
**eMethods.** Supplemental Methods

**eFigure 1.** Hierarchical Clustering of 142 Genes Significantly Different Between Black and White Patients After Adjusting for Age, Subtype, and Possible Batch Effects

**eFigure 2.** Clustering of Top 25 Proteins Significantly Different Between Black and White Patients After Adjusting for Age, Subtype, and Possible Batch Effects

**eFigure 3.** Numbers of Somatic Mutations in Coding Sequences (A) and Copy Number Alteration (B) by Breast Cancer Subtype and Race

**eFigure 4.** DNA Hypermethylated Probes Significantly Different Between Black and White Patients After Adjusting for Age, Subtype, and Tumor Purity

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**eFigure 6.** Kaplan-Meier Curves of Overall Survival for All Patients (A), Patients With Basal and Non-Basal Tumors (B), and Patients With Triple-Negative and Non-Triple-Negative Tumors (C). The analyses were performed using all followup time, though we plotted the curves for the first 10 years only.

This supplementary material has been provided by the authors to give readers additional information about their work.
Supplemental Methods and Online Figures

1. Genomic race determination

DNA samples from blood or normal breast were genotyped using Affymetrix SNP 6.0 arrays. For patients with both blood and normal breast samples, we utilized the genotype data from blood only. Uncorrelated single nucleotide polymorphisms from the TCGA cohort and the International HapMap Project were included in the principal component analysis using the eigenstrat package. The top two eigenvectors from principal component analysis were plotted and the three known continental ancestry groups from the HapMap Project (CEU, YRI, and ASN) were used as anchors. The proportion of ancestry relative to the reference continental groups for each patient was estimated by projecting the eigenvectors onto each of the three axes defined by the three anchors. According to the estimated proportion of ancestry, patients were grouped into genomic black (≥50% African ancestry) and genomic white (≥90% European ancestry). Figure below shows these ancestry proportions for the 930 patients included in this study. The cross table of reported and genomically defined ancestry groups are shown below. The mean African ancestry proportion for patients in the genomic black group was 0.801 (SD=0.106) and the mean European ancestry proportion for patients in the genomic white group was 0.990 (SD=0.019).

![Proportion of Ancestry](image)

<table>
<thead>
<tr>
<th>Reported Race</th>
<th>Genomic race: Black</th>
<th>Genomic race: White</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black</td>
<td>151</td>
<td>0</td>
<td>151</td>
</tr>
<tr>
<td>White</td>
<td>2</td>
<td>689</td>
<td>691</td>
</tr>
<tr>
<td>n/a</td>
<td>1</td>
<td>87</td>
<td>88</td>
</tr>
<tr>
<td>Total</td>
<td>154</td>
<td>776</td>
<td>930</td>
</tr>
</tbody>
</table>

2. HER2 status call

HER2 clinical status calls were made following CAP guidelines as described [1]. IHC-based clinical status calls were remade as described in our previous paper [2], and FISH status calls
were retained since there was a high consistency in this dataset between FISH status call and
FISH score at >99%. For cases without an IHC or FISH status call, DNA copy number data were
used to make the call. Of the 1098 cases, 1080 had DNA copy number (CN) data of which 780
overlapped with the 2012 dataset [2]. Despite the difference in the upper cap of the copy number
data which were 6.0 in the 2012 dataset and 3.657 in the current dataset, there was a high
consistency between the data points that were not capped with a Correlation=0.987 and P<2.2e-
16 where 2015 CN = 0.985 * 2012 CN. Using this transformation formula we derived double-
thresholds of 0.54 (from 0.55 for the 2012 dataset) and 1.66 (from 1.69 for the 2012 dataset)
such that a case with CN < 0.54 would be called HER2 negative, whereas a case with CN > 1.66
would be called HER2 positive. Using these thresholds, we observed that for cases with FISH
status calls in the original clinical table, CN-based HER2 negative status calls (n=319) were 93%
consistent with the original clinical FISH status calls (n=296), and CN-based HER2 positive
calls (n=46) were 100% consistent with the original clinical FISH status call (n=46). When
limiting to cases with both clinical FISH status and FISH score information, the consistency
levels were 96% (n=188 and 181 respectively) for CN-based HER2 negative cases and 100%
(n=19 and 19 respectively) for CN-based HER2 positive cases. The following table shows the
HER2 clinical status call for the 1080 cases with CN data.

<table>
<thead>
<tr>
<th>CAP: IHC and FISH</th>
<th>CN Calls</th>
<th>Final Call</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equivocal or N/A</td>
<td>387</td>
<td>26</td>
</tr>
<tr>
<td>Negative</td>
<td>554</td>
<td>335</td>
</tr>
<tr>
<td>Positive</td>
<td>139</td>
<td>26</td>
</tr>
<tr>
<td>Total</td>
<td>1080</td>
<td>387</td>
</tr>
</tbody>
</table>

Further comparison of the current HER2 final calls with the 2012 final calls show that, among
the 790 common cases in the current clinical dataset (n=1098) and the 2012 dataset (n=791),
there were only 7 cases whose CN-based HER2 status calls were changed, all from a non-
definitive status (Equivocal or N/A) to a definitive status (Positive or Negative) calls or vice
versa due to the new CN values or CN thresholds. On the contrary, in this same dataset of 790
cases, 12 had clinical status call changes by the BCR, including 7 from previous Negative calls
to current Positive calls.

3. DNA methylation analysis

We measured DNA methylation levels of breast tissue samples (1,000 breast tumor tissue
samples and 125 adjacent non-malignant breast tissue samples) using Illumina Infinium DNA
methylation HumanMethylation 27 (HM27) and HumanMethylation 450 (HM450) as previously
described [3]. To assess more genomic loci, we selected 124 AA cases and 517 White cases that
had been assayed on the HM450 for this study. Among 485,777 probes from HM450, 1% most
variable probes among 31 control cell line technical replicates were excluded to reduce technical
variations, resulting 480,721 probes.

Among 480,721 probes, we found 19,090 cancer associated hypermethylated probes in tumors
(beta value lower than 0.2 at least 95% of normal tissues and above 0.3 in at least 5% of tumors
without regard to race) after comparison with DNA methylation profiles of 79 normal breast
tissue samples (4 AA cases, 75 White cases). DNA hypermethylation at promoter region may
result in silencing of gene expression. Of the 19,090 hypermethylated probes, 13,811 probes located in promoter regions (< 1,500bp of transcription start site (TSS)) were used to fit linear regression model between DNA methylation and race adjusting age, purity, and PAM50 subtypes.

Next, we examined DNA hypomethylation associated with cancer and found 27,108 probes, which were hypomethylated in tumors compared to normal samples (beta value above 0.8 in at least 95% of normal tissues and below 0.7 in at least 5% of tumors without regard to race). Transcription factor binding at enhancer region may cause DNA hypomethylation, leading to changes in gene expression [4]. Therefore, we selected putative enhancer regions determined by ChromHMM in 9 cell types (GM12878, H1-hESC, K562, HepG2, HUVEC, HMEC, HSMM, NHEK, NLH) by ENCODE [5], identifying 9,436 hypomethylated probes in enhancer regions. To investigate effect of race on DNA methylation levels of these probes, linear regression model was used after adjusting age, purity and PAM50 subtypes.

After identifying statistically significantly associated probes (both hypermethylated promoter and hypomethylated enhancer probes) by race adjusting age, purity, and PAM50 subtypes (multiple testing adjusted p-value <0.05), we measured empirical p-values after generating 1,000 datasets with randomly scrambled labels on race to ensure the significant DNA methylation differences by race (empirical p-value <0.001). DNA methylation levels of 11 hypermethylated promoter probes and 8 hypomethylated enhancer probes were found to be statistically significantly associated with race (multiple testing adjusted <0.05, empirical p-value <0.001). We further excluded two hypermethylated promoter probes and one hypomethylated enhancer probes with known SNPs (dbSNP 142) inside the probe sequence, which can result in distorted DNA methylation measurements caused by disruption of probe hybridization. To compare DNA methylation levels of identified probes in breast tissues with in blood, we downloaded HM450 DNA methylation data for HapMap lymphoblastoid cell lines (73 YRI cases and 60 CEU cases) from GSE39672.

In order to assess the gene expression level associated with DNA methylation change of hypermethylated probes at promoter regions, level 3 RNA-seq RSEM data for 641 tumor cases and 66 normal cases, whose DNA methylation data were also available, were obtained from TCGA Data portal (http://tcga-data.nci.nih.gov/tcga). Level 3 RNA-seq RSEM data were log2 transformed (log2(RSEM+1)) to generate scatterplots (x-axis: DNA methylation, y-axis: gene expression).

4. Survival Analysis Outcomes

Breast cancer-free interval (BCFI) recurrence and overall survival (OS) analyses were used in this study [6]. Events for BCFI were defined as 1) locoregional recurrences, 2) distant metastases, or 3) new primary tumors in the breast. In this cohort there was no recorded death from breast cancer without a defined new breast tumor event. In the BCFI analysis, 17 patients with stage IV breast cancer were excluded since the patients had already developed distant metastasis at the time of diagnosis. Another 17 patients who died with tumor but without a defined new tumor event were also excluded from the analysis because there was no information whether the new tumor was in the breast (event) or not (censored). Since all the 17 patients were
white and 15 of them were from one site, we were concerned of a selection bias when cases were
supplied to TCGA Biospecimen Core Resource (BCR). Examining into the earlier paperwork, it
turned out that in earlier versions of the forms there was no specific instruction to indicate the
requirement that the tumor should refer to a breast tumor, so it was possible that some sites
selected “with tumor” regardless the tumor was related with breast tumor. BCR has no way to
find additional information to determine which of these tumors were breast tumors, thus they
could not be considered as Event (tumor in breast) or censored (tumor not in breast) and there
was no other choice but to exclude them from the BCFI analysis. There were also 12 patients
who had a new tumor event not in breast, and they were censored in this BCFI analysis. To
obtain a full picture how these 29 patients might affect the conclusions drawn from this study,
additional analyses were performed for different combinations of inclusion and exclusion of
these two groups of patients, resulting additional 4 sets of results (data not shown). In the final
models of these 4 analyses, only when both the 17 cases and the 12 cases were excluded was
there a significant racial disparity in survival. From these results, we draw the conclusion that
only by using strictly defined BCFI events could a significant racial disparity in survival be
detected in this dataset.

In this study we did not use the field “Tumor Status” in the main clinical table; instead we
carefully derived all the Events by using more original fields containing dates and recurrence or
vital status information. For a number of cases our derived tumor status was different from what
was shown in the “Tumor Status” field. Our observations seem to have extended the results
reported by Keenan et al in 2015 where an earlier version of the clinical data were available
reporting with only 35 recurrent events [7]. We were grateful that the authors provided us with
their dataset at our request when we tried to find out the cause why our conclusions were not
exactly the same as theirs. A careful evaluation of their dataset indicated that for a few cases our
Event calls were different from theirs, including 3 locoregional recurrences that we called as
Events which they did not. Perhaps partially because of such discrepancies and partially because
of a larger dataset we now have with a slightly longer follow-up time, the racial disparity for
BCFI in our analysis sustained adjustment for age, stage, and PAM50 subtypes, whereas in their
study race was significant in BCFI analysis after adjusting for age and stage but not after
adjusting for PAM50 subtypes.

Reference

American Pathologists guideline recommendations for human epidermal growth factor receptor 2
5. Ernst J, Kheradpour P, Mikkelsen TS, et al. Mapping and analysis of chromatin state dynamics in

Supplementary Figures

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