Germline variants and somatic mutation signatures of breast cancer across populations of African and European ancestry in the US and Nigeria

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Abbreviations: CADD: combined annotation dependent depletion; CCDS: consensus coding domain sequence; COSMIC: Catalog of Somatic Mutation in Cancer; ER: estrogen receptor; ExAC: Exome Aggregation Consortium; GRM: genetic relationship matrix; GWAS: genome-wide association study; HER2: human epidermal growth factor receptor 2; HR: hormone receptor; HRD: homologous recombination deficiency; HWE: Hardy–Weinberg equilibrium; indel: insertion and deletion; MAF: minor allele frequency; NER: nucleotide excision repair; NMF: non-negative matrix factorization; PCA: principal component analysis; PR: progesterone receptor; SKAT-O: optimal sequence kernel association test; SNPs: single nucleotide polymorphisms; SNVs: single nucleotide variants; TCGA: The Cancer Genome Atlas; VEP: variant effect predictor; WES: whole-exome sequencing; WGS: whole-genome sequencing

Additional Supporting Information may be found in the online version of this article.

Conflict of interest: K.P.W. serves as President, and is a shareholder of Tempus Labs Inc. O.I.O. serves as a board member of Healthy Life for all Foundation, is a co-founder, Chief Scientific Advisor and equity stockholder of CancerIQ, serves as Scientific Adviser and is a stockholder of Tempus. J.B. was a former Novartis employee and stock holder.

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Somatic mutation signatures may represent footprints of genetic and environmental exposures that cause different cancer. Few studies have comprehensively examined their association with germline variants, and none in an indigenous African population. SomaticSignatures was employed to extract mutation signatures based on whole-genome or whole-exome sequencing data from female patients with breast cancer (TCGA, training set, n = 1,011; Nigerian samples, validation set, n = 170), and to estimate contributions of signatures in each sample. Association between somatic signatures and common single nucleotide polymorphisms (SNPs) or rare deleterious variants were examined using linear regression. Nine stable signatures were inferred, and four signatures (APOBEC C>T, APOBEC C>G, aging and homologous recombination deficiency) were highly similar to known COSMIC signatures and explained the majority (60–85%) of signature contributions. There were significant heritable components associated with APOBEC C>T signature ($h^2 = 0.575$, $p = 0.010$) and the combined APOBEC signatures ($h^2 = 0.432$, $p = 0.042$). In TCGA dataset, seven common SNPs within or near GNB5 were significantly associated with an increased proportion (beta = 0.33, 95% CI = 0.21–0.45) of APOBEC signature contribution at genome-wide significance, while rare germline mutations in MTCL1 was also significantly associated with a higher contribution of this signature ($p = 6.1 \times 10^{-6}$). This is the first study to identify associations between germline variants and mutational patterns in breast cancer across diverse populations and geography. The findings provide evidence to substantiate causal links between germline genetic risk variants and carcinogenesis.

**What’s new?**

Women of African ancestry are more likely to be diagnosed with clinically aggressive breast cancer than women of European or Asian ancestry. Here, the authors examined associations between germline variants and mutational signatures in breast cancers across different ethnicities, especially in a unique sample set of indigenous African women. They identified four stable mutational signatures that explained the majority of tumor mutations, leading to a better understanding of the complex interplay between germline genetics and somatic mutations across different ethnicities.

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**Introduction**

Somatic mutation signatures act as the physiological readout of the biological history of a cancer, and provide a new bridge to connect cancer mutation to both exogenous and endogenous risk factors.1–3 Many signatures have been reported to be attributable to specific environmental or lifestyle mutagens.4 However, somatic mutations could also be influenced by heritable factors,5 and endogenous factors that influence cancer far more than previously considered.6,7 A few studies have examined the interplay between germline genetics and somatic mutations in carcinogenesis with signals identified for germline variants in RAD51B,8 MCIR,9 APOBEC3 regions10–12 and DNA mismatch repair genes in different types of cancers.13 In addition, a few genes driven by rare deleterious variants were reported to be significant, which include: polymerase epsilon, catalytic subunit (POLE) gene associated with the Catalog of Somatic Mutation in Cancer (COSMIC) signature 10 in colorectal and endometrial carcinomas;1 homologous recombination deficiency (HRD) genes PALB2 and BRCA1/2 associated with HRD signature in various cancers;14,15 and nucleotide excision repair (NER) genes associated with COSMIC signature 5 in urothelial tumors.16 The existing studies offered some evidences to the largely unexplored relationships between germline and somatic mutations.10,17,19

The burden and severity of breast cancer vary widely across populations. Women of African ancestry are more likely to be diagnosed with clinically aggressive disease and have a higher mortality rate than age-matched women of European or Asian ancestry.20 Emerging data from The Cancer Genome Atlas (TCGA) has identified mutation signatures that show differences across ancestry groups.21,22 These studies suggest modest differences between African and non-African ancestry groups but these datasets have relatively small number of women of African ancestry.24 To examine the association between common genetic variants, rare deleterious mutations and the contribution of mutational signatures across diverse populations, we used TCGA as the training set, and examined the whole-genome sequencing (WGS) and whole-exome sequencing (WES) from Nigeria as the validation set. We tested the hypothesis that biologically relevant germline variants associated with somatic mutation signatures in women of non-African ancestry would be validated in women of African ancestry as well.

**Methods**

**Ethics statement**

Our study was embedded within the Nigerian Breast Cancer Study (NBCS) and approved by the Institutional Review Board of all participating institutions. Informed consent has been obtained from the participants.

**Training dataset**

A total of 1,037 breast cancer patients from TCGA were used as the training set. Demographics of participants, including clinical characteristics across ethnicity are summarized in Supporting Information Table S1. Somatic mutation data (single nucleotide
variants [SNVs]) were generated as previously described.\textsuperscript{22} We included 1,035 WES and 84 WGS, and 82 patients owned both WGS and WES data in this analysis.

**Common germline variants from genotyping array.** Affymetrix Genome-Wide Human SNP 6.0 Array data for 2,270 breast cancer samples were downloaded from TCGA on November 6, 2015. A total of 1,134 samples were excluded for different reasons (12: missing ID; 1,110: solid tumor tissue; 12: male patients). Among the remaining 1,136 female samples (1,087 unique patients), we excluded samples for 33 more patients due to genotyping missing rate ≥5%, and worked with samples for 1,054 patients. Of the 906,600 single nucleotide polymorphisms (SNPs) included in the microarray data, 1,466 SNPs were excluded for lacking chromosome information or mapping to Y chromosome and 47,140 SNPs were removed due to genotyping missing rate ≥5% or being monomorphic, and 857,994 SNPs remained.

Genetic ancestry of TCGA patients was estimated using principal component analysis (PCA).\textsuperscript{21} According to the estimated proportion of ancestry, patients were grouped into genomic Black (≥50% African ancestry), genomic White (≥90% European ancestry) and genomic Asian (≥90% Asian ancestry) as previously described.\textsuperscript{21}

Genotype imputation was done using IMPUTE2 software with the 1,000 Genomes Project phase 3 integrated variant set as the reference panel.\textsuperscript{24} A total of 81,232,799 SNPs within autosomes were imputed, and 24,570,114 SNPs were kept after excluding singletons or SNPs with an imputation information score <0.7.

**Rare deleterious variants from germline exomes.** Using the Platypus (https://www.well.ox.ac.uk/research/research-groups/lunter-group/software/platypus-a-haplotype-based-variant-caller-for-next-generation-sequence-data) in single-sample mode, variants within exonic regions were called as described previously.\textsuperscript{22} Briefly, variants were considered rare and deleterious if (i) they had an allele frequency <0.05 in The Exome Aggregation Consortium (ExAC)\textsuperscript{23} and across TCGA blood germline exomes from various cancer types,\textsuperscript{22} and (ii) they were predicted by variant effect predictor (VEP) to have "HIGH" impact, cause protein loss-of-function (stop-gain, frameshift insertion and deletion [indel], etc.), or were missense mutations with a combined annotation dependent depletion (CADD)\textsuperscript{26} score >25. Only blood germline exomes were included. Additionally, variants were required to fall within consensus coding sequence (CCDS, version 17) exonic regions to be carried downstream. In total, 132,186 rare deleterious variants were found within 11,718 genes in 968 samples, of which 2,933 genes had only one mutation and 6,560 genes (56.0%) had less than five counts of rare coding deleterious variants among these samples. We therefore only focused on genes with at least five mutations (n = 5,158) in the downstream analyses. The Bonferroni corrected alpha levels for significance was 9.69 × 10^{-6}.

**Validation dataset**

A total of 172 women with breast cancer from Nigeria were evaluated. All Nigerian patients were assumed to be 100% African with little to no admixture with other populations.\textsuperscript{27} These patients were younger and had more aggressive subtypes of breast cancer with higher proportion of estrogen receptor negative (ER−), progesterone receptor negative (PR−), human epidermal growth factor receptor 2 negative (HER2−; triple-negative) subtypes. Characteristics of the Nigerian dataset were previously described.\textsuperscript{22}

**Common and rare genetic variants.** Hundred breast tumor samples for WGS and 129 breast tumor samples for WES from 172 unique patients were processed for this analysis. For the common SNP analysis, 98 WGS samples were involved, while 170 samples (129 WES, 41 WGS) were included for rare variants analysis. Rare coding deleterious variants were called as described above. In total, 22,035 rare coding deleterious variants were found within 5,439 genes in 170 samples, of which 2,196 genes had only one mutation among these samples and 3,876 genes (71.3%) had less than five rare coding deleterious counts. A total of 1,563 genes were included in the final analysis.

**Mutation signature calling and comparison with reported signatures**

**Mutational signature calling.** Somatic SNVs were called as described previously.\textsuperscript{22} Briefly, in order to be utilized in downstream analyses, SNVs needed to be called by both MuTect (https://software.broadinstitute.org/cancer/cga/mutect) and Strelka (https://github.com/Illumina/strelka) as well as be absent within a normal panel of exomes (n = 1,088) and genomes (n = 124) derived from blood. We employed a nonnegative matrix factorization (NMF) approach using SomaticSignatures\textsuperscript{28} to extract somatic mutational signatures and estimate their contributions to each sample.

The ability to reliably call mutation signatures depends on having sufficient number of mutations per sample. To this point, we used all high-quality somatic exome SNVs, regardless of their position in the coding or noncoding region. A pilot study was conducted to compare the stability of signatures among different number of minimum SNVs (24, 48, 72 and 96, data not shown), and found that a cut point of 24 is sufficient. This is consistent with recent finding that 20 mutations gave an average classification accuracy of 80% across signatures.\textsuperscript{29} Any sample containing at least 24 SNVs was included for downstream assessment in order to enlarge the sample size as much as possible.

In order to obtain more stable signature estimates and ensure comparability between the training and validation sets, we combined both datasets to call the signatures, including 84 WGS samples and 1,008 WES samples from TCGA as well as 98 WGS samples and 129 WES samples from Nigeria. For samples with both WGS and WES, both data types were included.
We first used the nonnegative matrix factorization without normalization to obtain the mutation matrix as described by Alexandrov et al., then calculated the percentage contributions of signatures for each sample by normalizing to the number of mutations of each sample. For subsequent analyses, we used the percentage of mutations assigned to each signature (contribution) rather than the total number of mutations attributed to a signature. The sum of mutation signatures’ contribution is 1. This distinction is important as high APOBEC contribution, for example, do not necessarily imply APOBEC hypermutation.

Nine signatures were retained at the end for further downstream analyses because nine signatures explained approximately 99% of variance in the current sample. For all nine signatures, we examined the correlation of contributions between exomes and genomes using 138 individuals who had both WES and WGS data. Each signature’s contribution was simultaneously called for WGS samples and WES samples for those 138 individuals. Signatures A1 (rho = 0.938), A2 (rho = 0.912) and A9 (rho = 0.866) all exhibited strong correlation. APOBEC C>T (Signature A1) and C>G (Signature A2) contributions were correlated (r = 0.43). We only focused on stable signatures between WGS and WES in order to reliably leverage all available WES samples.

Comparison with reported signatures. We compared our mutation signature matrices with the 30 previously reported signatures operative across a variety of cancer types downloaded from the COSMIC. Mean Kullback–Leibler Divergence was applied to evaluate the similarity between our signatures and those from COSMIC. The heritability was not calculated for Nigeria samples due to limited sample size. In the training dataset, among 24,570,114 SNPs, 1,573,566 were removed due to missing genotype data (missing proportion >5%), 5,301,200 variants were removed due to minor allele frequency (MAF) <0.001, and 188,864 variants were removed due to deviation from the Hardy–Weinberg equilibrium (HWE). The remaining 17,506,484 SNPs were used for downstream analyses. Among 1,054 samples with SNP chip data, 178 samples were removed due to missing genotype data (cut-off point as 1%), 77 were removed when pruning the GRM (relatedness <0.05) and 66 samples were excluded due to missing mutation signature. In the end, 733 samples were included in the model.

Association of common SNPs with signature contribution. For TCGA, we analyzed 7,177,790 common SNPs with MAF ≥0.05. The single-SNP association tests with signature contribution were conducted using linear regression as implemented in SNPtest, with adjustment for age and the eigenvectors from PCA. For the validation data, on the other hand, we only adjusted for age given that we do not anticipate any admixture with other ethnic. We applied genome-wide association study (GWAS) threshold (p = 5 × 10^{-8}) as our cut-off point in the discovery stage. We also filtered the association results of the GWAS-identified risk SNPs by its location within the autosomal regions to check their role in the signature contributions. All SNPs included were previously reported to be associated with breast cancer risk (https://www.ebi.ac.uk/gwas/). In Nigerians, we only assessed SNPs that were nominally significant in the TCGA cohort.

Association of rare deleterious variants with signature contribution. Gene-based association analyses for mutation signatures were conducted using the unified optimal sequence kernel association test (SKAT-O), as implemented in SKAT, as a linear combination of the burden and SKAT tests. SKAT-O achieves robust power whether a given gene has a high proportion of causal variants exerting effects in the same direction, or instead has many noncausal variants or variants exerting effects in opposite directions. We considered weighting parameter θ = 1 (burden) and θ = 0 (SKAT) tests and reported the optimal of the two tests. We included putatively deleterious variants with MAF ≤5%, and used the
beta distribution weights proposed by Wu et al., which upweights rarer variants, for both tests. Gene-based analyses were adjusted for age and the eigenvectors from PCA for TCGA data. For validation with Nigerian dataset, we only adjusted for age. Subgroup analyses by subtype were also evaluated for all significant results. We used a Bonferroni adjustment for multiple testing to assess the significance of the gene-based test results. We also calculate Benjamini–Hochberg’s false positive rate in the discovery dataset. For genes with evidences of association in the discovery phase, we pooled data from TCGA and Nigeria and conducted SKAT-O analysis after adjusting for age and proportion of African and Asian ancestry.

Two combined gene sets were examined to test their association with the contribution of HRD signature. One is a set of known breast cancer predisposition genes (BROCA gene panel: http://tests.labmed.washington.edu/BROCA), another one contained all homologous recombination related genes from Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database, including ATM, RAD51C, BARD1, BRCA1, BRCA2, BRIP1, FAM175A, MRE11A, NBN, PALB2, XRCC2, POLD1, RAD51B, BABAM1, BLM, BRE, EME1, RAD50, RAD52, RAD54B, RAD54L, RBBP8, RPA1, SYCP3, TOP3A, TOP3B, TOPBP1, UIMC1 and XRCC3. All statistical calculations were completed in R or Stata version 15.0 (College Station, TX). All p values are two-sided.

Results

Basic information

A total of 1,011 samples with somatic mutation count ≥24 (1,008 WES, 84 WGS) from TCGA were included as the training dataset, and 170 samples that satisfied the same criteria from Nigeria (129 WES, 98 WGS) were involved as the validation set (Fig. 1). The median (25th quartile and 75th quartile) for somatic mutation count per WES sample were 73 (50–144) for TCGA Caucasians, 100 (63–194) for TCGA African Americans and 131 (64–241) for Nigerians (Supporting Information Table S1 and Fig. S1). The number of mutation from WES was highly correlated with that from WGS (p < 0.001, Supporting Information Fig. S1C).

Nine mutational signatures were extracted (Supporting Information Fig. S2). Our signatures closely resembled breast cancer signatures from COSMiC: signatures A1 (S2: APOBEC C>T), A2 (S13: APOBEC C>G), A3 (S17: Unknown, breast cancer related), A4 (S10: Altered activity of polymerase POLE), A6 (S1: Aging),
A8 (S8: Unknown etiology) and A9 (S9: HRD; Supporting Information Fig. S3). The common signatures including S2 (APOBEC C>T), S13 (APOBEC C>G), S1 (Aging), S8 (Unknown etiology) and S9 (HRD), explained the vast majority of mutations regardless of ethnicity or subtype (Supporting Information Figs. S4). The correlation between exomes and genomes were presented in Supporting Information Figures S5—there was high correlation for S2, S13, S1 and S9. Given the robustness of signature calls between these two data types, we focused on these four signatures for subsequent analyses.

Heritability of selected signatures’ contribution

The estimates of array-based heritability, $h^2$, ranged from 0.040 to 0.575 in TCGA samples across four selected signatures (Table 1), with APOBEC C>T signature ($h^2 = 0.575$, $p = 0.010$) and combined APOBEC signature ($h^2 = 0.432$, $p = 0.042$) displaying the strongest heritable components. Of note, the heritability estimates of mutation signatures are unreliable due to limited sample size, although we showed that some of them were statistically significantly different from zero.

Association of common SNPs with signature contribution

Next, we analyzed the association of common SNPs with mutation signatures. Nine hundred seventy-four samples from TCGA and 170 samples from Nigeria with mutation signature results were further analyzed. No SNPs in TCGA showed significantly different from zero. The top seven SNPs in TCGA tagged a couple of SNPs having LD $r^2 ≥ 0.85$, and they are located in the region with several regulatory elements (Supporting Information Table S2, Fig. 2d). Using the Nigerian cohort as the validation set, 13 of 30 top SNPs were detected with nonsignificant $p$ values, but with consistent magnitude and direction in beta coefficients to those in TCGA (Table 2).

In addition, we evaluated the association between index SNPs identified in previous GWAS of breast cancer risk, and signatures contribution. We found a dozen SNPs associated at nominal significance level with either APOBEC signature or HRD signature (Supporting Information Table S3). The genes tagged by SNPs associated with APOBEC signature included ANKRD16, ZNF365, CHST9, ADRD3, FGFR2 and ESR1. Most of the genes associated with HRD signature function to decrease homologous recombination repair frequency, including RNF115, ANXA13, DNAJC1, DNAH11 and TFAP2A.

Associations between rare deleterious variants and signature contribution

Manhattan plots for the gene-based association analyses are shown in Figure 3a and Supporting Information Figure S8 for selected mutation signatures in TCGA cohort. Five genes showed evidence of association with contribution of APOBEC C>T signature or APOBEC C>G signature surpassing defined significance level (threshold as $9.69 \times 10^{-8}$, Table 3). MTCL1 is the top signal for combined APOBEC signature (pooled $p = 6.11 \times 10^{-8}$), and women with MTCL1 mutations had higher APOBEC signature contribution. Two additional genes (HIVEP1 and TMEM104) had marginal nominal significant association with contribution of combined APOBEC signature in both datasets. As the direction of the association for HIVEP1 was consistent across the TCGA and Nigerian datasets (pooled $p = 0.009$), while the TMEM104 association was positive in the TCGA cohort but negative in the Nigerian cohort (pooled $p = 0.079$) (Supporting Information Fig. S9), suggesting HIVEP1 is likely a true association but TMEM104 may be false negative. Mutations in three genes, including MTCL1, ERCI and HIP1, were associated with higher contribution of APOBEC C>G signature alone.
Germline mutations in four genes, BRCA1, BRCA2, COL15A1, and PIGO, were significantly associated with contributions from the HRD signature (all $p \leq 5.5 \times 10^{-6}$). The contribution of HRD signature was consistently increasing along with the number of rare deleterious variants in both BRCA1 and BRCA2 across different tumor subtypes (HER2+, HR+/HER2− and HR−/HER2+) and different datasets (Figs. 3b and 3c). We combined all mutations in BRCA1 and BRCA2 together. The combined gene set was significantly associated with the contribution to HRD signature in both TCGA ($p = 5.0 \times 10^{-12}$) and Nigeria samples ($p = 0.0036$). This association was also highly consistent across different tumor subtypes and datasets (Fig. 3d). Despite genome-wide significance, mutations in COL15A1 and PIGO are rare and further validation of their associations is desirable.

In addition, when we combined all mutations occurring in a set of known breast cancer predisposition genes (BROCA gene panel; 17 genes), or when we merged all homologous recombination related genes (29 genes), we found that both compounded gene sets were significantly associated with the contribution of HRD signature ($p = 2.6 \times 10^{-7}$ and $2.8 \times 10^{-5}$, respectively). The significance level remained in the validation set ($p = 0.0007$ and $0.014$, respectively). However, after excluding BRCA1 and BRCA2, neither of these gene sets remained significantly associated with HRD signature contribution. Two of HR related genes, BRIP1 and RAD50, probably contributed to the association signal since many Nigerian samples had HRD contributions present yet no germline mutation in BROCA or homologous recombination genes (Supporting Information Table S4).

Discussion

Using breast cancer data from 1,011 patients from TCGA (the training set) and 170 Nigerian women (the validation set), we extracted four stable mutational signatures including APOBEC C>T, APOBEC C>G, aging and HRD, which is consistent with previous studies.1,4,40,41 These signatures alone were able to explain the majority of the tumor mutations across both cohorts. Heritability analysis suggests that germline genetic factors could explain some of the heterogeneity in mutation signatures across patients with breast cancer. We confirmed the association between rare deleterious variants in BRCA1/2 and increased HRD signature activity. We found that common variants proximal to GB5 as well as rare exonic mutations within MITCL1 were associated...
Table 2. The top 30 signal for common SNPs in association tests with combined APOBEC signature contributions

| ID   | SNP                | Chromosome | Position (base) | Ref allele | ALT allele | TCGA Information score | MAF  | p value  | Beta   | Standard error  |
|------|--------------------|------------|----------------|------------|------------|------------------------|------|----------|--------|-----------------|
| 1    | rs66866642/rs578194564 | 15         | 52,444,832     | C          | CT         | 0.948                  | 0.169| 1.03E-08 | 0.353  | 0.062           |
| 2    | rs12902073          | 15         | 52,447,508     | G          | A          | 0.986                  | 0.170| 2.84E-08 | 0.334  | 0.060           |
| 3    | rs12440354          | 15         | 52,452,434     | T          | C          | 0.983                  | 0.175| 3.90E-08 | 0.328  | 0.060           |
| 4    | rs12901730          | 15         | 52,452,697     | T          | A          | 0.983                  | 0.171| 4.31E-08 | 0.330  | 0.060           |
| 5    | rs12901891          | 15         | 52,452,834     | T          | C          | 0.983                  | 0.171| 4.33E-08 | 0.330  | 0.060           |
| 6    | rs12438743          | 15         | 52,454,098     | C          | T          | 0.982                  | 0.171| 4.38E-08 | 0.330  | 0.060           |
| 7    | rs373292393         | 15         | 52,440,257     | C          | CAA        | 0.983                  | 0.155| 4.98E-08 | 0.340  | 0.062           |
| 8    | rs12910398          | 15         | 52,445,477     | C          | T          | 0.987                  | 0.176| 5.57E-08 | 0.322  | 0.059           |
| 9    | rs12906598          | 15         | 52,445,090     | A          | G          | 0.986                  | 0.176| 6.16E-08 | 0.321  | 0.059           |
| 10   | rs12909880          | 15         | 52,449,293     | T          | C          | 0.982                  | 0.173| 6.76E-08 | 0.323  | 0.060           |
| 11   | rs12902522          | 15         | 52,448,036     | A          | G          | 0.986                  | 0.177| 7.59E-08 | 0.319  | 0.059           |
| 12   | rs12901052          | 15         | 52,449,360     | T          | C          | 0.983                  | 0.178| 7.98E-08 | 0.318  | 0.059           |
| 13   | rs12903769          | 15         | 52,449,733     | G          | A          | 0.982                  | 0.167| 8.20E-08 | 0.327  | 0.061           |
| 14   | rs12908833          | 15         | 52,449,195     | G          | C          | 0.982                  | 0.174| 8.80E-08 | 0.320  | 0.060           |
| 15   | rs12908742          | 15         | 52,449,379     | A          | C          | 0.982                  | 0.174| 8.82E-08 | 0.320  | 0.060           |
| 16   | rs3794543           | 15         | 52,441,524     | C          | T          | 0.993                  | 0.158| 1.07E-07 | 0.327  | 0.061           |
| 17   | rs4776007           | 15         | 52,447,299     | A          | G          | 0.987                  | 0.185| 1.29E-07 | 0.308  | 0.058           |
| 18   | rs8034097           | 15         | 52,437,278     | A          | G          | 0.995                  | 0.151| 1.32E-07 | 0.330  | 0.063           |
| 19   | rs12438274          | 15         | 52,439,368     | T          | G          | 0.971                  | 0.188| 1.33E-07 | 0.311  | 0.059           |
| 20   | rs12438194          | 15         | 52,439,096     | T          | A          | 0.993                  | 0.152| 1.35E-07 | 0.330  | 0.063           |
| 21   | rs4636859           | 15         | 52,435,696     | T          | C          | 0.996                  | 0.151| 1.45E-07 | 0.329  | 0.063           |
| 22   | rs12438937          | 15         | 52,441,686     | C          | T          | 0.992                  | 0.170| 1.79E-07 | 0.313  | 0.060           |
| 23   | rs35709612          | 15         | 52,439,940     | A          | C          | 0.994                  | 0.169| 1.80E-07 | 0.313  | 0.060           |
| 24   | rs35448038          | 15         | 52,437,758     | GT         | G          | 0.981                  | 0.163| 1.89E-07 | 0.317  | 0.061           |
| 25   | rs12593041          | 15         | 52,443,107     | C          | T          | 0.988                  | 0.171| 2.49E-07 | 0.310  | 0.060           |
| 26   | rs62014722          | 15         | 52,434,338     | C          | G          | 0.995                  | 0.163| 2.52E-07 | 0.313  | 0.061           |
| 27   | rs62014721          | 15         | 52,434,284     | C          | G          | 0.994                  | 0.163| 2.53E-07 | 0.313  | 0.061           |
| 28   | rs62014721          | 15         | 52,436,334     | C          | T          | 1.000                  | 0.222| 2.75E-07 | 0.281  | 0.055           |
| 29   | rs71130134          | 15         | 52,437,673     | CA         | C          | 0.980                  | 0.157| 4.73E-07 | 0.313  | 0.062           |

Bold p values denote statistical significance at the p < 5 × 10^−8 (genome-wide association study threshold).

1A total of 17 SNPs were not covered in WES samples, so we filled the corresponding cell with “–”.

Abbreviation: MAF, minor allele frequency.
with increased APOBEC C>T/G activity, acting as plausible contributors to the observed heritability. Rare variants in HIVEP1 were also marginally associated with the signature although this did not reach genome-wide significance, probably because of the small sample size.

APOBEC signatures, the most widespread mutational signatures in human cancers, have been reported in 16 of 30 tumor types from TCGA, implicating APOBEC enzymes as some of the most pervasive mutators in human cancers. We found seven common SNPs in/near GNB5 to be significantly associated with APOBEC signature contribution in TCGA. However, this signal could not be replicated in Nigerians, possibly due to limited sample size. Previous studies suggested a potential role for GNB5 in cancer.

First, GNB5 encodes guanine nucleotide-binding protein beta-5, and this protein is expressed in breast tissue. Evidence from the Human Protein Atlas at the protein and mRNA levels also suggested the favorable prognostic role of GNB5 in renal cancer and endometrial cancer. Second, overexpression of GNB5 promoted a migratory phenotype in lung adenocarcinoma cells. However, it remains unclear whether GNB5 directly affects the transcription, translation or activity of APOBEC enzymes. Further molecular analyses are required to functionally characterize the observed relationship and its generalizability to other tumor types.

The association between HIVEP1 and APOBEC signature could be explained by their functional roles in viral defense. The presence of viral element can stimulate APOBEC expression through a complex network of innate immunity signaling, including components like Toll-like receptors, interferons, interleukins and even P53. It is possible that HIVEP1 proteins play a more direct role in APOBEC regulation as it binds enhancer elements containing the GGGACTTTCC motif. In the context of disease, downregulation of another family member, HIVEP2 gene, has already been shown as one of the genetic events responsible for breast cancer. We did not find prior evidence directly implicating MTCL1, also known as suppressor of glucose autophagy associated 2 (SOGA2), in APOBEC activity. Suggestive association between MTCL1 and breast cancer risk has been reported in women of Indonesian ancestry, further indicating the potential importance of germline variation proximal to this gene.

To further understand the biological basis of our observations, it will be

Figure 3. Associations between rare deleterious variants and HRD signature. (a) Plot of log-transformed p values from gene-based analysis for rare deleterious variants. (b–d) Comparison of contribution of HRD signature, grouped by breast cancer tissue subgroups, datasets, and the number of BRCA1 (b), BRCA2 (c) and BRCA1/BRCA2 mutations (d). [Color figure can be viewed at wileyonlinelibrary.com]
necessary to conduct functional studies to uncover the exact causal mechanisms that may ultimately inform innovative therapeutic approaches.

We found that the combined effects of rare, deleterious alleles in *BRCA1* and *BRCA2* were consistently associated with higher HRD signature contributions in both TCGA and Nigerian data. This finding was in accordance with other studies done with prostate and gastric cancers,\(^2,14,15\) and the mechanisms promoting HRD activity have been explored in several experimental studies.\(^2,14,15\) However, to the best of our knowledge, this is the first report of association in an indigenous African population in Nigeria, and our findings suggest that genetic ancestry may play an important role in mutational development and cancer progression. This highlights the need to accumulate more genetic data from diverse populations in order to better understand the heightened aggressive young onset breast cancer.\(^20\)

In previous work, Zhu and colleagues observed a significant inverse association \((p = 8.75 \times 10^{-6})\) between the risk allele in rs2588809 of the gene *RAD51B* and total somatic mutation count across 638 breast cancer patients of European ancestry from TCGA.\(^8\) In our study, rs2588809 was not associated with the contribution of any signatures, but rare deleterious variants within *RAD51B* were found to be nominal significantly correlated with the contributions of aging and HRD signatures. It has been shown that loss-of-function mutations in homologous recombination genes other than *BRCA1/2* can facilitate HRD signature activity.\(^41\) It is possible that *RAD51B* deficiency increases the mutational burden of tumors through HRD, potentially making these patients good candidates for immunotherapy due to their increased likelihood of harboring neoantigens.\(^53\) Taken together, these findings suggest that genetic ancestry may play an important role in mutational development and cancer progression. This highlights the need to accumulate more genetic data from diverse populations in order to better understand the heightened aggressive breast cancer risk observed in individuals of African ancestry.\(^54\)

There are multiple advantages in our study. First, samples from Nigeria added a substantial value in terms of validation, which expanded the findings from a primarily US European ancestry population to an indigenous African population.\(^55\)

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### Table 3. Significant results of gene-based analysis in the training and validation datasets

| Signatures | Gene   | TCGA data | Nigeria data | Pooled data |
|------------|--------|-----------|--------------|-------------|
|            |        | Nominal p value | False discovery rate | # of variants in the test | p value | # of variants in the test | p value |
| APOBEC C>T | *ATP13A1* | 9.04 × 10^{-6} | 0.006 | 1 | 5 | 0.580 | 5 | 0.0089 |
|            | *ERC1* | 0.186 | 1.000 | 1 | 9 | 0.442 | 1 | 0.282 |
|            | *HIP1* | 0.773 | 1.000 | 1 | 13 | 0.494 | 1 | 1.000 |
|            | *QARS* | 0.0023 | 1.154 | 0 | 6 | 0.434 | 1 | 0.0036 |
|            | *MTCL1* | 5.21 × 10^{-5} | 0.053 | 0 | 16 | 0.538 | 2 | 0.0005 |
|            | *HIVEP1* | 0.149 | 1.000 | 1 | 20 | 0.0657 | 9 | 0.0798 |
|            | *TMEM104* | 0.0002 | 0.061 | 0 | 15 | 0.156 | 7 | 0.0609 |
| APOBEC C>G | *ATP13A1* | 0.356 | 1.000 | 1 | 5 | 0.737 | 5 | 0.580 |
|            | *ERC1* | 4.45 × 10^{-7} | 0.0005 | 0 | 9 | 0.444 | 1 | 1.63 × 10^{-7} |
|            | *HIP1* | 3.46 × 10^{-6} | 0.005 | 0 | 13 | 0.219 | 2 | 9.18 × 10^{-7} |
|            | *QARS* | 6.64 × 10^{-7} | 0.001 | 0 | 6 | 0.740 | 1 | 2.86 × 10^{-5} |
|            | *MTCL1* | 1.35 × 10^{-6} | 0.001 | 0 | 16 | 0.360 | 2 | 4.21 × 10^{-7} |
|            | *HIVEP1* | 2.23 × 10^{-5} | 0.015 | 0 | 20 | 0.119 | 9 | 2.57 × 10^{-5} |
|            | *TMEM104* | 0.0095 | 0.417 | 1 | 15 | 0.0238 | 7 | 0.0445 |
| APOBEC C>G | *ATP13A1* | 0.1274 | 0.883 | 1 | 5 | 0.611 | 5 | 0.507 |
|            | *ERC1* | 0.0006 | 0.165 | 0 | 9 | 0.957 | 1 | 0.0014 |
|            | *HIP1* | 0.0154 | 0.452 | 0 | 13 | 0.808 | 2 | 0.0075 |
|            | *QARS* | 1.50 × 10^{-5} | 0.056 | 0 | 6 | 0.520 | 1 | 0.0002 |
|            | *MTCL1* | 1.91 × 10^{-6} | 0.012 | 1 | 16 | 0.393 | 2 | 6.11 × 10^{-6} |
|            | *HIVEP1* | 0.0024 | 0.253 | 1 | 20 | 0.054 | 9 | 0.0009 |
|            | *TMEM104* | 0.0004 | 0.189 | 1 | 15 | 0.041 | 7 | 0.079 |
| HRD        | *BRCA1* | 1.03 × 10^{-8} | 0.0001 | 1 | 25 | 0.139 | 6 | 7.02 × 10^{-9} |
|            | *BRCA2* | 1.05 × 10^{-5} | 0.008 | 1 | 50 | 0.014 | 8 | 7.93 × 10^{-7} |
|            | *COL15A1* | 5.15 × 10^{-6} | 0.005 | 0 | 10 | 0.391 | 1 | 5.50 × 10^{-6} |
|            | *PIGO* | 1.80 × 10^{-5} | 0.009 | 0 | 5 | – | 0 | 8.68 × 10^{-6} |

Bold p values denote statistical significance at the \(p < 9.69 \times 10^{-6}\) (Bonferroni corrected alpha level).

\(^1\)The weighting parameter theta indicates whether the SKAT test (theta = 0) or burden test (theta = 1) gave the smallest p value.

\(^2\)p value from optimal sequence kernel association test (SKAT-O).
Second, we harmonized data from a number of sources using the same pipeline, which avoided batch effect due to bioinformatics software. Third, we inferred mutations signatures across all samples using nonnegative matrix factorization and used the estimated signature contributions directly for analysis. This offered advantages over previous approaches which used the burden of mutations found in specific trinucleotide contexts as a proxy for APOBEC signature activity.3,8,56,57 Fourth, we comprehensively explored the association between common genetic variants, rare deleterious variants and the contribution of mutational signatures across different ethnicities, and provided insights to the relationship between germline variants and somatic mutational processes.10 Several study limitations should also be noted. First, we only focused on the substitutions, and did not consider other types of somatic mutation, such as indels and structural variants.3,41 Second, while WES allowed us to acquire a larger number of data, our analyses were confined to somatic mutations within protein coding regions. WGS could uncover microscale and macroscale somatic alterations58 and provide us more stable estimates of mutation signature contributions. As such, we only focused on the four signatures with strong concordance between WGS data and WES data. Although common practice,1,22 estimating mutation signatures from exome sequencing could cause potential biases due to sequence motif representation as compared to whole genome. APOBEC enzymes have been shown to preferentially target genic regions, which is likely due to the availability of ssDNA substrates during transcription.59–61 Additional work has demonstrated that APOBEC mutations are also enriched within early-replicating regions regardless of transcriptional activity.60 It is unclear if identical mutagenic processes affect all early-replicating and transcriptionally active regions. Even though we observed high mutation signature correlations between exomes and genomes, we cannot rule out the possibility that different molecular mechanisms generated similar mutational signatures in coding and noncoding regions. It is also possible that a single mutational process is responsible for generating somewhat different signatures in these regions. As such, it is important to remember that signature contribution estimates only serve as a phenotypic marker and may not fully capture the nuances of underlying mechanistic processes. Third, causal links between germline and somatic mutational processes are one explanation for the associations presented here, but other explanations cannot be ruled out.30

Conclusions
In summary, our study identified associations between germline variants and mutational patterns in breast cancer across different ethnicities, especially in African women for the first time. This finding may advance our understanding of breast cancer etiology with potential implications for prevention and treatment. However, further replications in larger and diverse populations are needed to validate our findings. Meanwhile, future work focused on understanding the biological basis of cancer susceptibility alleles will be instrumental in better understanding the complex interplay between germline genetics and somatic mutations.

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Data availability
Raw TCGA data used in this analysis were downloaded from TCGA Data Portal or Cancer Genomics Hub. Access to the harmonized variant calls that support the findings of our study are available on request from the corresponding author (O.I.O.). The raw sequencing data from Nigerian cases is available through dbGaP under Study Accession phs001687.v1.p1.
Germline variants and somatic mutation signatures of breast cancer

References

1. Alexandrov LB, Nik-Zainal S, Wedge DC, et al. Signatures of mutational processes in human cancer. Nature 2013;500:415–21.
2. Segovia R, Tam AS, Stirling PC. Dissecting genetic and environmental mutation signatures with model organisms. Trends Genet 2015;31:465–74.
3. Martincorena I, Campbell PJ. Somatic mutation in cancer and normal cells. Science (New York, NY) 2015;349:1483–9.
4. Alexandrov LB, Nik-Zainal S, Wedge DC. Deciphering signatures of mutational processes operative in human cancer. Cell Rep 2013;3:246–59.
5. Wu S, Powers S, Zhub W, et al. Substantial contribution of extrinsic risk factors to cancer development. Nature 2016;529:43–7.
6. Brucher BL, Jamali IS. Somatic mutation theory—why it’s wrong for Most cancers. Cell Physiol Biochem 2016;38:1663–80.
7. Alderton GK. Cancer risk: debating the odds. Nat Rev Cancer 2016;16:68.
8. Zhu B, Mukherjee A, Machida M, et al. An investigation of the association of genetic susceptibility risk with somatic mutation burden in breast cancer. Br J Cancer 2016;115:752–60.
9. Robles-Espinoza CD, Roberts ND, Chen S, et al. Germline MCIR status influences somatic mutation burden in melanoma. Nat Commun 2016;7:12064.
10. Middlbrooks CD, Banday AR, Matsuda K, et al. Association of germline variants in the APOBEC region with cancer risk and enrichment with APOBEC-signature mutations in tumors. Nat Genet 2016;48:1330–8.
11. Nik-Zainal S, Wedge DC, Alexandrov LB, et al. Association of a germline copy number polymorphism of APOBEC3A and APOBEC3B with burden of putative APOBEC-dependent mutations in breast cancer. Nat Genet 2014;46:487–91.
12. Starrett GJ, Luengas EM, McCann JL, et al. The DNA cytosine deaminase APOBEC3H haplotype I likely contributes to breast and lung cancer mutagenesis. Nat Commun 2016;7:12198.
13. Phipps AI, Ahnen DJ, Cheng I, et al. PIK3CA somatic mutation status in relation to patient and tumor factors in racial/ethnic minorities with colorectal cancer. Cancer Epidemiol Biomarkers Prev 2015;24:1046–51.
14. Decken B, Karyadi DM, Davis BW, et al. Bilallelic BRCA2 mutations shape the somatic mutation landscape of aggressive prostate Tumors. Am J Hum Genet 2016;98:818–29.
15. Sahasrabudhe R, Lott P, Bohorquez M, et al. Germline mutations in PALB2, BRCA1, and RAD51C, which regulate DNA recombination repair, in patients with gastric cancer. Gastroenterology 2017;152:983–6.e6.
16. Kim J, Mouw KW, Polak P, et al. Somatic ERCC2 mutations are associated with a distinct genomic signature in urothelial tumors. Nat Genet 2016;48:600–6.
17. Rahman N. Realizing the promise of cancer predisposition genes. Nature 2014;505:302–8.
18. Machiela MJ, Ho BM, Fisher VA, et al. Limited evidence that cancer susceptibility regions are preferential targets for somatic mutation. Genome Biol 2015;16:193.
19. Ding L, Bailey MH, Porta-Pardo E, et al. Perspective on oncogenic processes at the end of the beginning of cancer genomics. Cell 2018;173:305–320.e10.
20. Hau D, Ikpatt F, Khramtsov A, et al. Population differences in breast cancer: survey in indigenous African women reveals over-representation of triple-negative breast cancer. J Clin Oncol 2009;27:4515–21.
21. Hau D, Hu R, Rhie SK, et al. Comparison of breast cancer molecular features and survival by African and European ancestry in the cancer genome atlas. JAMA Oncol 2015;1:1654–62.
22. Pitt JJ, Riester M, Zheng Y, et al. Characterization of Nigerian breast cancer reveals prevalent homologous recombination deficiency and aggressive molecular features. Nat Commun 2018;9:4181.
23. Mccclanen JM, Lehner T, King MC. Gene discovery for complex traits: lessons from Africa. Cell 2017;171:261–4.
24. Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. PLoS Genet 2009;5:e1000529.
25. Lek M, Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature 2016;536:285–91.
26. Kircher M, Witten DM, Jain P, et al. A general framework for estimating the relative pathogenicity of human genetic variants. Nat Genet 2014;46:310–5.
27. Hau D, Zheng Y, Ogundiran TO, et al. Evaluation of 19 susceptibility loci of breast cancer in women of African ancestry. Carcinogenesis 2012;33:835–40.
28. Gehring JS, Fischer B, Lawrence M, et al. SomaticSignatures: inferring mutational signatures from single-nucleotide variants. Bioinformatics (Oxford, England) 2015;31:3673–6.
29. Temko D, Tomlinson I, Severini S, et al. The effects of mutational process and selection on driver mutations across cancer types. Nat Commun 2018;9:1857.
30. Helleyday T, Eshtad S, Nik-Zainal S. Mechanisms underlying mutational signatures in human cancers. Nat Rev Genet 2014;15:585–98.
31. Katrinaen R, Dave K, Pitkanen E, et al. CTCF-cohesin-binding sites are frequently mutated in cancer. Nat Genet 2015;47:818–21.
32. Yang I, Benyamin B, McEvoy BP, et al. Common SNPs explain a large proportion of the heritability for 23 quantitative and dichotomous traits. Nature 2010;463:1000–5.
33. Zamborszky J, Szikriszt B, Gervai JZ, et al. Loss of homologous recombination genes is associated with hereditary pharmacoresponse to platinum chemotherapy. Cancer. 2015;16:2231–5.
34. Cysme L, Carmichael S, Kikuchi K, et al. A general model for somatic mutation signatures in human cancers. Nat Genet 2013;45:970–6.
35. Baldwin AS Jr, LeClair KP, Singh H, et al. A large protein containing zinc finger domains binds to related sequence elements in the enhancers of the class I major histocompatibility complex and kappa immunoglobulin genes. Mol Cell Biol 1990;10:1406–14.
36. Fuji H, Gabrielson E, Takagaki T, et al. Frequent down-regulation of HIV2CR1 in human breast cancer. Breast Cancer Res Treat 2005;91:103–12.
37. Haruyono SJ, Datasena IG, Santosa WB, et al. A pilot genome-wide association study of breast cancer susceptibility loci in Indonesia. Asian Pac J Cancer Prev 2015;16:2231–5.
38. Powell SN, Kachnic LA. Roles of BRCA1 and BRCA2 in homologous recombination, DNA replication fidelity and the cellular response to ionizing radiation. Oncogene 2003;22:5784–91.
39. Ngew J, Nizelek E, Eng C. Into the eye of the storm: breast cancer’s somatic mutation landscape points to DNA damage and repair. Transl Cancer Res 2013;2:59–61.
40. Zamborszky J, Szikriszt B, Gervai JZ, et al. Loss of homologous recombination genes is associated with hereditary pharmacoresponse to platinum chemotherapy. Cancer. 2015;16:2231–5.
54. Ramakodi MP, Kulathinal RJ, Chung Y, et al. Ancestral-derived effects on the mutational landscape of laryngeal cancer. *Genomics* 2016;107:76–82.

55. Amar D, Izraeli S, Shamir R. Utilizing somatic mutation data from numerous studies for cancer research: proof of concept and applications. *Onco gene* 2017;36:3375–83.

56. Morganella S, Alexandrov LB, Glodzik D, et al. The topography of mutational processes in breast cancer genomes. *Nat Commun* 2016;7:11383.

57. Van den Eynden J, Larsson E. Mutational signatures are critical for proper estimation of purifying selection pressures in cancer somatic mutation data when using the dN/dS metric. *Front Genet* 2017;8:674.

58. Nagarajan N, Bertrand D, Hillmer AM, et al. Whole-genome reconstruction and mutational signatures in gastric cancer. *Genome Biol* 2012;13:R115.

59. Swanton C, McGranahan N, Starrett GJ, et al. APOBEC enzymes: mutagenic fuel for cancer evolution and heterogeneity. *Cancer Discov* 2015;5:704–12.

60. Kazanov MD, Roberts SA, Polak P, et al. APOBEC-induced cancer mutations are uniquely enriched in early-replicating, gene-dense, and active chromatin regions. *Cell Rep* 2015;13:1103–9.

61. Seplyarskiy VB, Andrianova MA, Bazykin GA. APOBEC3A/B-induced mutagenesis is responsible for 20% of heritable mutations in the TpCpW context. *Genome Res* 2017;27:175–84.