $HER2^+$, HER2 positive; TN, triple negative.

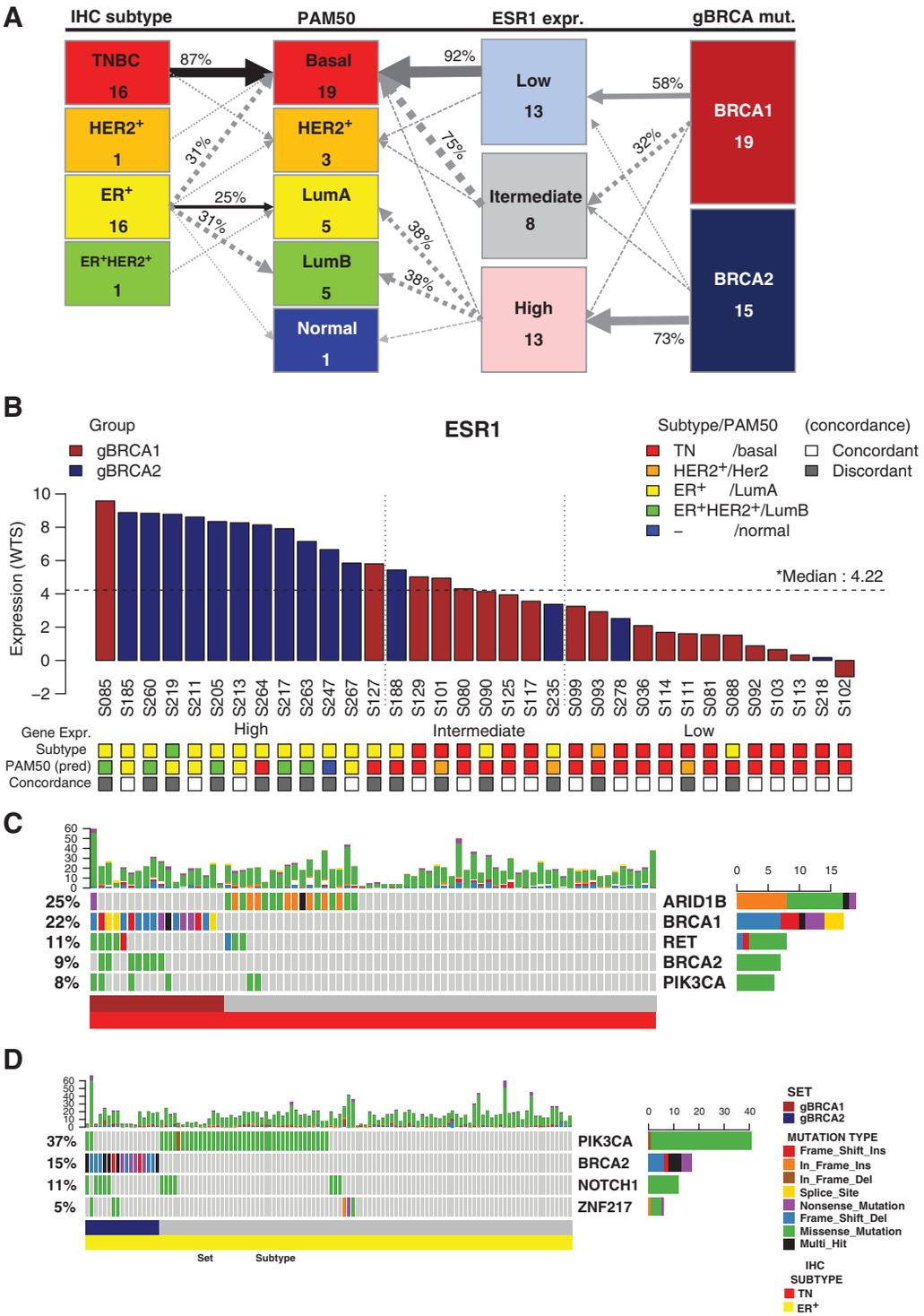



**Figure 3.**

**A,** Concordance between molecular subtypes identified by IHC and PAM50 in patients ($n = 34$) with data for whole-transcriptome sequencing. *ESR1* expression is arbitrarily classified as low ($n = 13$), intermediate ($n = 9$), and high ($n = 13$). **B,** *ESR1* expression profile of patients with WTS data ($n = 34$) is visualized on the basis of gBRCA mutation status as bar graph. In addition, subtypes analyzed by pathologic review and PAM 50 are marked at the bottom of each bar. **C,** Representative somatic mutation identified in triple-negative (TN) subtype in gBRCA1 mutant (mut). The list of genes that were either cooccurrent or mutually exclusive with gBRCA1, which satisfy $P < 0.05$ by Fisher exact test. **D,** Similarly, the list of genes in ER-positive subtype in gBRCA2 mutant showing statistically significant difference incidence ($P < 0.05$). ER$^+$, ER positive; HER2$^+$, HER2 positive; LumA, Luminal A; LumB, Luminal B.

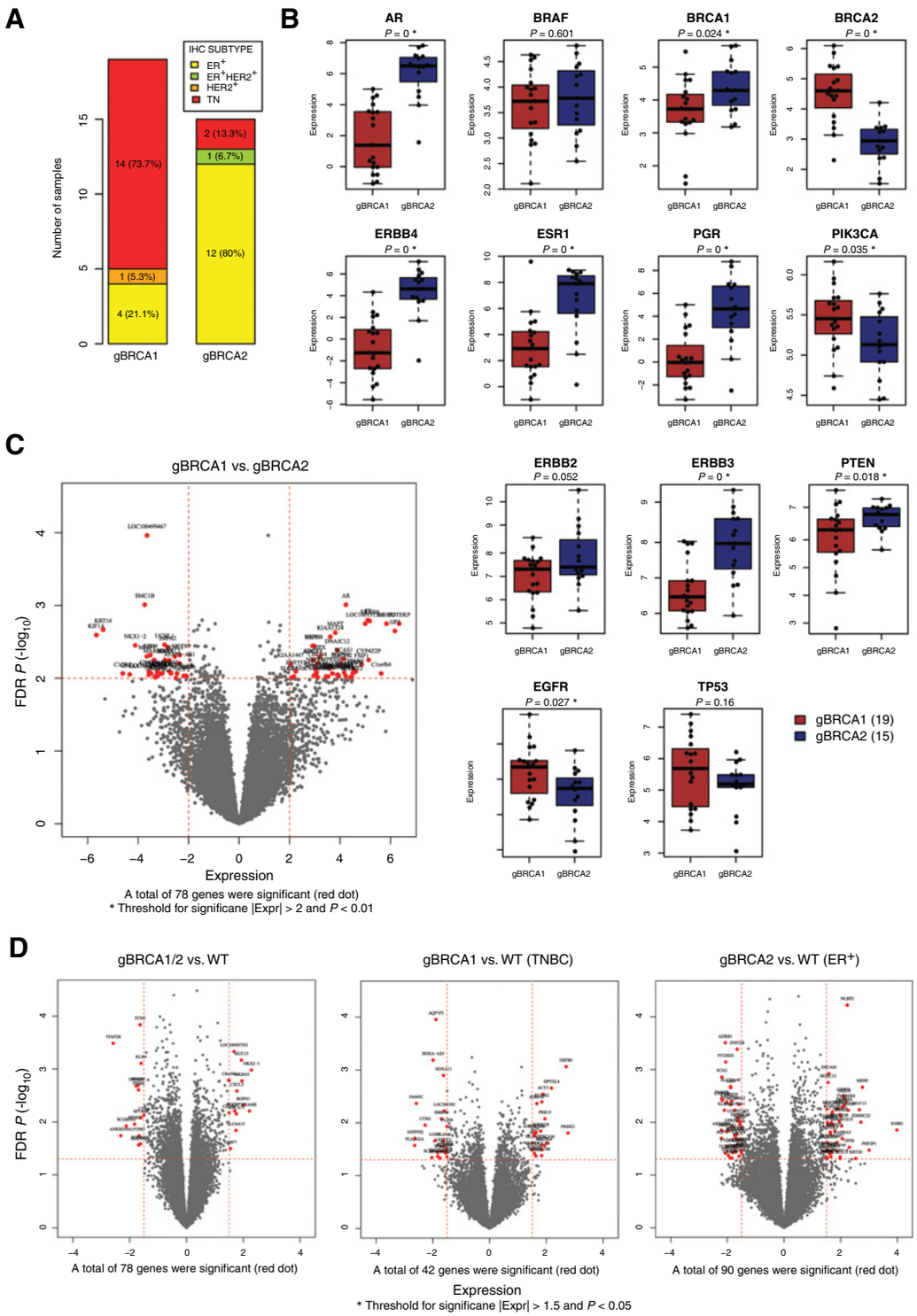



**Figure 4.**

**A,** Molecular subtype by IHC with gBRCA1 ($n = 19$) and gBRCA2 ($n = 15$) mutations with data for whole-transcriptome sequencing. **B,** Expression profile of preselected representative genes in gBRCA1 and gBRCA2 mutants. **C,** DEGs between gBRCA1 and gBRCA2. A red dot indicates a gene with significance. **D,** DEGs between gBRCA mutants and WT patients in merged population [gBRCA1/2 ($n = 34$) vs. WT ($n = 137$)], in triple-negative patients [gBRCA1 ($n = 14$) vs. WT ($n = 65$)], and in ER-positive patients [gBRCA2 ($n = 12$) vs. WT ($n = 78$)]. A red dot indicates a gene with significance. $ER^+$, ER positive; $HER2^+$, HER2 positive; TN, triple negative.

normal in those with a gBRCA2 mutation but without cancer (38). Normally, estrogen stimulation binds to the hormone receptor to promote transcription (39), which leads to the frequent formation of RNA–DNA hybrid (R-loop) structures. The abundant formation of R-loops is a replication stress status that has the potential to cause genomic instability, which is normally prevented by the action of BRCA1 or BRCA2. Therefore, by creating an environment with excess estrogen and the absence of damage control, gBRCA2 deficiency could be a pivotal element in carcinogenesis, which explains the high incidence of hormone receptor–positive breast cancer associated with gBRCA2 mutation. Interestingly, this explains the outcomes from a previous clinical trial, NSABP-P1, in which tamoxifen-treated gBRCA2 carriers showed a 62% reduction in breast cancer incidence compared with the placebo-treated group (40). This supposition should be confirmed through functional validation. As an extension of our analyses, we examined the difference between gBRCA1/2-mutant and WT breast cancer with the same molecular subtype. We found different patterns in gene expression and gene sets between patients with mutant gBRCA and those with the WT, although it was difficult to provide clear clinical implications (Supplementary Fig. S8; Supplementary Tables S7–S10).

Finally, we searched for the link between our results and cancer immunotherapy, which has become part of the standard treatment in breast cancer (41). Along with the clinical trials, there has been an effort to develop predictive biomarkers for immunotherapy. Among the candidates, genome-based biomarkers, such as those involved in microsatellite instability (42, 43) and the high TMB caused by DDR pathway alteration, seem to provide an early signal in patient selection for an immune checkpoint inhibitor (ICI; refs. 44, 45) therapy. In addition, the potential synergy between blocking DDR pathways, especially using PARP inhibitors, and ICI is under investigation in multiple clinical trials in breast cancer (46). This approach is based on a previous report showing that PARP inhibition can potentiate DNA damage and lead to inefficient repair, which could lead to the generation of immunologically relevant tumor antigens (47, 48). Unlike our assumption that alteration in the BRCA pathway could increase the total TMB, the median TMB was 6.53 per MB in gBRCA1-mutated patients and 6.55 per MB in gBRCA2-mutated patients, which is no different from the patients with WT BRCA in our dataset and previous reports (49). However, because of our limited number of samples, this finding should also be validated with larger cohorts.

Despite screening a relatively large number of patients, the number of paired samples for the genomic analyses was small and following hypotheses have been presented descriptively. In addition, the method for the genomic analyses was mostly restricted to the somatic alteration. Because this panel was based on tumor sample without matched normal, it was not feasible for the detail analyses, such as loss of WT allele, rearrangement burden, structural alteration, and rearrangement pattern, as previously evaluated by Nik-Zainal and colleagues in breast cancer (50). To elucidate the detail landscape of somatic mutation in germline BRCA mutant, in-depth analyses using the whole-genome sequencing should be conducted as a future work.

Nonetheless, to our knowledge, this is one of the largest sample sets ever used to comprehensively compare gBRCA-mutant samples and the first study to analyze genomic characteristics, including somatic-targeted sequencing, for a population of patients with gBRCA mutations, even retrospectively. Moreover, this study includes novel findings, such as discordance between IHC and PAM50 classification in ER-positive patients, which provides the clinical implication that ER-positive IHC patients with a gBRCA-mutant subset could have low expression of ESR1, which should be tested using an additional modality.

In conclusion, our results show that patients with breast cancer with gBRCA1 and gBRCA2 mutations have distinct genomic backgrounds and gene expression patterns compared with patients with WT gBRCA, and those genomic differences could affect the mechanisms of carcinogenesis in a way that leads to different molecular subtypes.

## Disclosure of Potential Conflicts of Interest

J.S. Ahn reports personal fees from Amgen, Pfizer, AstraZeneca, Menarini, Roche, Boehringer-Ingelheim, BMS-Ono, MSD, Janssen, and Samsung Bioepis outside the submitted work. Y.H. Park reports grants and non-financial support from Pfizer, AstraZeneca, Novartis, Merck, Eisai, Roche, and Hanmi, and personal fees and non-financial support from Alteogen outside the submitted work. No potential conflicts of interest were disclosed by the other authors.

## Authors' Contributions

**S. Park:** Conceptualization, resources, data curation, software, formal analysis, validation, investigation, visualization, methodology, writing-original draft, project administration, writing-review and editing. **E. Lee:** Resources, data curation, formal analysis, visualization, methodology, writing-original draft, writing-review and editing. **S. Park:** Resources, data curation. **S. Lee:** Resources, data curation. **S.J. Nam:** Resources, data curation. **S.W. Kim:** Resources, data curation. **J.E. Lee:** Resources, data curation. **J.-H. Yu:** Resources, data curation. **J.-Y. Kim:** Resources, data curation. **J.S. Ahn:** Resources, data curation. **Y.-H. Im:** Resources, data curation. **W.-Y. Park:** Software, supervision, project administration. **K. Park:** Data curation, formal analysis, validation, methodology. **Y.H. Park:** Conceptualization, resources, data curation, software, formal analysis, supervision, funding acquisition, validation, investigation, visualization, methodology, project administration, writing-review and editing.

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