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Differences in somatic *TP53* mutation type in breast tumors by race and receptor status

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Authors' contributions

NP, JR, and AET conceived the study idea. NP and JR performed literature and database reviews. NP curated *TP53* mutation status. PS downloaded data from publicly available databases. JM performed statistical analyses. HH, MT, KC, JJH, JLF, OIO, DH, EZ, and SN provided data for this study. NP, JR, and AET wrote the manuscript. All authors have read and approved the final manuscript.

Conflicts of Interest

HH is on the scientific advisory board for Invitae Genetics, Promega, and Genome Medical and has stock/stock options in Genome Medical and GI OnDemand. None of these are direct conflicts with this study of somatic *TP53* mutations in breast cancer.

Ethics Approval

This study was approved by the OSU Cancer Institutional Review Board. The City of Hope Institutional Review Board and the University of Chicago IRB approved study for participants enrolled at their respective sites.

Consent to Participate

Participants with previously unpublished data provided informed consent for this study. The remainder of data was de-identified and from publicly available databases or publications.

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Abstract

Purpose: Somatic driver mutations in *TP53* are associated with triple negative breast cancer (TNBC) and poorer outcomes. Breast cancers in women of African ancestry (AA) are more likely to be TNBC and have somatic *TP53* mutations than cancers in non-Hispanic White (NHW) women. Missense driver mutations in *TP53* have varied functional impact including loss-of-function (LOF) or gain-of-function (GOF) activity, and dominant negative (DNE) effects. We aimed to determine if there were differences in somatic *TP53* mutation types by patient ancestry or TNBC status.

Methods: We identified breast cancer datasets with somatic *TP53* mutation data, ancestry, age, and hormone receptor status. Mutations were classified for functional impact using published data and type of mutation. We assessed differences using Fisher's exact test.

Results: From 96 breast cancer studies, we identified 2964 women with somatic *TP53* mutations: 715 (24.1%) Asian, 258 (8.7%) AA, 1931 (65.2%) NHW, and 60 (2%) Latina. The distribution of *TP53* mutation type was similar by ancestry. However, 35.8% of tumors from NHW individuals had GOF mutations compared to 29% from AA individuals (p=0.04). Mutations with DNE activity were positively associated with TNBC (OR=1.37, p=0.03) and estrogen receptor (ER) negative status (OR=1.38; p=0.005).

Conclusions: Somatic *TP53* mutation types did not differ by ancestry overall, but GOF mutations were more common in NHW women than AA women. ER negative and TNBC tumors are less likely to have DNE+ *TP53* mutations which could reflect biological processes. Larger cohorts and functional studies are needed to further elucidate these findings.

INTRODUCTION

Tumor protein 53, encoded by *TP53*, is a transcription factor with tumor suppressive activity that regulates genes in response to cellular stress. Pathways regulated by TP53 include cell cycle check point, senescence, DNA repair, cell metabolism and apoptosis. Somatic mutations in *TP53* are the most common genetic abnormality in multiple cancers. *TP53* is mutated in 40–60% of breast cancers [1–3]. Mutated *TP53* is a negative prognostic factor and is associated with aggressive triple-negative breast cancers (TNBCs) and basal-like breast cancers [4, 5].

Over eighty percent of *TP53* mutations are missense mutations with consequences that differ depending on mutation position and amino acid change [6]. Pathogenic somatic mutations in *TP53* often disrupt DNA binding capability, impair transcriptional activity and result in other loss-of-function (LOF) effects. However, a subset of missense somatic variants demonstrate new gain-of-function (GOF) activities. GOF activity is typically mediated by the mutant protein binding to other tumor suppressive or oncogenic proteins or to novel regulatory regions [7]. GOF mutations result in accelerated tumor onset, metastasis, drug resistance and poorer survival outcomes [8, 9]. *TP53* missense mutations can also display dominant negative activity (DNE), in which a mutant TP53 protein disrupts the activity of non-mutant protein partners including TP63 and TP73 during tetramerization [10]. DNE is more common of hotspot mutations, sites where approximately 30% of somatic *TP53* mutations occur, and may contribute to accelerated loss of heterozygosity and tumor progression [11]. Because the importance of *TP53* mutations has been well-established for decades, there are abundant functional studies identifying LOF, GOF, and DNE activity for specific *TP53* mutations.

TNBCs, which are negative for estrogen receptor (ER) and progesterone receptor (PR) expression and lack HER2 amplification, have poorer prognoses compared to ER-positive breast cancer subtypes [12]. TNBCs are more likely to have somatic *TP53* mutations than other types of breast cancer such as Luminal A and Luminal B subtypes [13]. Interestingly, racial differences are observed between different breast cancer subtypes; TNBCs occur more frequently in women of African Ancestry (AA) (28–30%) or Latina ethnicity (17.5%) compared to non-Hispanic White (NHW) (12–15%) women [14–18]. AA women have a 42% higher breast cancer mortality rate [19–21] and a higher risk of tumor recurrence than NHW women (hazard ratio, 2.22; CI; 1.05–4.67)[22].

As TNBCs are more common in breast tumors from AA women than NHW women, and *TP53* mutations are more frequently observed in TNBC than other subtypes, it is not surprising that the proportion of all breast tumors with *TP53* mutations is 1.5- to 1.6-fold higher in AA than NHW women [22–24]. While there has been extensive research about overall *TP53* somatic mutation frequency by race, there has been little investigation to determine if there are differences by *TP53* mutation type. Given that *TP53* mutation effects can impact prognosis, mutation type is an important consideration [7, 9, 25]. Because of the differences in clinical outcomes between AA women and NHW women, even after accounting for subtype differences, and the literature supporting different outcomes for GOF versus LOF *TP53* mutations, we hypothesized that there would be frequency differences in types of *TP53* mutations across racial and ethnic groups. To test this hypothesis, we compared the racial distribution of *TP53* mutation type in breast cancer using existing published and unpublished datasets.

METHODS

Summary of data

This study was approved by the Ohio State Cancer Institutional Review Board. Data for this study were ascertained from multiple sources including The Ohio State University Total Cancer Care repository, existing data in publicly accessible databases, existing data

in publications, and unpublished data contributed by study authors. A description of all included studies is detailed in Supplementary Table S1.

Study inclusion and exclusion criteria

We included data from women with breast tumors with somatic *TP53* mutations and available ancestry information. For inclusion, all studies must have sequenced tumor DNA for *TP53* using any method (Sanger sequencing or next-generation targeted, exome, or whole genome sequencing) and at least included exons 5–8 which contain the majority of *TP53* mutations [26]. We excluded studies that used immunohistochemistry or other non-DNA-sequencing based methods to infer *TP53* mutation genotype. All likely invasive stages, grades, and morphologies of primary breast tumor were considered. Non-invasive ductal carcinoma in-situ tumors were excluded because only ~40% of these lesions progress to invasive cancer which could vary by *TP53* mutation status [27]. Data were annotated with race and ethnicity by the original authors or were from homogeneous populations. Studies were excluded if they lacked ancestry data or represented a unique population that was underpowered to detect differences. If available, patient age, tumor grade, stage, receptor status, and morphology data were collected. We considered studies of any design that fit the criteria, including population and clinical-based studies.

Data from unpublished datasets

Previously unpublished *TP53* somatic mutation data and self-reported race and ethnicity were ascertained from The Ohio State University's Total Cancer Care repository for 143 women (Supplementary Table S2). Other previously or partially unpublished data included 163 individuals with *TP53* mutant breast tumors enrolled through The Western New York Exposures and Breast Cancer (WEB) study, 24 through The Sylvester Comprehensive Cancer Center, and 21 through The City of Hope Comprehensive Cancer Center. Patients were enrolled through protocols approved by their respective institutional review boards.

Data from publicly accessible databases

We identified individuals and studies in databases with somatic mutation information that met inclusion criteria. From the International Agency for Research on Cancer (IARC) [28], 1254 individuals from 78 studies met inclusion criteria, as well as 333 from The Cancer Genome Atlas (TCGA), and 637 from the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) [29, 30] (Supplementary Table S1).

Data from literature review

To obtain additional data from previously published work, we conducted a literature search for studies in which individual level *TP53* data and race/ethnicity information were available. A PubMed search using "*TP53* and race" identified 277 articles. We excluded articles: 1) with samples already captured from database search; 2) of cancers other than breast; 3) on germline variants or polymorphisms in *TP53*; and 4) where study inclusion were not met. For studies without individual level race and/or ethnicity information, we contacted authors by e-mail to request this information. All studies included are listed in Supplementary Table S1.

Categorizing *TP53* missense variants

We used a standardized approach to evaluate findings from IARC and other *TP53* literature. All missense variant annotations were based on existing functional studies with cell culture, yeast, or animal experiments; we did not consider *in-silico* testing alone for inclusion. However, in cases of mutations with uncharacterized function, we utilized PHANTM (Broad Institute) to exclude variants with a predicted function close to wildtype TP53 (maximum PHANTM score <0, ~50 variants)[11]. We excluded well-established germline polymorphisms, such as p.P72R, and mutations with activity comparable to wildtype TP53. *TP53* mutations were categorized by function (GOF or LOF) and dominant negative activity (DNE+ or DNE−) as separate criteria.

We described function as GOF or LOF. GOF mutations resulted in significantly different activity from both TP53 null and TP53 WT proteins such as novel transcript activity, TP73 interference, growth advantage, and facilitation of oncogene activity. LOF mutations had evidence of protein truncation, loss of tetramerization, or activity comparable to TP53 null. When we found reports of both LOF and GOF activities, but not direct contradictions for the same TP53 function, we categorized variants as GOF. Variants with limited functional data available and PHANTM prediction scores that differed from wild-type were annotated as unknown. DNE+ variants were those with published evidence that the TP53 mutant protein interfered with TP53 WT function in heterozygous cells. DNE+ mutants formed heterotetramers with TP53 WT units and changed TP53 WT function, causing a dominant GOF or LOF effect. DNE+ mutations may also interfere with TP63 and TP73, and therefore may have unique biological impact beyond GOF [10]. Transcript-truncating mutations, such as nonsense, splicing, frameshifts, and large deletions, were assumed to be LOF without DNE because these mutations will activate nonsense-mediated decay and result in loss of a functional protein. Hotspot codons in the DNA binding domain may have different functional properties than missense mutations elsewhere [26, 31]. These sites were at positions 175, 245, 248, 249, 273 and 282, and CpG hotspot mutations were defined as C to T transitions at those codons plus R158H and P152L as described [7]. CpG hotspot mutations were studied separately because they may be part of a mutational signature [26, 32].

For tumors with multiple *TP53* somatic mutations, we considered the sum of multiple predicted effects. If any mutations were DNE+, the tumor was considered DNE+. GOF and LOF mutations were prioritized over unknown or functional mutations. Tumors with both GOF and LOF mutations were called GOF/LOF.

Statistical analyses

A Fisher's exact test for count data was used for comparisons between mutation categories (GOF/LOF, DNE/not DNE or CpG hotspot/not hotspot) and race, TNBC status, and ER status. For comparisons of mutation categories and age, a Welch Two Sample t-test was used. Analyses were run in R version 3.6.3 (2020-02-29) [33]. A comparison-wise p-value of 0.05 was considered significant.

RESULTS

Characteristics of study population

The study population is summarized in Table 1. We included somatic *TP53* mutation data from 2964 breast cancers from 96 studies for analysis (Supplementary Table S1). Patients were categorized into 4 racial/ethnic groups. The study population was 65.2% NHW (n=1931), 24.1% Asian (n=715), and 8.7% AA (n=258). Two percent (n=60) of patients had Hispanic or Latina ethnicity with European or undefined race (n=47 [1.6%] and n=13 [0.4%], respectively). Populations excluded from analysis due to low representation included Pacific Islander, Ashkenazi Jewish, Southwest Asian/North African, Indian Asian, and Latina AA women.

Ages at diagnosis were available for 1969 patients. Across the study population, ages ranged from 21 to 96 years, with a median age of 54 years and an average age of 55 years. By racial/ethnic group, median ages were 49 for AAs, 47 for Asians, 56 for NHW, and 52 years for Latina women.

Only a subset of tumors had receptor data available. ER status was available for 1481 tumors, with 47.5% ER+ (n=704) and 52.5% ER− (n=777). A smaller subset had additional tumor information. TNBC status was available for 1221 tumors with 36% classified as TNBC (n=439) and 64% as non-TNBC (n=782). Data were collected for morphology, grade, and stage, but were not used due to low availability across the datasets and the high number of categories.

Characteristics of mutations

Fifty-four tumors had multiple *TP53* mutations, for a total of 3024 *TP53* mutations across all 2964 patients (Supplementary Tables S3 and S4). A majority of mutations were missense (65%, n=1972). We identified 829 distinct alterations, including 427 missense, 63 nonsense, 209 frameshift, 58 in-frame insertion/deletions, 2 large insertion/deletions, and 56 unique splicing changes (Supplementary Tables S3 and S4). The most frequent missense mutations were p.R175H (n=138, 4.7%), p.R248Q (n=104, 3.4%), p.R273H (n=87, 2.9%), and p.R248W (n=73, 2.5%), all known hotspot mutations (Figure 1). Overall, mutations at reported hotspot sites (R175, G245, R248, R273 and R282) accounted for 20% of mutations (n=616). More specifically, mutations of CpG nucleotides at hotspots accounted for 17.5% of mutations (n=530).

For functional classification, tumors were analyzed based on the net effect of all *TP53* mutations per tumor (n=2964). Overall, we characterized 939 mutations as GOF and 1739 LOF; 286 mutations did not have sufficient information for classification. Evaluating mutations for DNE, there were 1246 DNE+, 1190 DNE−, and 528 uncharacterized mutations.

Association of race, tumor characteristics, and age with mutation type

To determine if there were associations between race and type of mutation, we conducted Fisher's exact test for racial and ethnic ancestry by mutation categories. No significant

associations were identified overall for GOF/LOF status (p = 0.15), DNE (p=0.62), mutation hotspots (p=0.32) or CpG sites (p=0.52) and race (Table 2). However, association of GOF/LOF status and race was significant when comparing GOF versus LOF in NHW and AA patients only, with NHW patients more likely to have GOF mutations (35.8% versus 29.2%, respectively, p=0.04). We additionally tested association between ER or TNBC status and mutation type (Table 3). We identified a significant association between DNE and TNBC (p=0.03) and related ER status (p=0.005). ER− tumors and TNBCs were less likely to have *TP53* somatic mutations that were DNE+. We did not identify associations between ER and GOF/LOF (p=0.51), with mutation hotspots (p=0.1514) or with CpG hotspots (p=0.24). Patients with hotspot mutations were slightly younger, with a mean age of 53.6 years versus 55.0 years for patients with non-hotspot mutations, at a level approaching significance (p=0.065) (Figure 2). We did not identify significant associations with age and GOF/LOF, with a mean age of 54.5 years for GOF and 55.0 years for LOF (p=0.52). We also found no significant association between age and DNE; the mean age was 54.5 years for DNE+, and 55.0 years for DNE− (p=0.49).

DISCUSSION

The goal of our study was to determine if the type of *TP53* somatic mutation (GOF or LOF, DNE− or DNE+, hotspot status, and CpG nucleotide position) varied in frequency between patients of different ancestry. Considering that the overall rate of somatic *TP53* mutations in breast cancer differs by race, this is an important concern for study of *TP53*-mutant breast tumors and differences in outcomes and treatment response by race [14–18]. We identified a modest difference between AA and NHW individuals, with NHWs slightly more likely to have GOF mutations.

Our finding that *TP53* mutations without DNE activity were associated with TNBC (p=0.03) and ER− status (p=0.005) is novel. This association could be due to complex interactions and shared regulation of apoptotic genes between *TP53* and ER. In ER+ tumors, estrogen receptor-beta (*ESR2*) activity has a pro-proliferative effect on *TP53*-wildtype tumors, but an anti-proliferative effect on *TP53*-mutant tumors [34]. DNE+ *TP53* mutations may have unique interactions with *ESR2* in ER+ tumors that drives higher DNE+ frequency. In this study, that only includes *TP53*-mutant tumors, we observed a higher proportion of ER− and TNBC tumors overall compared to unselected populations. This is consistent with previous studies that identified *TP53* somatic mutations in ~85% of TNBC versus 40–60% of unselected breast tumors [1–3, 35]. There has been some debate about the significance of mutant *TP53* DNE versus GOF activity, as many common somatic mutations, including hotspot mutations, are both DNE+ and GOF[36]. It is thus of great interest that the association with receptor status was only significant for DNE, not GOF/LOF, though functional studies are needed to better understand this phenomenon.

Our cohort included somatic *TP53* mutation data from TCGA, METABRIC, and IARC data-bases, studies identified for inclusion from literature, and 351 previously unpublished cases (Supplementary Table S1). The frequency of hotspot mutations observed in our study (20%) was slightly lower than previous studies finding that 28% of *TP53* mutations occurred at mutation hotspots [26]. We observed that 36% of tumors from NHW individuals had

GOF mutations compared to 29% in AA individuals (p=0.04). This is opposite of what we expected to find as GOF variants have been associated with poorer prognosis or worse outcomes and breast cancers in AA women have worse outcomes [7]. We considered that this effect may be an artifact of more NHW patients sequenced with earlier technology, such as Sanger, which could bias the *TP53* mutation detected to the exons more likely to have GOF mutations. However, there was no difference in use of Sanger vs NGS between these population groups, with 43.6% of NHW patients sequenced with Sanger, compared to 43.8% of AA patients. There also was no difference in the number of exons sequenced; 67.3% of NHW patients had at least exons 2–11 sequenced, compared to 68.6% of AA patients. Additionally, there was no difference in the percentage of unclassified variants between groups (7% in AA versus 9.9% in NHW for GOF/LOF, 14.3% in AA versus 17.3% in NHW for DNE). Thus, this difference is not likely due to mutation detection or classification. This paradox could be due to factors other than the *TP53* mutations or those that influence aggressiveness in addition to the *TP53* mutations that vary between ancestral groups such as differences in somatic mutation of other key driver genes or methylation pattern differences [23, 37]. Further studies of larger numbers of AA and NHW women are warranted to confirm this finding.

Participants with hotspot mutations were younger than those with non-hotspot mutations, with a mean age of 53.61 in hotspots versus 55.04 in non-hotspots, but this was not statistically significant (p=0.065). Age did not correlate with DNE or GOF/LOF. This finding is somewhat unexpected. Susceptibility to hotspot mutations is likely due to properties of the genetic sequence being vulnerable to mutation, rather than purely selective growth advantage of tumor cells [26]. A high proportion of hotspot mutations are CpG sites, a feature of mutation signature 1, which correlates with age, so it would seem more likely for somatic hotspot mutations at CpG sites to be associated with later age at diagnosis [32, 38]. However, a correlation for breast cancer has not yet been reported in the literature of which we are aware. Studies of *TP53* hotspot mutations, mutational signatures and breast cancer age of onset may reveal additional insight.

Strengths of this study include the large number of women included from multiple sources, including previously unpublished data. Previous studies characterizing *TP53* mutation types have not focused on race or ancestry. We limited the dataset to only include tumors with *TP53* somatic mutations and only included participants with race or ancestry data. There are a number of limitations to this study. Many of the studies used self-reported race and ethnicity information, which may not reflect genetic ancestry, and may have been categorized differently by study, such as distinguishing NHW and Ashkenazi Jewish ethnicity. There may be differences in *TP53* mutation types between ethnic groups within a racial group, such as between NHW individuals from Greece versus Finland. For studies in countries that are predominantly one racial group and for which detailed racial information was not available, we assumed that the individuals were of that racial group (e.g. Norway and European ancestry; China and Asian ancestry). Few studies only performed analyses of exons 4 through 8 which could miss more LOF variants that occur in other exons compared to GOF or DNE-associated missense variants that predominantly map to these exons. Because of the mixed data sources, this study did not include large *TP53* copy number changes or loss of heterozygosity data. There may be undetected effects by gene

copy number or loss of heterozygosity, either acting alone or modifying point mutation effects which could impact our findings. Finally, classifications of variants as GOF/ LOF and DNE were made based on literature. For some variants there was discordant information; we used the classifications from studies that tested more variants or included a larger number of assays. It is possible that some of the rarer missense variants were misclassified or have different effects in humans than in the system tested (e.g. yeast).

Summary

In this study, we found that somatic *TP53* mutation types did not differ by race overall, but GOF mutations were more common in NHW women when compared to AA women. We uncovered a modest association between DNE− and tumor receptor status. Functional studies are needed to understand this phenomenon. Additional tumor sequencing data from more racially diverse cohorts are needed to follow-up on these findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Availability of data

All data generated or analyzed during this study are included in this published article (Supplementary Tables S1–S4) or in the original publications (Supplementary File S5).

Abbreviation Term

AA	African ancestry
DNE	Dominant negative
ER	Estrogen receptor
ESR2	Estrogen receptor-beta

GOF	Gain of function
LOF	Loss of function
NHW	Non-Hispanic White
OR	Odds ratio
PR	Progesterone receptor
TCC	Total Cancer Care
TNBC	Triple-negative breast cancer
TP53	Tumor protein 53

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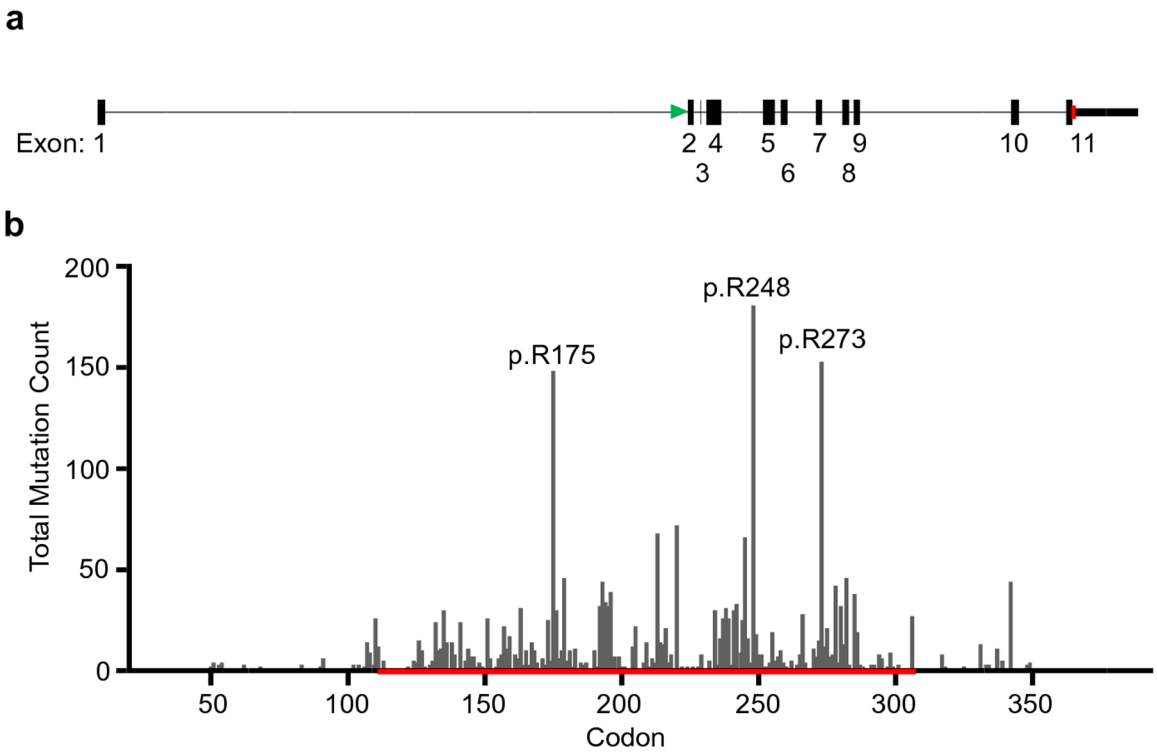

Figure 1: *TP53* Gene Structure and Mutation Frequency

(a). A diagram of the intron/exon structure of TP53 is drawn to scale. A green arrow denotes the exon containing the start codon and a red arrow denotes the exon containing the stop codon.(b). The frequency of somatic *TP53* mutations by codon is plotted. Exons 5 through 8 are denoted by a red line. Intronic mutations affecting splicing are not included. The figure was created in GraphPad Prism 8.

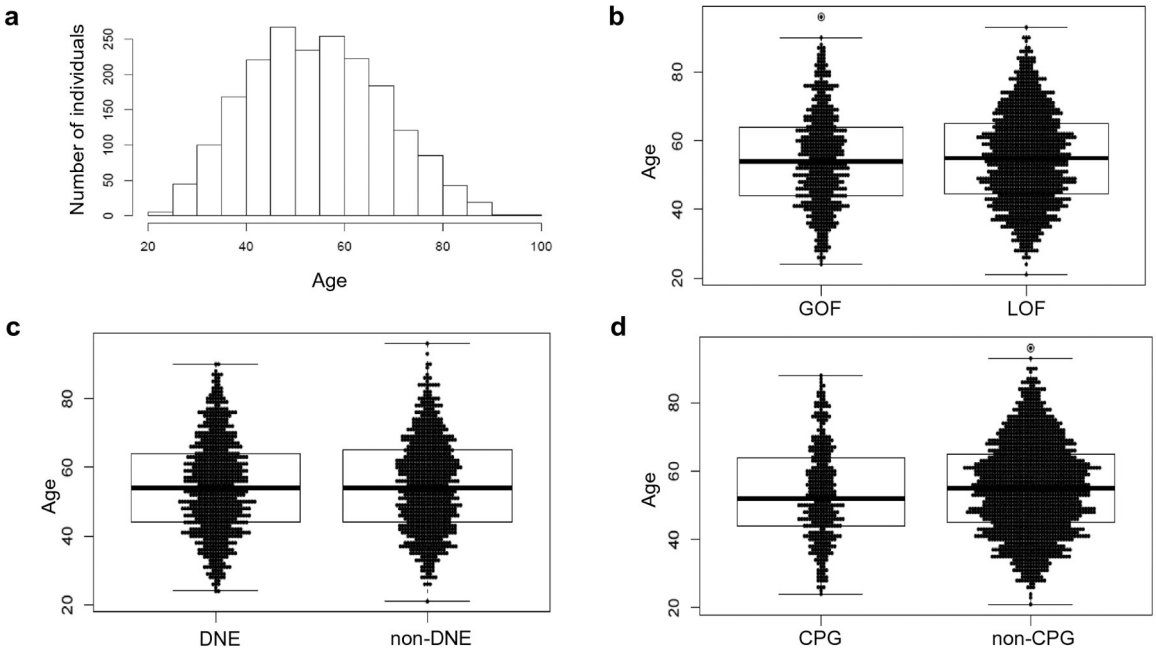

Figure 2: Association of age of breast cancer diagnosis with *TP53* mutation characteristics

The frequency of age of breast cancer diagnosis of all individuals included in the study was plotted by histogram (a). Age of diagnosis was not significantly associated with *TP53* GOF versus LOF, p-value =0.5 (b) or dominant negative effect, p-value 0.49 (c). Individuals with a *TP53* mutation at a CpG hotspot were slightly, but not significantly younger at age of diagnosis (53.6 years versus 55.0 years), p-value 0.065 (d). P-values were calculated using a Welch two sample T-test. P-values < 0.05 were considered significant. The figure was created in R.

Table 1:

Study Population Demographics and Tumor Characteristics

Study Population Demographics	**NHW n (%)**	**AA n (%)**	**Asian n (%)**	**Latina n (%)**	**Total n (%)**
Individuals[1]	1931 (65%)	258 (9%)	715 (24%)	60 (2.0%)	2964
Breast Cancer dx age available[2]	1461 (76%)	162 (63%)	286 (40%)	60 (100%)	1969 (66%)
Age range (years)	21– 96	24 −84	26–90	31–72	21–90
Median age (years)	56	49	47	52	
p-value	[Ref]	<0.0001	<0.0001	0.0161	
Tumor Characteristics [2]					
ER+	553 (49%)	54 (36%)	60 (42%)	37 (62%)	704 (47.5%)
ER−	575 (51%)	95 (64%)	84 (58%)	23 (38%)	777 (52.5%)
No ER data	803	109	571	0	1483
Non-TNBC	660 (64%)	47 (51%)	31 (69%)	44 (77%)	782 (64%)
TNBC	366 (36%)	46 (49%)	14 (31%)	13 (23%)	439 (36%)
No TNBC data	905	165	670	3	1743

NHW, non-Hispanic White, AA, African ancestry; n, number; % percentage; ref, reference population; dx, diagnosis

[1]Percentages were calculated for participants in the study for each racial/ethnic group category

[2]Percentages for age at diagnosis and tumor characteristics were calculated only including individuals for whom marker information was available within each racial/ethnic group category

Table 2:

TP53 Mutation Frequency by Type and Population

	Populations				Total
Mutation type	**NHW (n=1931)**	**Asian (n=715)**	**AA (n=258)**	**Latina (n=60)**	**n=2964**
GOF	623 (32.2%)	222 (31%)	70 (27.1%)	24 (40%)	939 (31.7%)
LOF	1116 (57.8%)	419 (58.6%)	170 (65.9%)	34 (56.7%)	1739 (58.7%)
Unknown function	192 (10%)	74 (10.3%)	18 (7%)	2 (3.3%)	286 (9.6%)
p-value	0.15				
DNE+	829 (43%)	286 (40%)	105 (40.7%)	26 (43.3%)	1246 (42%)
DNE−	767 (39.7%)	280 (39.2%)	116 (45%)	27 (45%)	1190 (40.1%)
Unknown	335 (17.3%)	149 (20.2%)	37 (14.3%)	7 (11.7%)	528 (17.8%)
p-value	0.62				
Hotspot	416 (21.5%)	140 (19.6%)	44 (17%)	13 (21.7%)	613 (20.7%)
Non-hotspot	1515 (78.5%)	575 (80.4%)	214 (83%)	47 (78.3%)	2351 (79.3%)
p-value	0.32				
Hotspot CpG	353 (18.3%)	125 (17.5%)	39 (15.1%)	13 (21.7%)	530 (17.9%)
Non-hotspot CpG	1578 (81.7%)	590 (82.5%)	219 (84.9%)	47 (78.3%)	2434 (82.1%)
p-value	0.52				

n, number; GOF, gain-of-function mutation; LOF, loss-of-function mutation; DNE+, dominant negative activity present; DNE−, no dominant negative activity; NHW, non-Hispanic White, AA, African Ancestry

Table 3:

TP53 Mutation Frequency by Age and Tumor Characteristics

	Tumor Subtypes				
Mutation type	**ER− (n=777)**	**ER+ (n=704)**	**TNBC (n=439)**	**Non-TNBC (n=782)**	**Mean age**
GOF	216 (27.8%)	216 (30.7%)	126 (28.7%)	230 (29.4%)	54.5
LOF	514 (66.1%)	424 (60.2%)	291 (66.3%)	485 (62%)	55.0
Unknown	47 (6%)	64 (9.1%)	22 (5%)	67 (8.6%)	NA
p-value	0.10		0.51		0.52
DNE+	288 (37.1%)	297 (42.2%)	162 (36.9%)	317 (40.5%)	54.5
DNE−	384 (49.4%)	285 (40.5%)	226 (51.5%)	331 (42.3%)	55.0
Unknown	105 (13.5%)	122 (17.3%)	51 (11.6%)	134 (17.1%)	NA
p-value	0.0045		0.029		0.49
Hotspot	143 (18.4%)	151 (21.4%)	81 (18.5%)	164 (21%)	53.6
Non-hotspot	634 (81.6%)	553 (78.6%)	358 (81.5%)	618 (79%)	55.0
p-value	0.15		0.20		0.065
Hotspot CpG	126 (16.2%)	131 (18.6%)	75 (17.1%)	135 (17.3%)	54.0
Non-hotspot CpG	651 (83.8%)	573 (81.4%)	364 (82.9%)	647 (82.7%)	55.0
p-value	0.24		1.0		0.30

n, number; GOF, gain-of-function mutation; LOF, loss-of-function mutation; DNE+, dominant negative activity present; DNE−, no dominant negative activity; ER, Estrogen receptor; TNBC, Triple negative breast cancer