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Profile of the somatic mutational landscape in breast tumors from Hispanic/Latina women

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ABSTRACT

Breast cancer causes the most cancer deaths among Hispanic/Latinas (H/L). However, limited tumor-sequencing data from H/L are available to guide treatment. To address this gap, we performed whole-exome sequencing of DNA from 140 HL germline and 146 matched breast tumors and RNA-seq for the tumors. We generated somatic-mutation profiles, identified copy-number alterations (CNAs), and compared results to non-Hispanic White (White) women in The Cancer Genome Atlas. Similar to Whites, *PIK3CA* and *TP53* were the most commonly mutated genes in breast tumors from H/L. We found 4 common COSMIC mutation signatures (1, 2, 3, 13) and signature 16 not previously reported in other breast-cancer datasets. We observed recurrent amplifications in breast-cancer drivers including *MYC*, *FGFR1*, *CCND1*, and *ERBB2*, and a recurrent amplification on 17q11.2 associated with high *KIAA0100* gene expression, implicated in breast-cancer aggressiveness. Expanded research is required to determine how these characteristics of H/L tumors impact treatment response and survival.

Key words: Hispanic/Latino (H/L); Non-Hispanic Whites (Whites); copy-number alterations; breast cancer; somatic mutations; expression outlier; disparities

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INTRODUCTION

Sequencing studies of breast cancer have identified recurrently mutated genes and somatic copy number alterations (SCNAs) affecting tumor suppressors and oncogenes[1-3]. Both somatic mutations and CNAs may be useful in determining prognosis. Currently, therapies for breast cancer can be selected based on particular somatic mutations (i.e., alpelisib for *PIK3CA*[4]), SCNAs (i.e., Trastuzumab for *HER2*), and germline mutations in genes in the homologous recombination repair (HRR) pathway (polyADPribose polymerase inhibitors - PARPi's).

Genetic ancestry is associated with specific somatic mutations in many cancer types. *EGFR* mutations are approximately four-fold more common in lung cancer from women and men of East-Asian ancestry compared with lung cancer from women and men of other populations[5] with self-reported Hispanic/Latinos (H/L) representing an intermediate group [6, 7]. *FOXA1* mutations in prostate cancer also are substantially more common in East-Asian ancestry populations compared to European and African ancestry populations[8]. Comprehensive analyses of The Cancer Genome Atlas (TCGA) have demonstrated that many mutations and CNAs are more common in specific ancestral populations[9, 10]. In breast cancer, previous studies have demonstrated that women of African ancestry have higher rates of *TP53* mutations and lower rates of *PIK3CA* mutations, likely related to a higher incidence of a basal-like breast-cancer subtype in African-American women[11, 12]. However, the genomic landscape of breast cancer has not been well-characterized in H/L groups.

H/L represent the largest minority population in the US and have diverse origins, with the largest subpopulations including Mexican Americans and Puerto Ricans. Genetically, H/L are a population of mixed European, Indigenous-American (IA) and African ancestries with those ancestry proportions varying widely depending on country of origin and regions within a country. Although breast cancer is less common overall among H/L compared to self-reported non-

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Hispanic White (White) women due to both environmental [13] and genetic factors[14], there is a higher proportion of breast cancers diagnosed under age 50 years than in Whites[15]. Moreover, outcomes are usually worse among H/L compared to White women[16]. In some studies, IA ancestry was associated with poorer outcomes among H/L with breast cancer[17]. Human epidermal growth factor (HER2) amplifications are over-represented among H/L and are more common among H/L with more IA ancestry compared to those with more European ancestry[18]. Few studies have investigated the distribution of somatic mutations and SCNAs in breast tumors from H/L. In TCGA, out of 1,096 breast-cancer cases, only 39 are self-reported H/L. A recent study analyzed data including whole-exome sequencing (WES) and gene expression data from 109 Mexican women living in Mexico [19]. However, no similar-size study has been conducted in H/L in the United States (US). To investigate the somatic-mutational spectrum in breast cancer among H/L, we generated whole-exome sequencing (WES) and RNA sequencing (RNA-seq) data from 146 tumors from 140 H/L from Southern California and performed analyses of somatic mutations, SCNAs, and gene expression.

METHODS

Participants. One hundred and forty breast-cancer patients seen at City of Hope (COH) in Duarte, California were included in this study. All participants signed a written informed consent approved by the COH Institutional Review Board. Inclusion criteria were: 1) self-identified as H/L; 2) tumor tissue from surgery was available and the sample contained more than 40% tumor based on examination by a single breast pathologist (D. Schmolze). The percentage tumor ranged from 40% to 90% with an average of 64% and a median of 65% tumor. An exclusion criterion was neo-adjuvant therapy as treatment could change the mutation profile. Clinical data were abstracted from medical records including date at diagnosis, date at surgery, tumor stage, grade, histological estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor (HER2) status, second cancers, breast-cancer recurrence, parity, history of breast

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feeding, age at menarche, and cause of death, if applicable. Six of the 140 breast-cancer patients had two primary contralateral breast cancers with tissue available for study for a total of 146 tumors.

DNA and RNA sequencing

DNA extraction. Germline DNA was extracted from peripheral blood cells or from formalin-fixed paraffin-embedded (FFPE) normal breast tissue adjacent to tumor tissue from surgery. Peripheral blood cell DNA was extracted using a standard phenol chloroform method. For FFPE tissue, DNA and RNA were extracted from ten 30-µm sections from each tumor using the QIAmp DNA FFPE Tissue Kit (Qiagen) and miRNeasy Kit (Qiagen) according to manufacturer's instructions. DNA was quantified with the Quant-iT PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific, MA). After extraction and quantification, DNA was sent to The National Cancer Institute (NCI) Cancer Genomics Research Laboratory (CGR) for WES. For RNA sequencing, 500 ng total RNA was sent to the COH Integrative Genomics Core (IGC).

DNA library construction, hybridization, and massively parallel sequencing. Library production and sequencing for 146 tumors and 140 matching normal samples was performed at CGR. The KAPA HyperPlus Kit (Kapa Biosystems, Inc., Wilmington, MA) was used to generate libraries from 300ng DNA according to the KAPA-provided protocol. Libraries were pooled and sequence capture was performed with NimbleGen's SeqCap EZ exome v3 (Roche NimbleGen, Inc., Madison, WI, USA), according to the manufacturer's protocol. The resulting post-capture enriched multiplexed sequencing libraries were used in cluster formation on an Illumina cBOT (Illumina, San Diego, CA, USA) and paired-end sequencing was performed using an Illumina HiSeq 4000 following Illumina-provided protocols for 2 × 100 bp paired-end sequencing to an average-fold coverage of 80X for the tumors and 30X for the germline samples. Paired□end reads from each sample were aligned to human reference genome (hg19) using Novoalign

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(v3.00.05), and the aligned binary format sequence (BAM) files were sorted and indexed using SAMtools (1, 2). The sorted and indexed BAMs were processed by Picard (v1.126, https://broadinstitute.github.io/picard/) to remove duplicate sequencing reads. Local realignment around suspected sites of indels was performed using Genome Analysis Toolkit (GATK) IndelRealigner (v3.3-0-g37228af). These mapped sequence reads were then base-recalibrated before being used for somatic mutation calling by MuTect2 in GATK (v4.0.11.0).

RNA-seq. In the COH IGC, sequencing libraries were prepared with Kapa RNA HyperPrep kit with RiboErase (Roche) and sequenced on a HiSeq 2500 (Illumina) with 40 million reads per sample. The RNA-seq reads were aligned to hg19 genome assembly using Tophat2 (v2.0.8) with default settings. The gene-expression levels were counted by obtaining raw counts with HTSeq (v0.6.1p1) against Ensembl v86 annotation. The counts data were normalized using the trimmed mean of M values (TMM) method implemented in R package edgeR[20]. Log2-transformed counts were used to assign PAM50 subtypes based on the subgroup-specific gene centering method developed by Zhao, *et al.*[21]. We estimated Z-scores based on the corrected median absolute deviation (MAD) implemented by the *robStandardize* R function in the robustHD R package and defined expression outliers as gene-sample data points with robust Z-scores greater than three. Raw counts of RNA-seq data for 1,189 TCGA samples (including both tumor and matched normal samples) were downloaded from the Genomic Data Commons (GDC) using the GDCRNATools[22] R package. RNA-seq data for H/L tumor samples and TCGA samples were processed and analyzed separately.

Data analysis

Germline variant calling. Germline variant calling from the BAM files was performed in the COH IGC using GATK HaplotypeCaller (https://software.broadinstitute.org/gatk). Variants with a call quality less than 20, read depth less than 10, or allele fraction ratio less than 20% were removed. Variants in variant call format files were evaluated for pathogenicity using Ingenuity

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Variant Analysis (IVA) version 4 (Qiagen Inc, Alameda, CA) and American College of Medical Genetics and Genomics (ACMGG) guidelines were applied using the IVA ACMGG calling algorithm[23]. Pathogenic or likely pathogenic variants were individually evaluated by the research team using the available literature and ClinVar to make a final determination[24].

Genetic ancestry analysis. We performed genetic ancestry estimation for each of the 140 women using the germline whole-exome sequencing data. We used 90 European (1000 Genomes), 90 African (1000 Genomes), 90 East-Asian (1000 Genomes) and 71 IA ancestry[25] reference samples. We identified the SNPs that overlap all data sets (N=9,935). We combined all SNPs and dropped SNPs that did not match based on reference and alternate alleles. To estimate the ancestry for each sample, we used ADMIXTURE 1.3.0 setting the K parameter to 4 and running the unsupervised algorithm[26]. In addition, we used principal components analysis, calculated using PLINK 1.9[27] as a complementary method to assess ancestry.

Somatic variant calling. We identified somatic single nucleotide variants (SNV) using MuTect2 in GATK4 (v4.0.11.0) suite with default parameters[28] and indels using GATK Indelocator. Using the SNV and indel filtering method described in Pereira et al.[3], we focused on frameshift, non-synonymous, canonical splicing site, and stop gain mutations. Briefly, somatic mutations were manually curated and considered true positives in a sample if the mutation was observed in >10% of reads or with a frequency of 5-10% if in frequently mutated breast-cancer genes or seen in COSMIC database[29]. Because the tumors include both tumor and normal stromal cells, it is expected that the proportion of reads will have less than the expected 50% if 100% tumor. Mutations in <5% of reads, in segmental duplication regions, or indels that overlapped homopolymer stretches of six or more bases were considered false positives. We did visual checking using the Integrative Genomics Viewer (IGV) to assess the quality of all somatic mutations. We performed Sanger sequencing on a subset of samples to confirm specific

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mutations in *AKT1, BARD1, MAP3K1,* and *MET*. Using the filtered and annotated somatic mutations, we performed a somatic-mutation significance analysis via MutSigCV[30] (version 1.3.5) on Genepattern (https://www.genepattern.org/modules/docs/MutSigCV). Genes with false discovery rate (FDR) $q < 0.05$ are considered to be significantly mutated genes.

We compared the significant somatic mutations in our analysis with the mutations from the Romero-Cordoba dataset[19]. Using the publicly available somatic-mutation data from the Romero-Cordoba study of the Mexican patients, we combined our somatic-mutation data and performed a MutSigCV analysis to identify the common significant genes. Similarly, to investigate if these significantly mutated genes were associated with ancestry, we performed the same analysis on breast tumors from Whites in TCGA. Using 2% as the mutation frequency threshold, we performed Fisher's exact test for each frequently mutated gene for comparison.

Copy-number analysis using FACETS. We used FACETS implemented in R package facets version 0.6.1[31] to calculate CNAs. The counts of reads with the reference (ref) allele, alternate (alt) allele, errors (neither ref nor alt), and deletions at a specific genomic position were generated using BAM files from the 146 matched tumor-normal sample pairs using the application snp-pileup in the facets package. The segmentation of each tumor sample was then estimated with the critical value (cval) 150.

The segmentation files generated by facets served as input files for the GISTIC2.0[32] on the GenePattern server (https://genepattern.broadinstitute.org/gp) to identify significant SCNAs using a q-value cutoff < 0.05. A gene was considered as copy number altered with GISTIC2-thresholded scores of -2 (deep loss), -1 (shallow loss), 1 (low-level gain) and 2 (high-level gain). The GISTIC2 copy-number results and clinical data for 816 TCGA tumor samples were downloaded from the cBioPortal database[33] (https://www.cbioportal.org). Expression outliers (defined by Z-scores greater than 3.0) were considered as driven by copy-number changes if

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greater than 90% expression outliers in a gene had a GISTIC2-thresholded copy-number score of 2 (high-level gain) or 1 (low-level gain). Fisher's exact test was used to identify genes with frequency difference in expression outliers, driven by copy-number alterations, between 146 tumor samples from H/L and 452 TCGA Whites (determined as having > 95% European ancestry as described below).

Mutation-signature analysis

Using the previously called SNVs, we performed a mutational signature analysis via the MutationalPatterns R package[34]. Hg19 was used as the reference genome. SNVs were parsed and classified into six mutation patterns (C>T, T>A, C>G, T>C, C>A and T>G) and 96 trinucleotide changes. Then a non-negative matrix factorization algorithm was implemented to extract mutation signatures. And we compared the similarities of these mutation signatures with the COSMIC mutation signatures and each mutation signature could be treated as a linear combination of the 30 COSMIC mutation signatures. The 30 COSMIC mutation signature percentage contribution was then computed for each tumor and a contribution heatmap was generated. Within these tumor samples, we performed a signature contribution comparison using the two-sided Wilcoxon rank-sum tests among the five tumor subtypes (Luminal A, luminal B, basal-like, HER2-enriched and normal-like).

We also compared the mutation-signature analysis with the breast tumors in the Romero-Cordoba dataset and the breast tumors from Whites in TCGA SNV dataset. For the significant COSMIC mutation signatures identified in our dataset, we performed two-sided Wilcoxon rank-sum tests among the three datasets to test if the signature was enriched in Mexican patients.

RESULTS

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Clinical/demographic data and germline pathogenic variants. Characteristics of the 140 participants are shown in Table 1. The mean age at diagnosis was 48.7 years with a range from ages 31 to 75 years. Nearly all of the 140 H/L were of mixed European (Eur) and IA ancestry. The mean ancestry composition was 50.6% Eur, 40.8% IA, 5.9% African, and 2.7% Asian although the range of ancestry proportion varied widely from <1% to 96% IA at the extremes (Figure 1). As shown in the Principal Component Analysis (PCA) plots in Figure 1A, H/L samples are not well-represented in TCGA project. For the six individuals with two primary tumors (in the contralateral breasts), the tumors were considered independent tumors (Supplemental Table 1) which was borne out by different somatic-mutation profiles. The majority of the women were diagnosed with Stage I (44%) or II (43%) tumors (Table 1). There were 22 recurrences and 10 deaths during the time of follow-up. Of the 146 tumors, 83% were ER-positive, 72% were PR-positive, and 17% were HER-2 positive and these proportions were similar to White women in TCGA [1]. Germline pathogenic variants in breast cancer predisposition genes were identified in six participants including one *BRCA1* exon 9-12 deletion, four *CHEK2* L236P, and one *NF1* Y408X variants of which the *BRCA1* and *CHEK2* variants are of Indigenous-American ancestry[35].

Somatic mutations. We observed a total of 4510 true somatic mutations in 3391 genes in the 146 primary breast tumors (Supplemental Table 2). The number of mutations per individual varied from 2 to 225. Using MutSigCV, we found that mutations in *PIK3CA, TP53, GATA3, MAP3K1, CDH1, CBFB, PTEN,* and *RUNX1* were significant (FDR < 0.05) cancer driver mutations. To identify additional, potentially significantly mutated genes in H/L, we merged the mutation data from our cohort with a previously published study of Mexican breast-cancer patients (N = 135)[19]. Within the aggregated mutation data of this combined cohort (N = 281), we re-ran MutSigCV and identified one more significantly mutated gene, *AKT1*, which only occurred twice in our 146 primary breast tumors. Using the statistically significantly mutated genes

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obtained from the aggregated cohort, we visualized the mutational profiles within our cohort (Figure 2a) and the variant locations for *PIK3CA* and *GATA3* (Figure 2b). For *MAP3K1* and *RUNX1*, at least one tumor harbored multiple mutations in the same gene. Furthermore, in *GATA3*, eight tumors had the identical splice mutation (NM_002051.2:exon5:r.spl;NM_001002295.1:exon5:r.spl) that affected expression (data not shown). Other genes of interest that did not meet the significance threshold (FDR < 0.05) but which have been identified as significant in prior studies and were mutated in our dataset included *MLL3* (aka *KMTC2*) (6%), *PTPRD* (3%), *MAP2K4* (2%), *PIK3R1* (2%), *NF1* (1%), *RB1* (1%), *TBX3* (1%), *FOXA1* (1%), *PADI4* (1%), *CDKN1B* (1%), *CTCF* (1%), and *NCOR1* (1%). In addition, we found mutations in *MET* (4.1%) which is not generally considered a breast-cancer gene but is a known driver in other cancer types[36].

The frequency of mutations in genes known to be significantly mutated in breast cancer, including *PIK3CA, MAP3K1, GATA3, CBFB, and MLL3/KMT2C,* were not significantly different in tumors from H/L compared to tumors from White women in TCGA (FDR q > 0.05, Supplemental Table 3). Similar to tumors from Whites, *PIK3CA* and *TP53* were the most commonly mutated genes. We identified *AKT1* mutations in 2 of 146 tumors (1.4%), including the E17K hotspot mutation which was found to be mutated in 8% of patients among Mexican women[19]. After correction for multiple hypothesis testing, we found no somatic mutations significantly associated with genetic ancestry.

Mutational signature analysis. To investigate the mutational processes in H/L breast-cancer tumors and the association between PAM50 subtypes and mutational patterns, we adopted the non-negative matrix factorization approach as proposed by Alexandrov et al.[37] for mutational signature analysis of tumors. Signature calling revealed five major contributing signatures in the

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146 tumors corresponding to the COSMIC signatures 1,2,3,13 and 16 (Figure 3a; Supplemental Table 4). Signature 1 was detected in all 146 tumors. The contribution of COSMIC signature 1 was greater in luminal A and B subtypes than HER2 and basal subtypes ($p < 0.05$, two-sided Wilcoxon rank sum test) (Figure 3b). Signatures 2 and 13, attributed to activity of the AID/APOBEC family of cytidine deaminases, were found in tandem in 16% (n = 23) of the tumors and were more common in tumors with HER2 subtype compared to luminal A and B subtypes (Figure 3b). We found that 13 tumors were homozygous and 29 tumors were heterozygous for a common 29.5kbp germline deletion spanning most of *APOBEC3B*. Tumors with the deletion had a higher proportion of COSMIC signatures 2 ($p = 0.0005$, Wilcoxon rank sum test) and 13 ($p = 0.0008$, Wilcoxon rank sum test). Signature 3, attributed to defects of homologous recombination double-stranded DNA break-repair, was found significantly more often in basal subtypes than the other PAM50 subtypes ($p < 0.05$, two-sided Wilcoxon rank sum test) including the tumor with the germline *BRCA1* exon 9-12 deletion. We observed a group of tumors (N=40, 27.4%) with more than 5% COSMIC signature 16 contributions. Since this was not previously reported in other breast tumor studies, we re-examined other datasets, using the same analytic pipeline used herein. We found that signature 16 was present in 20 (19.6%) tumors in a previous study of Mexican breast-cancer patients[19] which was not significantly different than the proportion in our dataset ($p = 0.18$, Fisher's exact test). The proportion in tumors from TCGA White women (N=75; 8.9%) was significantly lower than in our dataset ($p < 0.001$, Fisher's exact test) (Figure 3c) and in the Romero-Cordoba et al. dataset ($p < 0.0001$, Fisher's exact test). The percentage of this signature was significantly higher in luminal A and B subtypes compared to HER2 and basal tumors ($p < 0.05$, two-sided Wilcoxon rank sum test) (Figure 3b).

Somatic CNAs (SCNAs)**.** Using GISTIC2, we identified chromosome arm-level SCNAs that were significantly ($q < 0.05$) amplified at 1q, 8q, 6p, 1p, 6q, 16p, 20q, 8p, 12q and deleted at 22q,

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16p, 17p, 8p (Supplemental Table 5). In addition to these broad SCNAs, we identified significantly ($q < 0.05$) amplified or deleted focal regions including 29 peak regions of amplification and 48 regions of deletion (Figure 4A). Seven recurrently amplified regions contain common oncogenes (*FGFR1*, *MYC*, *CCND1*, *MDM2*, *IGF1R*, *ERBB2*, and *ZFP217*); one recurrently deleted region contains *TP53* (Figure 4A). By integrative analysis of RNA-seq gene-expression data and copy-number data, we observed that greater than 90% of expression outliers (defined by robust Z-score greater than 3.0) in *ERBB2, FGFR1, IGF1R,* and *MDM2* were associated with copy-number gain (Figure 4B). Therefore, we sought to identify expression outliers from 1,121 genes contained in the 29 copy-number amplification peak regions for the 146 H/L breast tumor samples and 452 White TCGA breast tumor samples. Of 1,121 genes in the 29 regions, over 90% of expression outliers were associated with copy-number gain in 214 genes, including 88 genes from the 146 H/L samples, 62 genes from the 452 TCGA White samples, and 64 genes from both sample groups (Supplemental Table 6). Eighteen of 214 genes had significant (FDR < 0.05) difference in frequency of expression outliers copy number between the 146 H/L and 452 TCGA White tumor samples (Table 2 and the top 18 rows in Supplemental Table 6). Expression outliers from those genes were more prevalent in the 146 H/L than in the 452 White tumors because we focused on the 29 copy-number regions (Figure 4A) found in H/L (Table 2; Supplemental Table 6).

Using this combined copy-number and gene-expression analysis approach, we identified *KIAA0100,* also known as Breast Cancer Overexpressed Gene 1 (*BCOX1*), as the top gene that was systematically different between Whites (TCGA) and our H/L cohort. (Figure 5A). Since this gene is within ~11 megabases of *ERBB2* on chromosome 17q, we investigated whether it was part of the ERBB2 GISTIC amplification peak. We observed that the peaks for copy-number amplifications (Figure 5B) were distinct for *KIAA0100* and *ERBB2* are at 17q11.2 and 17q12.

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Discussion

We analyzed tumor-germline sequencing data combined with RNA-seq data from 146 tumors from 140 self-identified H/L recruited from a single center in the Los Angeles region. As expected, the majority were of mixed European and IA ancestries. Since TCGA has extremely limited samples of breast cancer from H/L and particularly of H/L of mixed IA ancestry, our report fills a critical gap in the landscape of somatic mutations and copy-number alterations in this increasing US population. Together, our analyses and the recent paper focused on Mexican women living in Mexico [19] substantially enhance the data in the public domain for women of H/L heritage.

The most commonly mutated gene in our population was *PIK3CA* which is the most commonly mutated gene in TCGA White samples. For women with advanced ER+/HER2- breast cancers, alpelisib is a currently approved therapy, and our results suggest that this therapy should be useful in a large fraction of H/L women. The Romero-Cordoba et. al. study identified a high frequency (8%) of the E17K activating *AKT1* mutation indicating such women may benefit from AKT inhibitors. We only identified two tumors with mutations in *AKT1* and only one with the E17K mutation. The difference between our results and those of Romero-Cordoba may be due to chance, differences in selection criteria between the two cohorts, and/or differences in environmental exposures between the two cohorts. Since the ancestry of our population is similar, it is unlikely that the differences we observed are due to germline-genetic differences between the two cohorts.

We performed analyses of the somatic-mutational signatures and compared them to the TCGA dataset. Our analysis identified COSMIC signature 16 (contribution > 5%) in a significant fraction of tumors (27.4%) in our dataset with similar rates in the data from Romero-Cordoba et

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al. who analyzed breast tumors from Mexican patients. Because Romero-Cordoba et al. used a contribution cutoff in their mutation-signature-analysis pipeline, they did not report this signature. However, in our analysis, we implemented the non-negative matrix factorization algorithm and no contribution cutoff was applied such that signature 16 was observed. There were significantly lower rates of this signature in TCGA White women ($p< 0.001$). We do not believe our finding is a technical artifact from FFPE because this signature was found in frozen tissue in the Romero-Cordoba et al data. No known genetic or environmental exposures that predispose to this signature have been reported and prior studies have not found this mutational signature in breast cancer, although it has been reported to be common in liver cancers[37].

Other COSMIC signatures were the same as those previously reported in TCGA. We found signatures 2 and 13 associated with APOBEC loss as a relatively common finding, associated with HER2-amplified tumors and specifically with the germline APOBEC copy-number variant similar to previous reports[38]. The APOBEC*3B* common 29.5-kbp germline deletion results in the fusion of *APOBEC3A* and the 3'UTR of *APOBEC3B*[39]. This fusion generates a more stable *APOBEC3A* mRNA, resulting in increased expression of *APOBEC3A*, higher overall mutation burden, and a higher odds ratio of developing breast cancer[40, 41]. We also found Signature 3, associated with defects in homologous recombination repair as a common signature, which is over-represented in basal-like tumors as previously reported[37, 42].

Our copy-number analyses identified copy-number gains, i.e., 1q, 8q, 17q which are common in breast cancer in other populations[1, 2]. We also identified several known CNAs which were recurrently gained in our dataset. In combined analysis of copy-number alterations and gene expression, we identified *KIAA0100* (*BCOX1*) as a recurrently amplified region with high gene expression which was more common in tumors from H/L than tumors from White women in TCGA. KIAA0100 was originally identified in a screen for genes that were more frequently found

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391 in breast tumor than in normal breast tissue[43] and increased expression was associated with
392 poor prognosis[43, 44]. Knock-down of *KIAA0100* by siRNA in the breast-cancer cell line MDA-MB-
393 231 reduced cell aggregation, reattachment, cell metastasis and invasion[45]. Thus, KIAA0100
394 may be of interest for further study in understanding the biology of tumors in H/L and stratifying
395 women for risk of recurrence.
396
397 Our study has several limitations. We included only women who did not have neoadjuvant
398 therapy prior to surgical resection. We chose this subset of women to avoid effects possibly
399 induced by neoadjuvant chemotherapy such as new mutations and/or selection for resistant
400 subclones. However, because neoadjuvant therapy is more likely to be given to patients with
401 large tumors and/or tumors with poor prognosis[46], tumors included in our study may have some
402 differences in comparison with prior studies due to these selection criteria. For example,
403 because most triple-negative breast tumors are first treated with neoadjuvant therapy, the
404 proportion of triple-negative tumors in our study was lower than previously reported[47]. Our
405 analysis of tumor copy-number alterations was based on WES data. Although WES and other
406 forms of targeted sequencing are used for CNA analysis, it makes it difficult to conduct one-to-
407 one comparisons to array-based or whole genome sequencing-based analyses. Therefore, we
408 limited our analyses to copy-number events that also demonstrated gene-expression
409 differences across populations. Finally, although our study substantially increases the number
410 of tumors analyzed by WES in H/L, the overall numbers are still substantially lower than in
411 White women. In particular, we are likely underpowered to discover low frequency, ethnic
412 and/or ancestry-specific drivers that may be unique to this population. There also were too few
413 recurrences and deaths for statistical analyses.
414
415 In summary, we conducted a comprehensive characterization of somatic mutations, CNAs, and
416 gene expression in 146 breast tumors from 140 H/L from Los Angeles County, California. We

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found that COSMIC signature 16 was more common in our dataset and a recently published dataset of Mexican women living in Mexico, suggesting that this signature may be important in self-reported H/L/Hispanic women and potentially useful to understand differences at diagnosis and for outcome. Finally, our combined CNA and gene-expression analysis suggested that KIAA0100 may be a possible driver of breast-cancer aggressiveness in a subset of our sample. These results should be useful to understanding the biology and guiding therapy for breast cancer among H/L.

Acknowledgments:

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Conflicts of interest. JNW is a speaker for the Bureau for AstraZeneca, and an employee at Natera. No other conflicts of interest from authors

Data are being deposited in dbGAP and will be made available at the time of acceptance.

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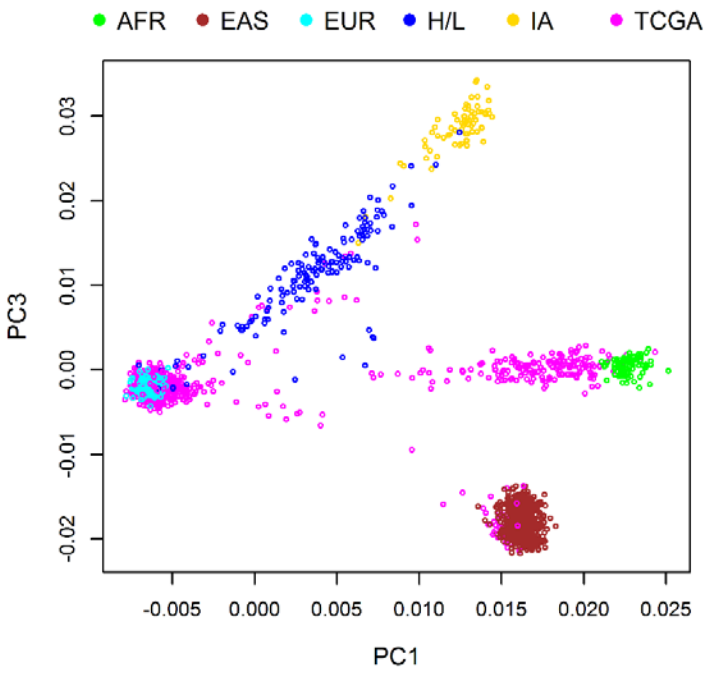
Figure 1

A.



B.

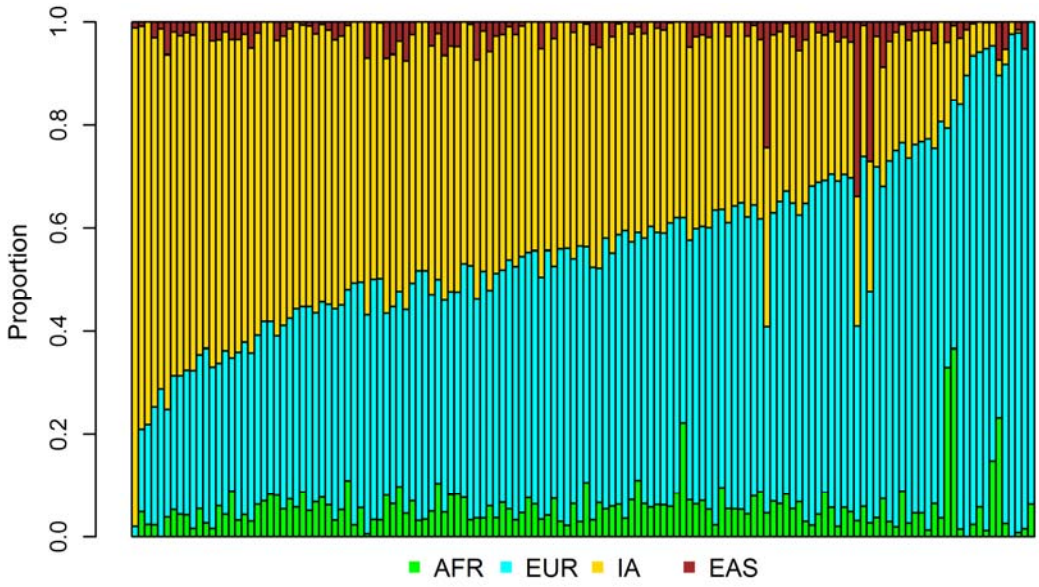


Figure 1. Ancestry of the cohort. Results of principal components analysis comparing the values for samples on principal component (PC) 1 (x-axis) and PC3 (y-axis) (A). Each dot represents the results from one individual: Hispanic/Latina (H/L) (dark blue); TCGA (pink); and

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reference populations including African (AFR), Yoruban individuals from Nigeria from HapMap (light green); East Asians (EAS), Han Chinese from HapMap (brown); European American (EUR), CEBP from HapMap (light blue); and Indigenous American (IA) (yellow) from Mexico. PC2 (not shown) captures individuals of Asian and Indigenous-American ancestry. (B). Results from ADMIXTURE analysis. Each vertical bar represents estimate of ancestry from one individual. Ancestry is assigned for each individual as a fraction of either African (green), Asian (brown), European (light blue) or Indigenous American (yellow) ancestry.

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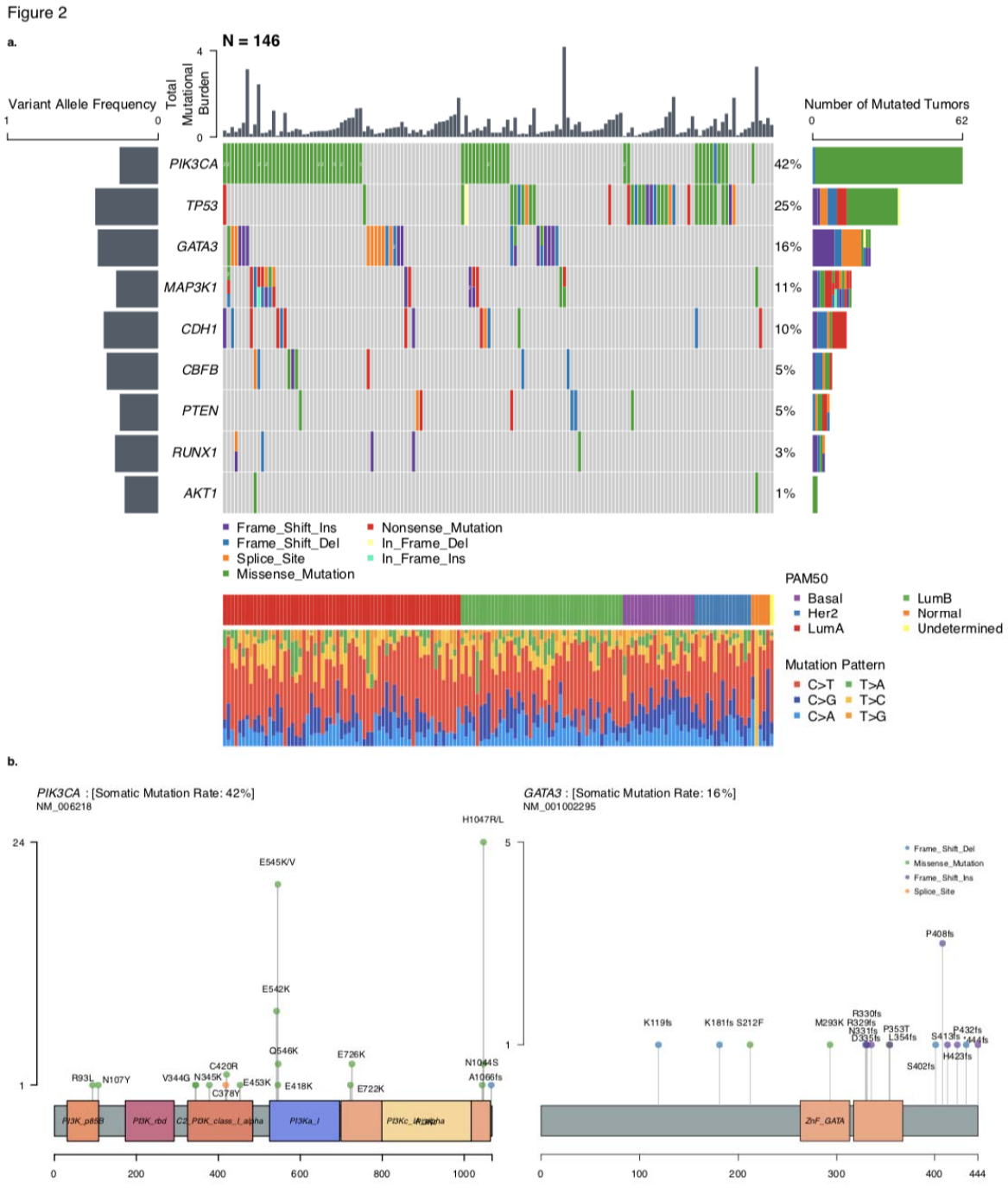
459 **Figure 2**



460

461 **Figure 2**. Tumor mutational burdens and somatic-mutational profiles. a. Mutation plot of nine

462 significantly mutated genes in the 146 tumors. Different mutation classifications are color-coded.

463 Numbers are shown where multiple mutations of the same classification were detected. Total

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464 mutational burden for each tumor is shown as a bar chart on top. The mean variant allelic
465 frequency is shown for each gene on the left. PAM50 subtype and mutation pattern for each
466 tumor are shown at the bottom. b. Lollipop plot of *PIK3CA* and *GATA3* mutations within the 146
467 tumors. Mutation classifications are color coded and amino-acid changes are specified for each
468 mutation.
469
470
471

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Figure 3

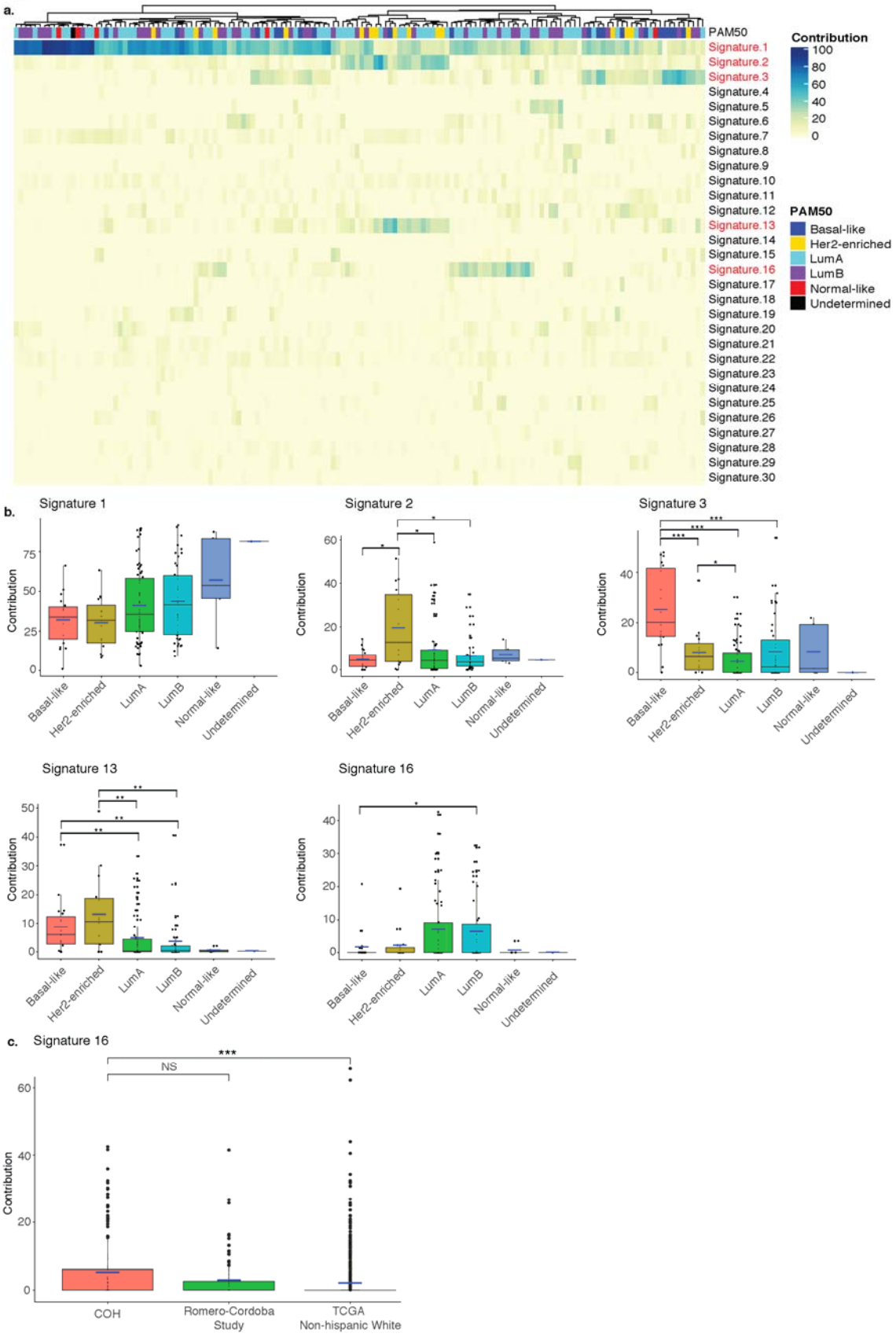

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Figure 3. Mutational Signatures. a. Unsupervised clustered heatmap of contributions from each mutational signatures for the 146 tumors. Significant signatures are highlighted in red. PAM50 subtype for each tumor is shown on top of the heatmap. b. Box-plots comparison of the contributions of the five significant mutational signatures (Signature 1, 2, 3, 13, 16) across the PAM50 subtypes. Statistical significance levels are indicated within the box plots (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$, Wilcoxon Rank-sum test). c. Box plot of Signature 16 contributions in the 146 tumors from the Hispanic-Mexican cohort (COH), Romero-Cordoba Study and the Non-Hispanic White tumors in the TCGA dataset. Statistical significance levels are indicated within the box plot (NS: not significant, $p > 0.05$; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$, Wilcoxon Rank-sum test).

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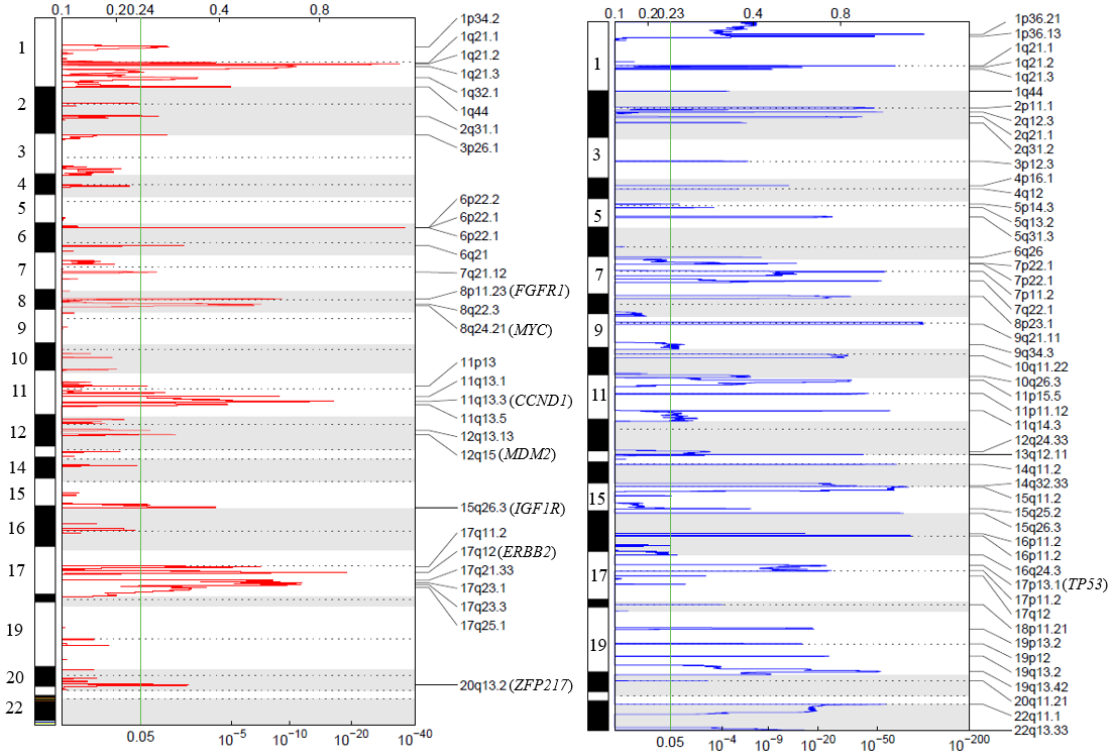
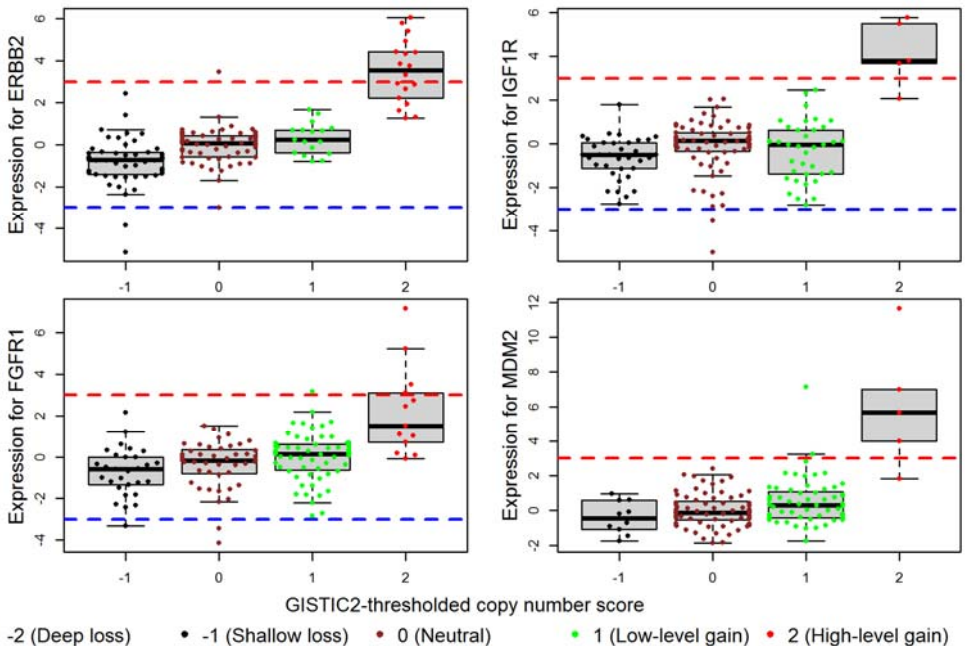
Figure 4.

A.



B.



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506 **Figure 4**: Copy-number alterations.

507 a. Genomic regions of significant copy-number gain (left) and loss (right) identified by GISTIC2.
508 Common oncogenes and tumor suppressor genes are in parentheses next to the corresponding
509 cytobands. The green vertical line marks the GISTIC2 q value of 0.05 (bottom x-axis). b.
510 Outlying gene expression and copy-number gain in four genes in 146 H/L breast tumor
511 samples. Gene-expression values on the y-axis are Z-scores estimated by robust
512 standardization; the red dash line of Z-score = 3 and blue dash line of Z-score = -3 are cutoff
513 values for outliers of over-expression and under-expression, respectively.

514

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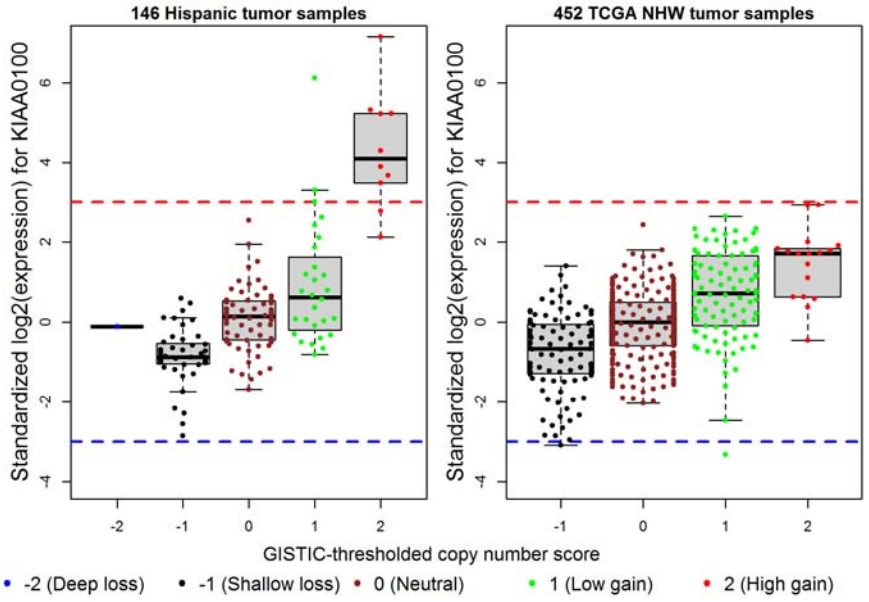
Figure 5.

A.



B.

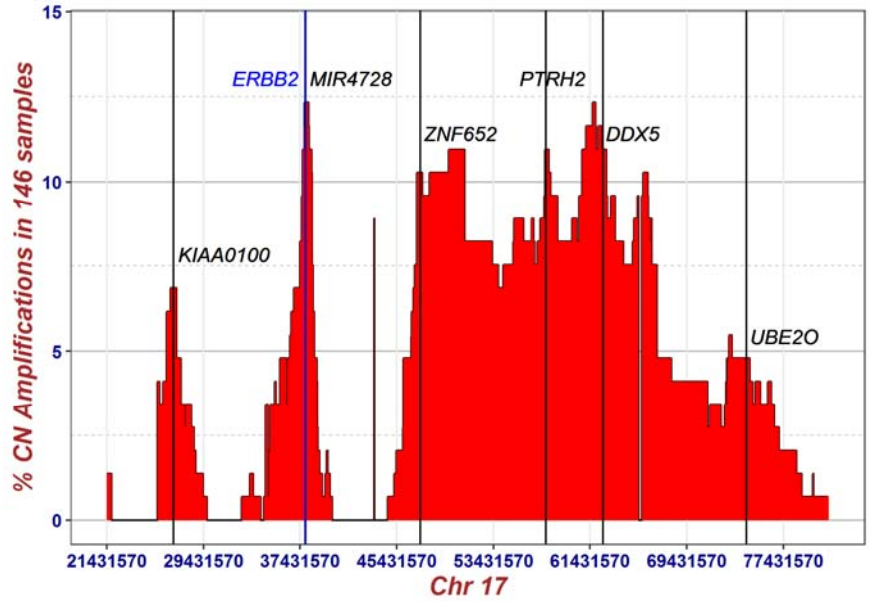


Figure 5. Expression outliers and copy-number gain in KIAA0100.

a. Distribution of gene expression and GISTIC2-thresholded copy-number scores in *KIAA0100* for 146 breast tumor samples from Hispanic whites and 452 breast tumor samples from TCGA Non-Hispanic whites (A). The y-axis is standardized gene-expression values (Z-scores)

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525 estimated robustly based on the corrected median absolute deviation (MAD). Red and blue
526 dashed lines represent Z-score of 3 and -3, respectively. b. Distribution of proportion of high-
527 level copy-number gain for 950 genes spanning the 6 amplified regions of 17q11.2, 17q12,
528 17q21.33, 17q23.1, 17q23.3, and 17q25.1. Y-axis is the percentage of the 146 Hispanic
529 samples with GISTIC2-thresholded copy-number score of 2; x-axis is genomic boundaries
530 (Chr17: 21431570 – 81188573, hg19) for the six significantly amplified regions determined by
531 GISTIC2. The vertical lines mark the genomic locations of *KIAA0100* (*BCOX1,* 17q11.2) at
532 Chr17:26941457 – 26972177, *ERBB2* (17q12) at Chr17: 37844336 – 37873910, *MIR4728*
533 (microRNA 4728, 17q12) at Chr17: 37882747 – 37882814, *ZNF652* (17q21.33) at Chr17:
534 47366567 – 47439476, *PTRH2* (17q23.1) at Chr17: 57774666 – 57784959, *DDX5* (17q23.3) at
535 Chr17: 62494371 – 62503156, and *UBE2O* (17q25.1) at Chr17: 74385612 – 74449288.
536
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538

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539 **Table 1**. Patient and tumor characteristics of 140 H/L breast-cancer cases and their 146 breast
540 tumors

Patient characteristics	Mean	Range	Median	
Age at diagnosis (years)	48.7	31-75	48	
Breastfeeding (months)	7.2	0-84	2	
Parity (number children)	2.3	0-8	2	
Age at menarche (years)	12.6	9-18	12	
Tumor characteristics	Positive	Negative	Unknown	Equivocal
Estrogen receptor	120 (82%)	25 (17%)	1 (0.7%)	
Progesterone receptor	104 (72%)	41 (28%)	1 (0.7%)	
HER2	25 (17%)	116 (80%)	1 (0.7%)	4 (3%)
Stage at diagnosis	I	II	III	IV
	63 (44%)	63 (43%)	17 (12%)	3(2%)

541
542

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Table 2: Frequency difference in expression outliers driven by copy number gain between 146 tumors from H/L and 452 tumors from TCGA White

Gene	GISTIC2 gain region	Specific to H/L*	GISTIC2 q value	# of Outliers in 146 H/L	Freq of Outliers in 146 H/L	# of Outliers in 452 White	Freq of Outliers in 452 White	Fisher Exact p value**	BH adjusted p value
KIAA0100	17q11.2	yes	7.85E-08	11	0.08	0	0	1.37E-07	2.93E-05
DSCC1	8q24.21	yes	1.18E-06	7	0.05	0	0	4.63E-05	4.95E-03
C4BPA	1q32.1	yes	6.24E-04	10	0.07	4	0.01	2.31E-04	9.88E-03
C4BPB	1q32.1	yes	6.24E-04	6	0.04	0	0	1.96E-04	9.88E-03
RNF169	11q13.5	yes	1.90E-05	12	0.08	6	0.01	1.41E-04	9.88E-03
POLDIP2	17q11.2	yes	7.85E-08	10	0.07	5	0.01	5.48E-04	1.95E-02
FOXJ3	1p34.2	yes	8.16E-03	7	0.05	2	0	1.05E-03	2.94E-02
MIR4728	17q12	no	1.02E-19	12	0.08	9	0.02	1.10E-03	2.94E-02
MYBPH	1q32.1	yes	6.24E-04	8	0.05	4	0.01	2.13E-03	3.95E-02
SAP30BP	17q25.1	yes	2.60E-04	10	0.07	7	0.02	2.22E-03	3.95E-02
SDF2	17q11.2	yes	7.85E-08	8	0.05	4	0.01	2.13E-03	3.95E-02
UBE2O	17q25.1	yes	2.60E-04	12	0.08	10	0.02	1.91E-03	3.95E-02
AHCTF1	1q44	no	1.16E-05	5	0.03	1	0	3.96E-03	4.71E-02
GSDMC	8q24.21	yes	1.18E-06	10	0.07	8	0.02	3.95E-03	4.71E-02
MTF1	1p34.2	yes	8.16E-03	4	0.03	0	0	3.44E-03	4.71E-02
PIGS	17q11.2	yes	7.85E-08	6	0.04	2	0	3.49E-03	4.71E-02
QSER1	11p13	no	3.38E-02	9	0.06	6	0.01	3.13E-03	4.71E-02
UNC13D	17q25.1	yes	2.60E-04	5	0.03	1	0	3.96E-03	4.71E-02

H/L:Hispanic/Latino; White: Non-hispanic White; Freq: frequency; GISTIC2: GISTIC2 algorithm for copy number analysis.

*GISTIC2 gain regions are identified in the 146 HW samples, but not in the 663 TCGA Caucasian samples based on GISTIC2 results published by Romero-Cordoba SL, et al[9]; the 8q24.21 region was identified in both groups, however, the wide-peak boundary for the 663 TCGA Caucasian samples (chr8:128657453-128779930) was narrower than that for the 146 HW samples (chr8:114449162-130760646), therefore, DSCC1 and GSDMC are included in 8q24.21 from the 146 H/L samples, but not in the 8q24.21 from the 663 TCGA Caucasian samples.

**frequency difference in the number of expression outliers between H/L and White group was tested using the Fisher's exact method.

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