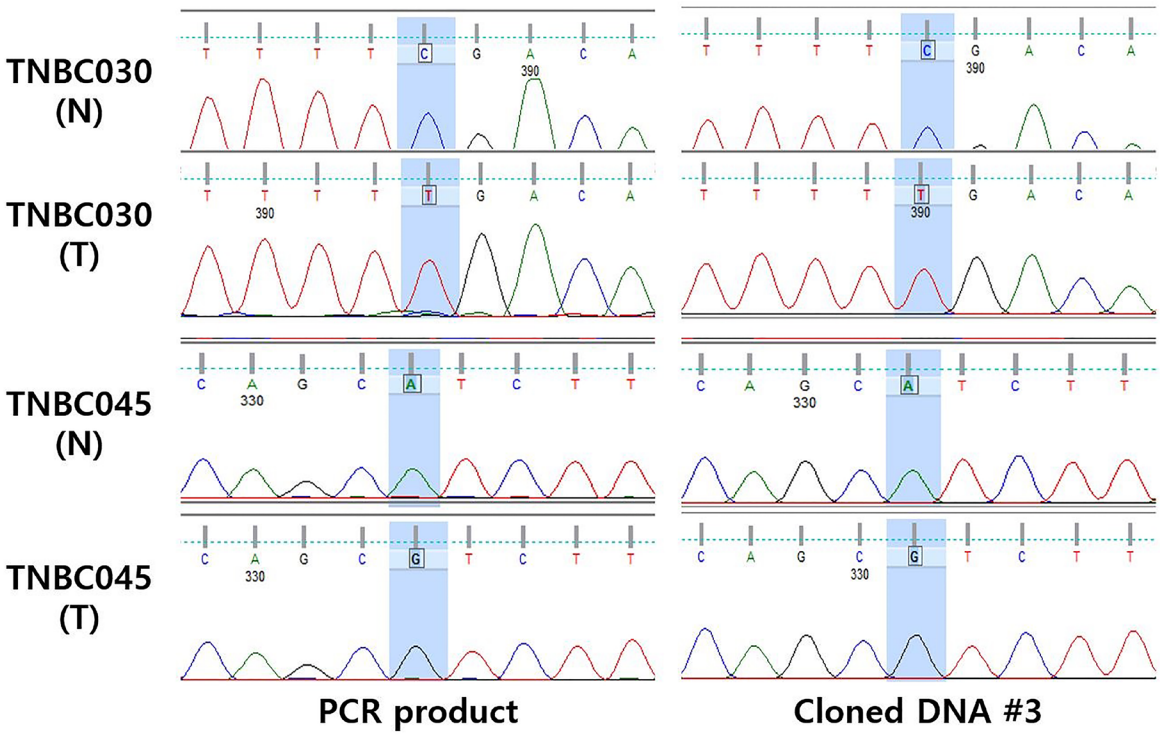
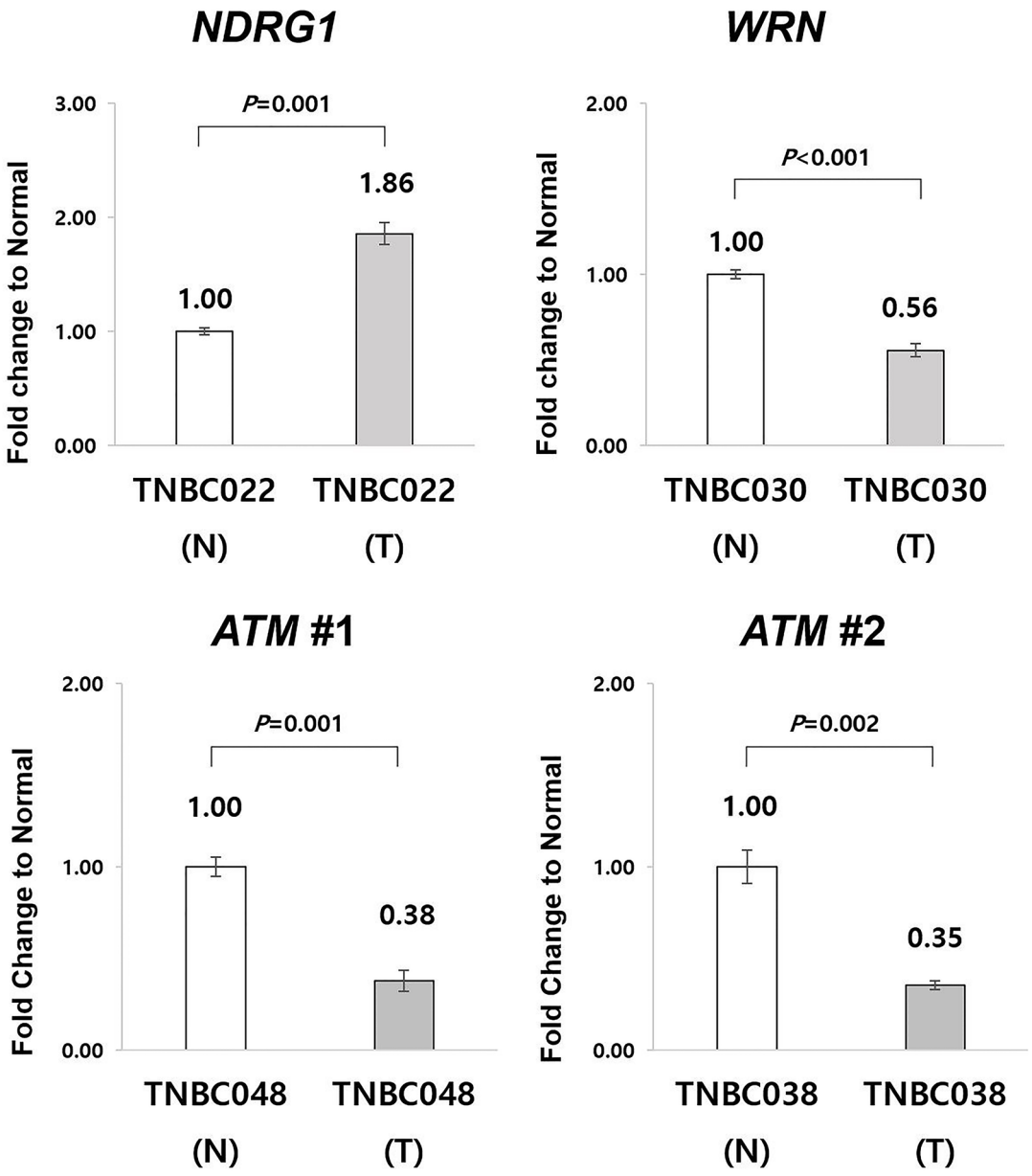
# Targeted exome sequencing of Korean triple-negative breast cancer reveals homozygous deletions associated with poor prognosis of adjuvant chemotherapy-treated patients

## SUPPLEMENTARY MATERIALS

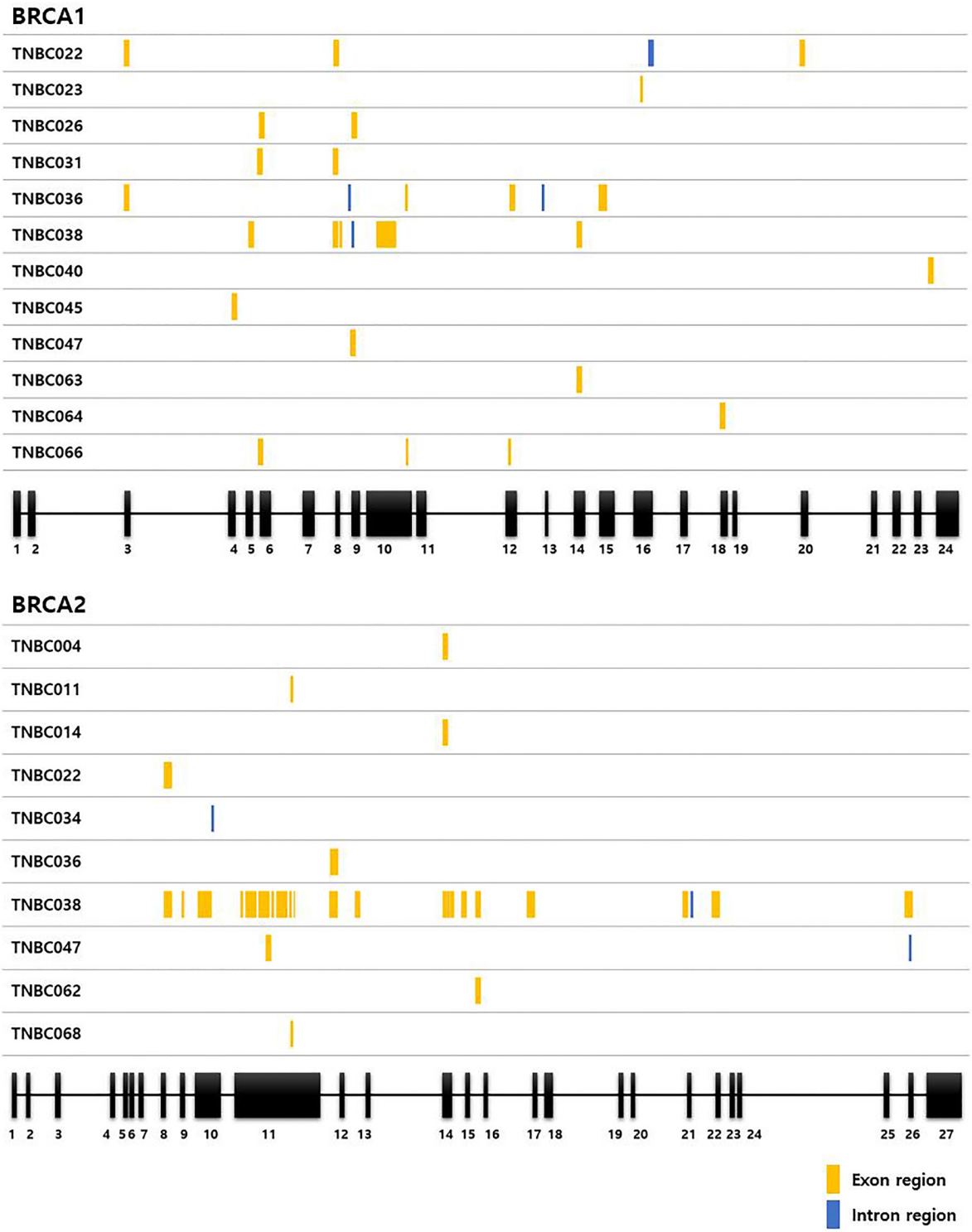
| Sample | Targeted NGS | Sanger sequencing | | | | | |
|---|---|---|---|---|---|---|---|
| | | PCR product | Cloned DNA | | | | |
| | | | #1 | #2 | #3 | #4 | #5 |
| TNBC030 (N) | 637C | 637C | 637C | 637C | 637C | 637C | 637C |
| TNBC030 (T) | 637C>T | 637C>T | 637C | 637C>T | 637C>T | 637C>T | 637C>T |
| TNBC045 (N) | 578A | 578A | 578A | 578A | 578A | 578A | 578A |
| TNBC045 (T) | 578A>G | 578A>G | 578A>G | 578A>G | 578A>G | 578A>G | 578A>G |




**Supplementary Figure 1: Single nucleotide variant (SNV) validation by Sanger sequencing.** Experimental validation of targeted exome-sequencing data. Two frequently occurring *TP53* mutations (637C>T and 578A>G) were validated by Sanger sequencing. A single 637C>T clone (clone #1) was identified as wild type, perhaps because of the contamination of the cryopreserved tumor sample.




**Supplementary Figure 2: Copy number variation (CNV) validation by quantitative PCR (qPCR).** Frequently occurring amplifications of *NDRG1* and deletions of *WRN* and *ATM* were validated by qPCR. All experiments were performed in triplicate and demonstrate significant alterations in gene expression in tumor samples.




**Supplementary Figure 3: Localization of deleted regions of *BRCA1* and *BRCA2* in individual patients.** Homozygous deletions of regions of *BRCA1* and *BRCA2* in each corresponding patient are displayed in detail. Deletions in individual patients were observed in a single exon or in multiple exons.

| Gene | *TP53* | *UBR5* | *MYC* | *EXT1* | *NDRG1* | *BRD4* | *WRN* | *NOTCH3* | *NOTCH4* |
|---|---|---|---|---|---|---|---|---|---|
| ***TP53*** | --- | <0.000001 | <0.000001 | <0.000001 | <0.000001 | <0.000001 | <0.000001 | 0.000133 | 0.49133 |
| ***UBR5*** | | --- | <0.000001 | <0.000001 | <0.000001 | 0.009468 | 0.000013 | 0.039225 | 0.082807 |
| ***MYC*** | | | --- | <0.000001 | <0.000001 | 0.000004 | 0.016019 | 0.164945 | 0.343477 |
| ***EXT1*** | | | | --- | <0.000001 | 0.000022 | 0.051449 | 0.168537 | 0.174497 |
| ***NDRG1*** | | | | | --- | 0.000016 | 0.082249 | 0.199102 | 0.167127 |
| ***BRD4*** | | | | | | --- | 0.055443 | <0.000001 | 0.469896 |
| ***WRN*** | | | | | | | --- | 0.432464 | 0.077401 |
| ***NOTCH3*** | | | | | | | | --- | 0.000001 |
| ***NOTCH4*** | | | | | | | | | --- |

*p*-values <0.05, as derived via Fisher's Exact test are outlined in red.
*p*-values are not adjusted for FDR.

| Legend |
|---|
| Strong tendency towards mutual exclusivity (0 < Odds Ratio < 0.1) |
| Some tendency towards mutual exclusivity (0.1 < Odds Ratio <0.5) |
| No association (0.5 < Odds Ratio < 2) |
| Tendency toward co-occurrence (2 < Odds Ratio < 10) |
| Stron tendency towards co-occurrence (Odds Ratio > 10) |
| No events recorded for one or both genes |

**Supplementary Figure 4: Mutual exclusivity analysis using The Cancer Genome Atlas (TCGA) breast cancer database.** Significantly co-occurring mutations were observed in the DNA damage response-related genes, *TP53*, *MYC*, *WRN*, *NDRG1*, *NOTCH3*, *UBR5*, and *BRD4*.

**Supplementary Table 1: Associations between clinicopathological features and disease-free survival (DFS) or distant metastasis-free survival (DMFS)**

See Supplementary File: 1

**Supplementary Table 2: Targeted exome-sequencing statistics**

| Target Sequencing Statistics | Tumor Sample | Normal Sample |
|---|---|---|
| Target Territory (bp) | 2,364,198 | 2,364,198 |
| Average Target Coverage (X) | 130.36 | 139.71 |
| % of 1x Target Bases | 4.25 | 4.35 |
| % of Target Bases ≥ 2x | 93.35 | 93.12 |
| % of Target Bases ≥ 10x | 86.23 | 86.69 |
| % of Target Bases ≥ 20x | 78.47 | 79.56 |
| % of Target Bases ≥ 30x | 71.67 | 73.13 |
| % of Target Bases ≥ 40x | 65.65 | 67.36 |
| % of Target Bases ≥ 50x | 60.33 | 62.21 |
| % of Target Bases ≥ 100x | 40.64 | 42.57 |

Target sequencing statistics of 140 samples (70 pairs of tumor and normal samples). The distribution of read coverage depths was similar in tumor and normal samples with average target coverage greater than 130×, which is sufficient for mutation analysis.

**Supplementary Table 3: Number of genes with somatic variants or copy number variations (CNVs)**

| Somatic Variants | | Mutation Number | Total | Number of Mutated Genes |
|---|---|---|---|---|
| Somatic SNVs | Novel | 220 | 292 | 157 |
| | COSMIC or dbSNP | 72 | | |
| Somatic Deletions | Novel | 11 | 21 | |
| | COSMIC or dbSNP | 10 | | |
| Somatic Insertions | Novel | 7 | 9 | |
| | COSMIC or dbSNP | 2 | | |
| Copy Number Alterations Genes | | | | Number of Altered Genes |
| Amplification | | | | 365 |
| Homozygous deletion | | | | 346 |

Patients with triple-negative breast cancer (TNBC) were found to have 157 mutated genes, 365 amplified genes, and 346 deleted genes. Most of the somatic variants were novel single nucleotide variants (SNVs).

**Supplementary Table 4: Complete list of somatic mutations identified in this study, along with their chromosomal positions, frequency, and mutation type**

See Supplementary File: 1

**Supplementary Table 5: *BRCA1* and *BRCA2* germline mutations**

See Supplementary File: 1

**Supplementary Table 6: List of all genetically altered genes**

See Supplementary File: 1

**Supplementary Table 7: Result of Cox proportional hazard ratio analysis**

| Gene Name | Homozygous Deletion Event | n (%) | Recurrence | | DFS | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | yes (%) | no (%) | HR | low CI | high CI | p-value | Benjamini -Hochberg adjusted p-value |
| *EPHA5* | No | 59 (88.1) | 8 (13.6) | 51 (86.4) | | | | | |
| | Yes | 8 (11.9) | 6 (75.0) | 2 (25.0) | 7.0154 | 2.4197 | 20.340 | 0.0003 | 0.0355 |
| *MITF* | No | 61 (91.0) | 9 (14.8) | 52 (85.2) | | | | | |
| | Yes | 6 (8.96) | 5 (83.3) | 1 (16.7) | 9.2915 | 2.9141 | 29.625 | 0.0002 | 0.0349 |
| *ACSL3* | No | 63 (94.0) | 10 (15.9) | 53 (84.1) | | | | | |
| | Yes | 4 (5.97) | 4 (100) | 0 (0.00) | 7.7495 | 2.3721 | 25.317 | 0.0007 | 0.0494 |
| Total | | 67 (100) | 14 (20.9) | 53 (79.1) | | | | | |
| | | | Distant metastasis | | DMFS | | | | |
| | | | yes (%) | no (%) | HR | low CI | high CI | p-value | Benjamini -Hochberg adjusted p-value |
| *MITF* | No | 61 (91.0) | 4 (6.56) | 60 (93.4) | | | | | |
| | Yes | 6 (8.96) | 4 (66.7) | 2 (33.3) | 26.346 | 4.796 | 144.72 | 0.0002 | 0.0355 |
| Total | | 67 (100) | 8 (11.9) | 59 (88.1) | | | | | |

Homozygous deletions of three genes were significantly associated with disease-free survival (DFS), and *MITF* was significantly associated with distant metastasis-free survival (DMFS).

CI, confidence interval; DFS, disease-free survival; DMFS, distant metastasis-free survival; HR, hazard ratio.

**Supplementary Table 8: Comparisons of frequently altered genes in this cohort of Korean patients with triple-negative breast cancer (TNBC) and Western European-North American (WENA) patients with TNBC**

See Supplementary File: 1

**Supplementary Table 9: Full list of the 368 target genes analyzed in this study**

See Supplementary File: 1

**Supplementary Table 10: Validation regions and primer sequences**

See Supplementary File: 1