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Genome-wide Copy Number Alterations in Subtypes of Invasive Breast Cancers in Young White and African American Women

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Abstract

Genomic copy number alterations (CNA) are common in breast cancer. Identifying characteristic CNAs associated with specific breast cancer subtypes is a critical step in defining potential mechanisms of disease initiation and progression. We used genome-wide array comparative genomic hybridization (aCGH) to identify distinctive CNAs in breast cancer subtypes from 259 young (diagnosed with breast cancer at <55 years) African American (AA) and Caucasian American (CA) women originally enrolled in a larger population-based study. We compared the average frequency of CNAs across the whole genome for each breast tumor subtype and found that estrogen receptor (ER)-negative tumors had a higher average frequency of genome-wide gain ($p < 0.0001$) and loss ($p = 0.02$) compared to ER-positive tumors. Triple negative (TN) tumors had a higher average frequency of genome-wide gain ($p < 0.0001$) and loss ($p = 0.003$) than non-TN tumors. No significant difference in CNA frequency was observed between HER2-positive and negative tumors. We also identified previously unreported recurrent CNAs (frequency >40%) for TN breast tumors at 10q, 11p, 11q, 16q, 20p and 20q. In addition, we report CNAs that differ in frequency between TN breast tumors of AA and CA women. This is of particular relevance because TN breast cancer is associated with higher mortality and young AA women have higher rates of TN breast tumors compared to CA women. These data support the possibility that higher overall frequency of genomic alteration events as well as specific focal CNAs in TN breast tumors might contribute in part to the poor breast cancer prognosis for young AA women.

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Keywords

breast cancer; triple negative; genomic alteration; array comparative genomic hybridization; young women

Introduction

Breast cancer is a heterogeneous disease consisting of five major breast tumor subtypes, basal, human epidermal growth factor receptor 2 (HER2)-expressing/estrogen receptor (ER)-negative, luminal A, luminal B and normal-like. These subtypes have been shown to have distinct expression patterns, based on microarray profiling, and clinical outcomes [1–4]. Protein expression of ER, progesterone receptor (PR), and HER2, detected by standard immunohistochemical (IHC) assays, has been used to approximate the gene expression subtypes. Triple negative (TN) breast cancer (ER⁻, PR⁻, HER2⁻) overlaps with basal cancer, ER-positive cancers closely define luminal cancers and the ER-negative, PR-negative and HER⁺ cancers approximate the HER2-expressing subtype.

The TN breast cancer subtype is not synonymous with the basal phenotype and expression of basal markers such as epidermal growth factor receptor (EGFR) and/or basal cytokeratins highlight the heterogeneity of the TN grouping [5]. However, the TN subset of breast cancers is highly enriched for the basal phenotype [6,7,1,3]. As is the case for basal cancers, TN tumors arise at an earlier age than non-TN cancers, and are almost exclusively high grade tumors [8].

One of the most striking findings related to TN breast cancer is that African American (AA) women exhibit an almost two-fold higher prevalence of this breast cancer subtype than Caucasian American (CA) women. The prevalence of TN breast cancer has been reported as high as 40% in AA women [9,1,10]. Additionally, TN incidence rates have now been reported and are also nearly twice as high among AA compared to CA women [11]. Even though AA have an overall lower incidence of breast cancer than CA women, the higher likelihood of developing TN breast cancer might contribute to the higher mortality from breast cancer experienced by AA women compared with CA women [12–17,10].

In addition to the differentiation of breast cancer subtypes by gene expression profiling, genome-wide array comparative genomic hybridization (CGH) techniques have demonstrated that breast cancer subtypes are associated with characteristic copy number alteration (CNA) profiles [18–21]. These characteristic genomic alterations serve as useful markers for subtype classification and can be applied towards the identification of critical genes that are consistently lost or gained in the specific subtypes to impact tumor biology.

The acquisition of genomic alterations is a critical event in cancer formation, potentially impacting the expression patterns of individual genes or entire biological pathways. For this study of young women we used genome-wide array CGH to identify CNAs that are high frequency and/or different in frequency between breast cancer subtypes defined by protein expression of ER, PR and HER2. We also compared CNA profiles of TN breast cancers in young CA and AA women to identify biological factors that might contribute to the poor breast cancer prognosis for young AA women.

Materials and Methods

Tumor Specimens

Primary breast tumors were from AA or CA women ages 20–54 years previously enrolled in the population-based Atlanta Women’s Interview Study of Health (WISH) cohort, which included 950 women diagnosed with breast cancer between 1990 and 1992 [22]. When diagnosed, these women were residents of a 3-county metropolitan region of Atlanta, Georgia. The 259 breast tumors analyzed in the present study are the subset of the women in the WISH study with sufficient tumor tissue for testing. All tumors were reviewed and scored by an expert pathologist (P.L.P.) for tumor grade, ER, PR, and HER2 status [23].

Flow Cytometry Cell Sorting and DNA Extraction and Labeling

Flow cytometry was performed on macro-dissected and dissociated formalin-fixed, paraffin-embedded tumor samples to enrich for tumor cells by removing contaminating stromal and lymphocytic cells as previously described [24].

Genomic DNA was extracted from the flow sorted tumor cells (minimum of 100,000 cells per tumor) as previously described [25]. We quantified tumor genomic DNA by real-time PCR (Applied Biosystems, Foster City, CA) using two chromosome 2 specific probes at 2p25.3 (29,907–30,162) and 2q31.1 (21,407,882–21,408,181) with normal human female genomic DNA (Promega, Madison, WI) as the reference.

Whole-genome amplified and labeled samples were prepared as previously reported [21]. Briefly, ten nanograms each, of tumor and DpnII digested normal female reference DNA (Promega, Madison, WI), were random amplified and labeled with a Cy5- or Cy3-labeled primer, respectively, according to the method of Lieb et al., with modifications [26]. Labeled PCR products purified and combined with blocking agents (50 µg of human Cot-1 DNA and 100 µg of yeast tRNA) and hybridized to the microarray.

Array CGH

The array consists of 4320 human bacterial artificial chromosomes (BAC) with a median spacing of 413 kb when pericentric heterochromatic regions and the short arms of acrocentric chromosomes are excluded [21]. BAC clone locations are based on NCBI Build 36.1 of the human genome.

The Cy3- and Cy5- labeled genomic DNA and blocking agents were hybridized to the BAC array as described previously [21]. Arrays were scanned with a GenePix 4000A scanner (Axon Instruments Inc., Union City, CA); fluorescence data were processed with GenePix 3.0 image analysis software (Axon Instruments, Inc.). For each spot, \log_2 ratio = $\log_2(\text{Cy5}/\text{Cy3})$ and average \log_2 intensity = $[\log_2(\text{Cy5}) + \log_2(\text{Cy3})]/2$ were calculated, where Cy5 and Cy3 refer to the median foreground fluorescent signals of the tumor and reference DNA, respectively. The \log_2 ratios on each array were normalized and corrected for intensity-based location adjustment with a block-level lowess algorithm [27].

Statistical Analysis

Normalized aCGH data were processed using wavelet along the genome [28]. The processed aCGH values were then categorized into copy number loss, no change, and gain events using the cut-off \log_2 ratio -0.34 and 0.38 , for loss and gain respectively, where the cut-off values were chosen based on X-chromosome titration experiments, previously reported [21].

Sampling weights were incorporated into the analysis based on the larger cohort of 950 cases for analysis of the 259 cases that were analyzed by aCGH. (See Supplemental Data 1

for additional details of statistical analysis; Supplemental Data Table 1). We calculated the weighted average overall genome-wide frequencies of copy number gain or loss by race (AA/CA) and the following tumor subtypes: ER status (positive/negative), triple negative (yes/no), and HER2 status (positive/negative), where the genome-wide copy number gain (or loss) for a tumor was defined as number of clones showing gains (or losses) divided by the total number of clones. To adjust for possible confounding effects of age and stage, weighted multivariable logistic regression was performed to examine whether each comparison group differs in gains and losses at each of the 4320 clones, respectively. Given some clones may have no or few events of gains or losses, the *p*-values based on asymptotic distributions of the test statistics would be biased. To correct for this bias, the bootstrap method was used to obtain exact *p*-values. A total of 1000 bootstrap samples were used for each comparison.

Hierarchical clustering was performed using clones that show statistical significance in any of the comparisons to identify whether subtypes of tumors would cluster based on the profiles of copy number alterations. For the heatmap clustering, we used the euclidean distance as the dissimilarity function and complete linkage.

All the analyses were done using statistical software R version 2.6.0. The wavelet smoothing required package of 'waveslim'; the weighted logistic regression required package of 'survey' (<http://www.r-project.org/>). Throughout the paper, a *p*-value < 0.05 is considered statistically significant.

Results

Breast Tumor Characteristics of Young African American and Caucasian American Women

The individual and tumor characteristics of the 259 women (AA n=53 and CA n=206) such as age, vital status, ER, PR, HER2 expression status, grade, and stage are shown for all tumors and separately by race (Table 1). The samples in the present study, as observed in previous reports of this population-based Atlanta WISH cohort, show a significant racial difference in the distribution of tumors based on ER, PR, and HER2 expression status, as well as vital status, stage and grade for young AA compared to CA women [10,23,29] and, as described in the methods and Supplemental Data, a weighted analysis was performed to more accurately reflect the make-up of the original cohort.

Genome-wide Copy Number Alterations in Subtypes of Breast Cancer and Racial Groups

We compared the average genome-wide frequency of copy number gain and loss for individual tumors of IHC-defined breast cancer subtypes (Table 2). In the comparison between ER-positive and ER-negative tumors, ER-negative tumors had a significantly higher frequency of both copy number gain (gain: 6.9% versus 4.9% $p < 0.0001$) and loss (8.5% versus 7.3% $p = 0.02$) events. TN breast tumors similarly had a significantly higher overall frequency of both genome-wide copy number gain (7.3% versus 5.2%; $p < 0.0001$) and loss (8.9% versus 7.4%; $p = 0.003$) than non-TN tumors, respectively. There was no statistically significant difference in the overall genome-wide frequency of copy number gain (5.6% versus 5.8%; $p = 0.68$) and loss (7.3% versus 7.9%; $p = 0.37$) when comparing HER2-positive to HER2-negative tumors, respectively. When we compared overall frequency of genomic alterations in breast tumors of AA to CA women, we observed higher frequencies of both gain (6.9% versus 5.2%; $p = 0.0002$) and loss (9.0% versus 7.3%; $p = 0.003$) in AA than CA women, respectively. This difference is consistent with the higher percentages of ER negative and TN breast tumors in AA compared to CA women (Table 1),

suggesting that breast tumor subtypes differ with respect to frequency of genome-wide alteration events.

Frequent Copy Number Alterations in Breast Cancer Subtypes and Racial Groups

To identify the specific genomic regions that were more frequently altered (copy number gain or loss) in the breast cancer subtypes, we classified CNAs as a “high-frequency” event if the gain or loss events for the specific probe on the array occurred in over 40% of the tumors in the specified subtype. We mapped these probes indicating high-frequency CNAs for the specific breast cancer subtypes to chromosome arms (Table 3) and cytogenetic bands (Supplemental Data Table 2) to profile the distribution of high frequency events. We identified high-frequency CNAs that were shared by the subtypes, as well as high-frequency CNAs that differed by subtype. The high frequency events common to all subtypes included gain events on chromosomes 1q and 8q, and high-frequency loss events on chromosomes 8p, 10q, 11q, 12q, and 16q. Subtype specific high-frequency CNAs included copy number loss events on 4p and 11p, which were observed in ER-negative, but not ER-positive tumors. Interestingly, HER2-positive tumors had the widest distribution of high-frequency loss events across the genome, i.e., the most chromosome arms with high-frequency loss events. We also observed high-frequency loss on 13q and 20q in TN breast cancers that were not observed in other non-TN tumors. Taken together, these results support the presence of high-frequency subtype-specific CNA events.

Differential Copy Number Alterations in Breast Cancer Subtypes

In addition to the CNA frequency differences at the genome-wide and chromosomal arm level, we identified subtype specific CNA events (gain and loss) at individual BAC clones. Differential CNAs (CNAs that differ in frequency between groups ($p < 0.05$)) by individual BAC clones were identified based on comparisons of age- and stage-adjusted tumors in each comparison and restricted to clones with the difference of CNA frequency 0.20 (Table 4 and Supplemental Data Table 3). In the comparison between ER-positive ($n = 154$) and ER-negative ($n = 105$) breast cancers, we found 90 (79 loss and 11 gain) differential CNAs. ER-positive tumors were characterized by differential gain events on 1q, and loss events on 11q and 16q, whereas, ER-negative tumors were characterized by differential gain events on 8q and 10p, and loss events on 3p, 4p, 5q, 9q, 10q, 12q, 13q, and 14q (Figure 1). In HER2-positive ($n = 35$) vs. HER2-negative ($n = 224$; including both ER-positive and ER-negative) tumors, a total of 49 (25 loss and 24 gain) CNAs were identified to be differential. HER2-positive tumors were characterized by differential gain on 17q and 20q, and loss on 4p, 8p, and 13q. HER2-negative tumors exhibited differential loss only on 16q (Figure 1). When we compared TN ($n = 65$) to all non-TN tumors ($n = 194$), we found a total of 203 (145 loss and 58 gain) differential CNAs. Triple-negative tumors were characterized by differential gain events on 1q, 2p, 8q, 10p, 12p, and 18p, and loss events on 3p, 4p, 5q, 9q, 10q, 12q, 13q, 14q, 15q, and 20q. Non-TN tumors had differential gain on 1q, and loss on 11q and 16q (Figure 1).

When we compared CNA frequencies by race in breast tumors from AA ($n = 53$) and CA ($n = 206$) women, we found a total of 22 (20 loss and 2 gain) differential CNAs. Breast tumors from AA women, regardless of subtype, were characterized by differential gain at 8q, and loss at 5q, 9q, 10q, 14q, 15q. In this comparison, there were no differential gain or loss events characteristic for CA women. When we compared CNA events in TN only breast tumors of AA ($n = 22$) and CA women ($n = 43$), we found 216 CNAs (130 loss and 86 gain) with a frequency difference $> 20\%$ by race (see Materials and Methods; Table 4). Overall, these data support the observation that there are differences in frequency and location of CNA events for TN tumors in AA and CA women.

Hierarchical Clustering of Breast Cancer Subtypes Based on Differential Copy Number Alterations

Hierarchical clustering was performed with the 320 statistically significant differential BAC clones that were identified in the subgroup comparisons. We were interested in determining if breast cancer subtypes would cluster based on patterns of their characteristic CNAs. The dendrogram shows that the 259 tumors clustered into two major groups, one enriched for ER-negative tumors (Figure 2. Group A) and the other enriched for ER-positive tumors (Figure 2. Group B), regardless of HER2 status. The Group A cluster contained the majority of TN breast tumors, 52 of the 65 TN tumors (80%) TN tumors were characterized by gains in 1q, 8q, and 10p, and loss in 4p, 5q, 14q, and 15q. Gains at 10p15-10p12 and loss at 14q32 were the most predominant differentiating regions of CNA associated with the TN tumors. Group B on the dendrogram contained the majority of ER-positive tumors (115/154, 75%) and HER2-positive tumors (24/53, 69%).

Discussion

In the current study, we used a genome-wide aCGH approach to profile CNAs from breast cancers for 259 young women from a previously reported population-based case-control study in Atlanta, GA [22]. We identified characteristic CNAs associated with breast cancer subtypes (ER+/-, HER2+/-, TN) and found statistically significant differences in the average overall frequency of genome-wide CNAs in subtype comparisons, as well as frequency differences in CNAs occurring at specific genomic sites. We also observed differences in the frequency (>20%) and genomic locations of CNA events for TN tumors in AA and CA women.

Our results demonstrate that TN tumors had marked genomic instability with the highest average frequency of genome-wide CNAs compared to the other breast cancer subtypes. Chin et al. and Bergamaschi et al. also reported similar findings, with TN/basal tumors having the highest frequency of both copy number gains and losses compared to other subtypes [18,19]. Fridlyand et al. observed a subset of ER- tumors associated with poor outcomes and extensive genomic instability, classifying this molecular subtype as the “complex” subtype. These “complex” tumors were found to have a high degree of similarity for CNA profiles when compared to BRCA1 hereditary tumors [20]. We also observe our TN tumor samples to have CNAs in genomic regions that are characteristically altered in BRCA1 hereditary tumors specifically at 5q, 10p, 12p, 12q, and 20q [30,31].

Copy number gain at 10p has been reported to be a distinguishing CNA for TN/basal tumors compared to other breast cancer subtypes [32,18,33,19]. We observed a copy number gain in the region of 10p spanning 10p15-10p12 in our set of TN tumors. There are numerous genes spanning this region, with several confirmed to have increased protein expression associated with TN/basal tumors. Up-regulation of gene expression for several genes in this region (10p13), specifically, C10orf7, UPF2, HSPA14, RPP38 and CAMK1D has been confirmed to correlate with copy number gain [32,34]. The region of 10p13 also contains the gene for vimentin (VIM) that has been associated with increased expression with TN/basal tumors and plays a role in the epithelial-mesenchymal transition [35]. Although we do not present corresponding gene expression data for our samples, we see a significantly higher frequency of copy number gain at 10p13, corresponding to the genomic region containing the gene for VIM in TN tumors.

Amplification at 8q24 is common in breast cancer and has been previously observed in TN/basal and BRCA1 breast tumors and associated with poor outcomes [36,37]. We observed a significant difference in the frequency of gain events in the genomic region (8q24) containing the C-MYC gene in ER-negative, primarily TN tumors, with >50% of the TN

tumors compared to non-TN tumors (30%) having copy number gain in this region. We also compared the frequency of CNAs in TN tumors of AA and CA at 8q24, and observed a negligible CNA frequency difference between AA women (54%) and CA women (52%) for gains in this region, indicating that copy number gain in this region, containing the C-MYC, is not a distinguishing feature between tumors of AA and CA women.

There was twice the frequency of copy number gain in 13q31-13q34 for TN tumors for AA (20%) versus CA (9%) women. Amplification in the region of 13q31-13q34 has been previously reported to be associated with TN/basal tumors (20%) and BRCA1-associated breast tumors (8.1%) in a study reported by Melchor et al. [38]. They identified two “driver” genes in 13q34 that facilitate tumor progression, cullin4A (CUL4A) and transcription factor Dp-1 (TFDP1). Both were demonstrated to have increased protein expression in tumors with amplification at 13q34. Both CUL4A and TFDP1 overexpression in breast cancers have been associated with shorter overall and disease-free survival [39,40]. The study conducted by Melchor et al. included a total of 188 familial and 277 sporadic breast cancer samples, most of which came from cancer centers of predominantly Latino/Hispanic patients in Spain and Ecuador. Both AA and Hispanic women with TN/basal tumors have poorer outcomes compared to CA women [9,41]. Additional studies are needed to evaluate events associated with amplification of 13q31-13q34 in relation to race and ethnicity and clinical outcome for AA, Latino/Hispanic, and Caucasian women.

ER-negative, specifically TN tumors, had a statistically significant differential frequency of copy number loss at 14q32.2 ($p=0.001$) when compared to the ER-positive and non-TN tumors, respectively and rarely occurred in HER2-positive tumors (5%) (Table 4). In addition, this CNA occurred more than twice as often in TN tumors of AA women compared to TN tumors of CA women (59% vs. 21%, respectively). The 14q32.2 region contains the gene for the microRNA, miR-342. MiR-342 has a critical role in proliferation, differentiation, development, and metabolism (reviewed in [42]) and functions as a pro-apoptotic tumor suppressor in colon tumors [43]. For breast cancer, a recent study demonstrated that miR-342 expression was highest in ER-positive and HER2-positive breast tumors and lowest in TN/basal tumors. This expression pattern is consistent with the CNA profiles they we found at 14q32.2 for the breast tumor subtypes, suggesting that copy number loss at 14q32.2 in TN tumors may lead to the downregulation of miR-342 expression, particularly in tumors of AA women.

Although the current literature has been inconsistent with respect to biological differences between tumors of AA and CA women, two recent reports support the hypothesis that biological differences exist and find that in women with breast tumors of similar ER status, AA women have poorer survival than CA women, even after adjustment for socioeconomic factors [44,45]. In addition, a separate study showed that there are biological differences impacting angiogenesis, chemotaxis, and immunobiology pathways in breast tumors of AA and CA women based on the comparison of gene expression profiles of tumor and stromal tissue from breast tumors of these two racial populations [46]. Our preliminary findings of differences in CNA frequencies in TN tumors from AA and CA women support the observations that there may be biological differences in the TN tumors. It is still unknown how these differences contribute to prognosis for AA and CA women.

One potential study limitation was selection bias in the array-tested samples. Therefore, we conducted a weighted analysis to address the issue of selection bias, but cannot be certain that this weighting completely addressed that issue. In addition, although there were limitations in the use of the moderate resolution BAC array for the identification of CNAs, we successfully demonstrated that we could confirm previously identified CNAs associated

with specific breast cancer subtypes and identify additional novel CNAs not previously reported, particularly for the TN/basal subtype.

In this report we found characteristic genomic alterations associated with subtypes of breast cancer. The breast cancer samples included in this study were a part of a larger cohort of young women, and included the largest aCGH study on both breast tumors from young women and on number of TN tumors analyzed by aCGH. Further replication studies will need to be performed to confirm these findings. These results can be applied to future studies to increase our understanding of the biology of the different breast cancer subtypes, particularly TN tumors, and differences by race, ultimately leading towards the identification of improved targeted therapeutic strategies and breast cancer survival.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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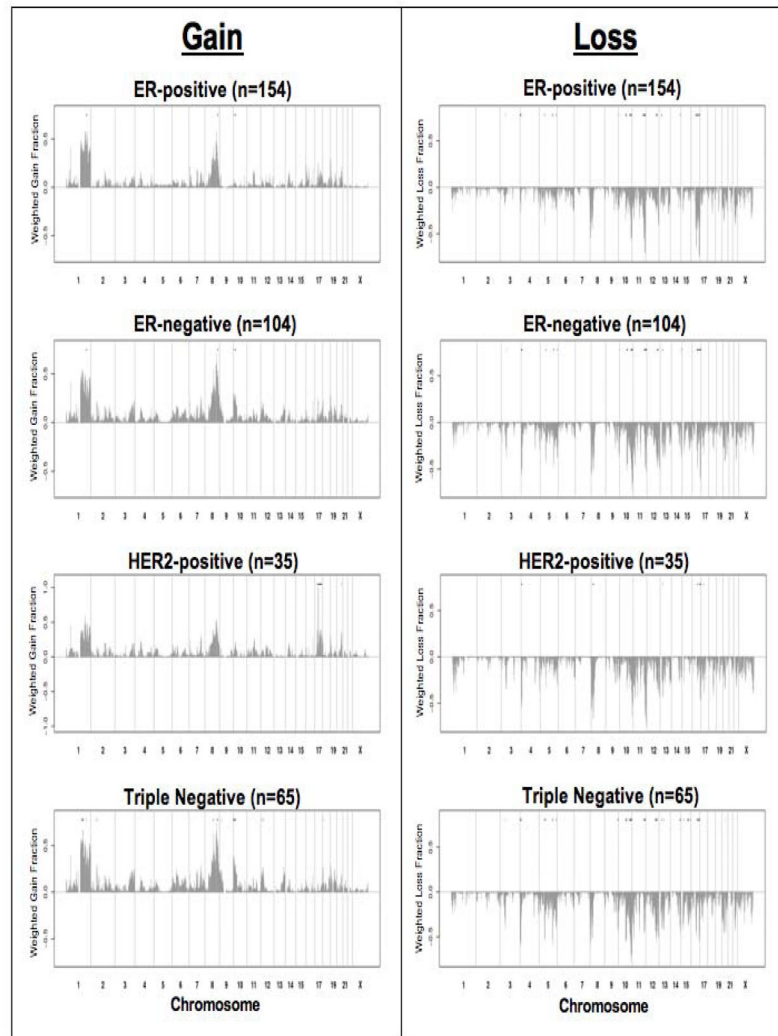


Figure 1. Genome-wide copy number alteration (gain and loss) frequencies by subtype. The whole-genome plots show the frequency (y-axis) of gain (gray bars above the x-axis) and loss (gray bars below the x-axis) events for breast cancer subtypes. The black circle at the top of each plot represents the genomic location of a BAC clone demonstrating a differential copy number alteration for the specific breast cancer subtype.

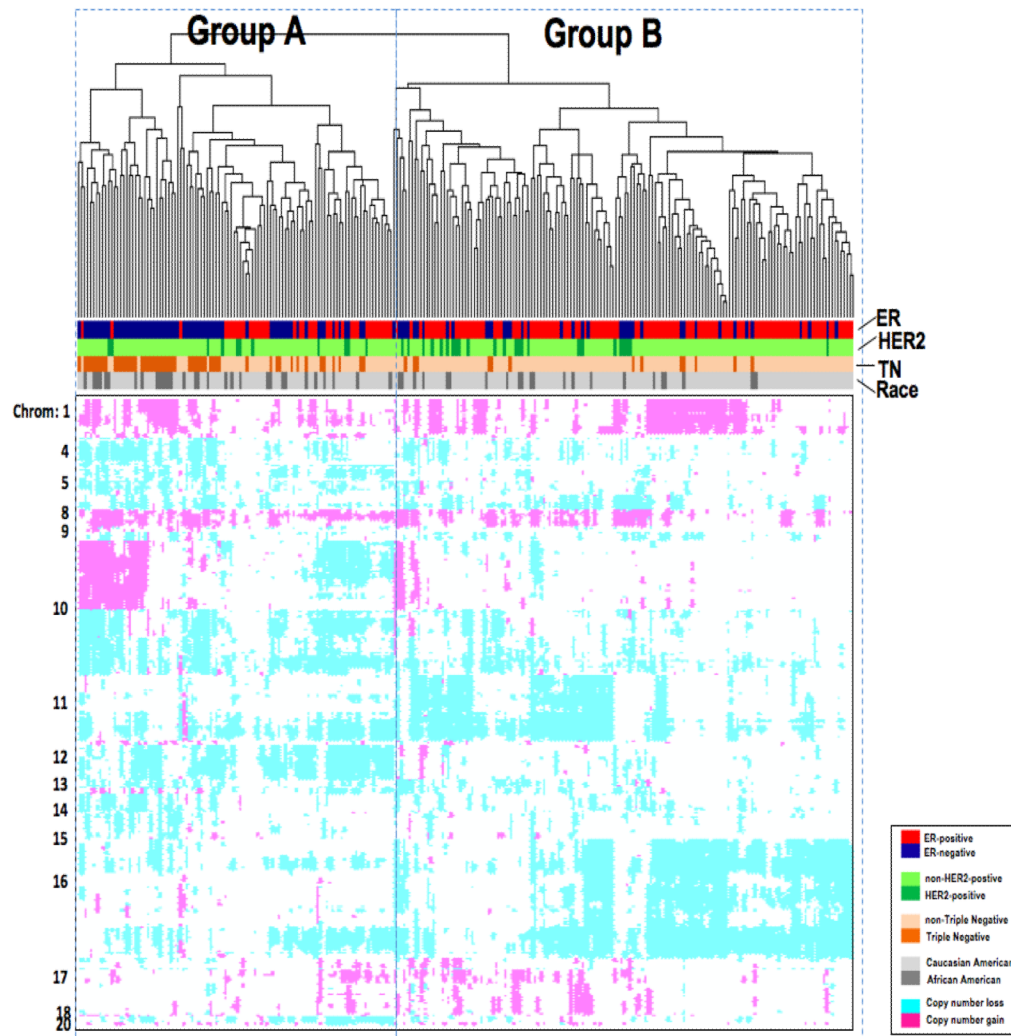


Figure 2. Hierarchical clustering based on differential copy number alterations. Individual tumors (columns) clustered into two major groups (A and B). Tumor characteristics such as estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER2) and racial background are indicated in the legend. Chromosome locations of copy number alteration events (gain in pink and loss in turquoise) are indicated to the left of the heatmap.

Table 1

Tumor Characteristics of Cases Analyzed with aCGH

	All aCGH n=259	African American n=53	Caucasian American n=206	<i>p</i> -value*
	n	n (%)	n (%)	
Estrogen Receptor				
negative	105	36 (68)	69 (34)	<.0001
positive	154	17 (32)	137 (66)	
Progesterone Receptor				
negative	99	32 (60)	67 (33)	0.0004
positive	160	21 (40)	139 (67)	
HER2				
negative	224	44 (83)	180 (87)	0.5467
positive	35	9 (17)	26 (13)	
Triple Negative				
Yes	65	22 (42)	43 (21)	0.004
No	194	31 (59)	163 (79)	
Tumor grade				
Low	50	1 (2)	49 (24)	<.0001
Intermediate	96	15 (28)	81 (39)	
High	113	37 (70)	76 (37)	
AJCC Stage				
I	89	7 (13)	82 (40)	0.0009
IIA	79	17 (32)	62 (30)	
IIB	54	16 (30)	38 (18)	
III/IV	37	13 (25)	24 (12)	

* *p*-values were determined with a chi-square test

Table 2

Comparison of the Weighted Average Frequency* of Genome-wide Copy Number Alterations

	ER-negative (n=105)	ER-positive (n=154)	<i>p</i> -value**
Gain	0.069	0.049	<0.0001
Loss	0.085	0.073	0.02

	HER2-positive (n=35)	HER2-negative (n=224)	<i>p</i> -value
Gain	0.056	0.058	0.68
Loss	0.073	0.079	0.37

	Triple Negative (n=65)	Non-Triple Negative (n=194)	<i>p</i> -value
Gain	0.073	0.052	<0.0001
Loss	0.089	0.074	0.003

	African American (n=53)	Caucasian American (n=206)	<i>p</i> -value
Gain	0.069	0.052	0.0002
Loss	0.090	0.073	0.003

* Genome-wide gain or loss events were calculated for each tumor then averaged (weighted) across tumors in each comparison group.

** *p*-values were generated with a weighted t-test

Table 3

High Frequency Copy Number Alterations in Subtypes of Breast Cancer*

Chromosomal arm	ER-positive (n=154)	ER-negative (n=105)	HER2-positive (n=35)	Triple Negative (n=65)
1p	Gain	Gain	Gain/Loss	
1q	Gain	Gain	Gain/Loss	Gain
2p			Loss	
2q			Loss	
3p			Loss	Loss
3q			Loss	
4p		Loss	Loss	Loss
4q			Loss	
5p				
5q		Loss		Loss
6p			Loss	
6q			Loss	
7p			Loss	
7q			Loss	
8p	Loss	Loss	Loss	Loss
8q	Gain	Gain	Gain/Loss	Gain
9p			Loss	
9q			Loss	
10p			Loss	
10q	Loss	Loss	Loss	Loss
11p		Loss	Loss	Loss
11q	Loss	Loss	Loss	Loss
12p			Loss	
12q	Loss	Loss	Loss	Loss
13q				Loss
14q			Loss	Loss
15q			Loss	Loss
16p			Loss	
16q	Loss	Loss	Loss	Loss
17p			Loss	
17q			Gain/Loss	

Chromosomal arm	ER-positive (n=154)	ER-negative (n=105)	HER2-positive (n=35)	Triple Negative (n=65)
18p				
18q			Loss	
19p			Loss	
19q			Loss	
20p		Loss		Loss
20q				Loss
21p				
21q				
22p				
22q		Loss	Loss	
Xp				
Xq			Loss	

* High-frequency CNA (weighted fraction >40% of tumors) mapped by chromosomal arm.

Table 4
Differential Copy Number Alterations in Breast Cancer Subtypes and Racial Groups

Cytogenetic Band*	ER-Negative vs ER-Positive	HER2 Positive vs HER2 Negative	Triple Negative (TN) vs Non-Triple Negative	African American (AA) vs Caucasian American (CA)	African American (AA) vs Caucasian American (CA) in Triple Negative Only**
1p36					AA
1p36					CA
1p21			TN		CA
1q23			TN		AA
1q24			TN		AA
1q25			TN		AA
1q31					AA
1q32	ER-pos		non-TN		AA
2p25					AA
2p16			TN		
2p15			TN		
3p21	ER-neg		TN		AA
4p16	ER-neg	HER2 pos	TN		CA
4p15			TN		AA
4q13					AA
4q31					AA
5p15					CA
5p14					AA
5p13					CA
5q11	ER-neg		TN		
5q13			TN		
5q31	ER-neg		TN	AA	
5q35	ER-neg		TN		AA
6p21					CA
6q11					AA

Cytogenetic Band*	ER-Negative vs ER-Positive	HER2 Positive vs HER2 Negative	Triple Negative (TN) vs Non-Triple Negative	African American (AA) vs Caucasian American (CA)	African American (AA) vs Caucasian American (CA) in Triple Negative Only**
6q12					AA
7q31					CA
7q36					CA
8p21		HER2 pos			
8q21			TN		AA
8q22					AA
8q23				AA	CA
8q24	ER-neg		TN	AA	AA
9p21			TN		
9q32					CA
9q33					CA
9q34	ER-neg		TN	AA	AA
10p15	ER-neg		TN		
10p14			TN		
10p13	ER-neg		TN		
10p12	ER-neg		TN		
10q22	ER-neg		TN	AA	CA
10q23					CA
10q25	ER-neg		TN	AA	CA/AA
10q26	ER-neg		TN	AA	AA
11p15					AA
11p11					AA
11q13					CA
11q13					AA
11q23	ER-pos		non-TN		
11q24	ER-pos		non-TN		
11q25	ER-pos		non-TN		CA

Cytogenetic Band*	ER-Negative vs ER-Positive	HER2 Positive vs HER2 Negative	Triple Negative (TN) vs Non-Triple Negative	African American (AA) vs Caucasian American (CA)	African American (AA) vs Caucasian American (CA) in Triple Negative Only***
12p13			TN		AA
12p12			TN		AA
12q12					CA
12q14					CA
12q15					CA
12q21					CA
12q23	ER-neg		TN		CA
12q24	ER-neg		TN		CA
13q12	ER-neg	HER2 pos	TN		Ca
13q14			TN		AA
13q32					AA
13q33					AA
13q34					AA
14q32	ER-neg		TN	AA	AA
15q12			TN		AA
15q21					CA
15q22			TN		AA
15q24			TN	AA	AA
15q25			TN		
15q26			TN		AA
16p13					AA
16p13					CA
16q12	ER-pos	HER2 neg			AA
16q12					AA
16q13	ER-pos	HER2 neg			AA
16q21	ER-pos		non-TN		
16q22	ER-pos	HER2 neg	non-TN		
16q23	ER-pos	HER2 neg	non-TN		

Cytogenetic Band*	ER-Negative vs ER-Positive	HER2 Positive vs HER2 Negative	Triple Negative (TN) vs Non-Triple Negative	African American (AA) vs Caucasian American (CA)	African American (AA) vs Caucasian American (CA) in Triple Negative Only**
16q24	ER-pos	HER2 neg	non-TN		AA
17q11		HER2 pos			AA
17q12		HER2 pos			AA
17q21		HER2 pos			
17q22		HER2 pos			
17q23		HER2 pos			
17q24		HER2 pos			
17q25		HER2 pos			
18p11			TN		CA
18q23					CA
19p13					CA
20p11					AA
20q12			TN		AA
20q13		HER2 pos			
22q11					AA
22q12					AA
Xp22					CA
Xq13					AA

* Cytobands containing differential BAC clones with p-values < 0.05 & weighted fraction difference ≥ 0.20 (with the exception of the race comparison in TN only)

** Cytobands containing differential BAC clones with weighted fraction difference ≥ 0.20

Copy Number Loss

Copy Number Gain