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# Genetic Mutations Associated with Hormone-Positive Breast Cancer in a Small Cohort of Ethiopian Women

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**Abstract:** In Ethiopia, a breast cancer diagnosis is associated with a prognosis significantly worse than that of Europe and the US. Further, patients presenting with breast cancer in Ethiopia are far younger, on average, and patients are typically diagnosed at very late stages, relative to breast cancer patients of European descent. Emerging data suggest that a large proportion of Ethiopian patients have hormone-positive (ER<sup>+</sup>) breast cancer. This is surprising given 1) that patients have late-stage breast cancer at the time of diagnosis, 2) that African Americans with breast cancer frequently have triple negative breast cancer (TNBC), and 3) these patients typically receive chemotherapy, not hormone-targeting drugs. To further examine the similarity of Ethiopian breast tumors to those of African Americans or of those of European descent, we sequenced matched tumor and normal adjacent tissue from Ethiopian patients from a small pilot collection. We identified mutations in 615 genes across all three patients, unique to the tumor tissue. Across this analysis, we found far more mutations shared between Ethiopian patient tissue and White patients (103) than we did comparing to African Americans (3). Several mutations were found in extracellular matrix encoding genes with known roles in tumor cell growth and metastasis. We suggest future mechanistic studies on this disease focus on these genes first, toward finding new treatment strategies for breast cancer patients in Ethiopia.

Keywords: metastasis, BRCA, TCGA, MAPK, extracellular matrix

## Introduction

Women in Ethiopia present with late-stage breast cancer, often at a very young age (2). The most comprehensive study followed 1,070 women and determined that two key contributors to an increased hazard ratio were 1) being diagnosed younger than 50 years old, and 2) being diagnosed with stage 3 breast cancer. In the US, women are determined to be high risk patients if they have family history of breast cancer (3, 4), high breast density (5), or a mutation in a known high-risk gene, such as BRCA1/2 (6). While the lack of information pertaining to family history and breast density (access to mammograms) is a public health issue faced by many populations around the globe, only 30% of familial breast cancers can be traced to a known genetic mutation (7). That number is even lower in non-western populations: e.g. a Korean study found less than 22% of familial breast cancer was traced to a BRCA1/2 mutation (8).

Breast cancer is the most common form of cancer diagnosed in Ethiopian women (31.5 - 33% of cancers in women) (9, 10). Kantelhardt *et al.* analyzed 352 patients

treated at Addis Ababa University hospital and found that only 31% of tumors were hormone receptor negative (9). This is in stark contrast to related publications on patients from West Africa, which were up to 76% hormone receptor negative (11). This is an important point, as studies in the US have largely focused on the fact that metastatic African American disease is largely triple negative (TNBC). The overarching challenge here is the limited information on this hormone-positive East African disease (2). This is critically important, as Ethiopian women with breast cancer from rural areas have a 50% survival rate after 2 years (12). There are many possible reasons why Ethiopian women present with metastatic, hormone-positive breast cancer at an early age, such as environmental factors, age of menarche, socioeconomic status, healthcare resources, stigma, and distance to major hospitals (13, 14).

A recent study found that the overall mean age at diagnosis was 43 years old, and 40% of patients were under 40 years old (15). Amongst this cohort, patients with Luminal B breast cancer had the youngest median age at diagnosis at 35, followed by Her2-enriched at 41, Triple negative at 46, and Luminal A at 47. The age of

diagnosis across breast cancer subtypes is consistently younger for those born in Africa compared to those born in the US (**Figure 1B**) (1, 16). This is not accounted for by overall mortality rates alone (**Figure 1A**). Also, at the time of diagnosis, Ethiopian patients had a significantly higher chance of having a higher-grade pathology compared to white and African Americans, across all subtypes (16). Second, a recent study from Jemal and Fedewa demonstrated that breast cancer cases in East Africa (74% of these are from Ethiopia) are more likely to be ER<sup>+</sup> compared to West African or African American cases (**Figure 1C**) (1).

There is a paucity of studies on the genetics of breast tumors of African women in general, and even less of those of East Africans. Most studies have focused on documenting mutations of the BRCA1/2 genes (17). This is particularly important when trying to relate this disease to that of TNBC in African Americans. However, we are not aware of any studies that have performed whole exome sequencing on patients within an East African or Ethiopian community. This is the critical data that we need to find the driver genes of breast cancer in Ethiopian women, and to determine if these driver genes are unique to this population or not.

## Results

### Whole exome sequencing reveals unique mutations in Ethiopian breast tumors.

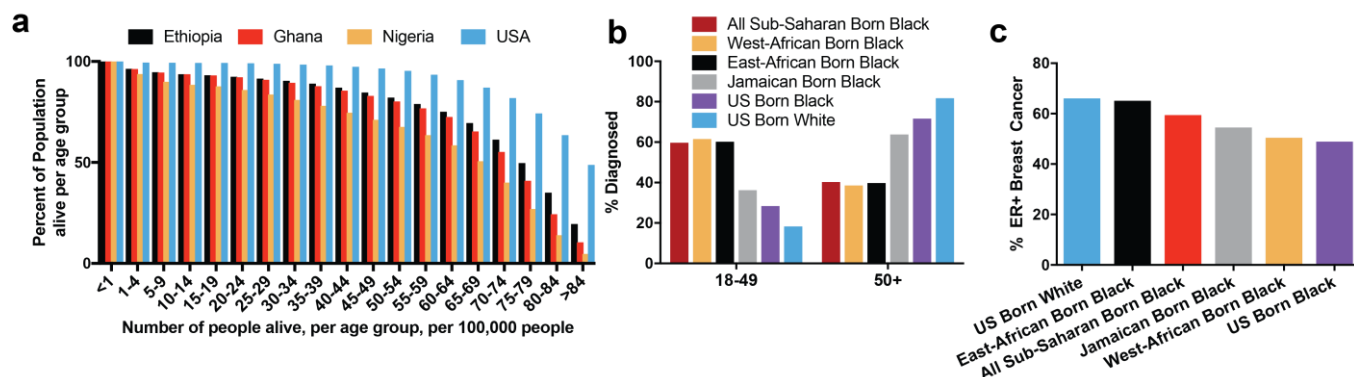
We performed DNA sequencing on three tumors and healthy tissue from matched patients. Each of the three tumors were identified as ER<sup>+</sup> using immunohistochemical subtyping. We identified mutations in 615 genes across all three patients, unique to the tumor tissue (**Table S1, S2**). Two patients had a mutation in a different spot in the BRCA2 gene, which is not surprising based on the number of variants of uncertain significance in BRCA2 (18).

Additionally, data from this population shows that patients with a family history were more likely to be ER<sup>+</sup> than patients without a family history (19), suggesting that many of these patients' cancers are due to somatic mutations. We also searched for mutations in genes related to breast cancer metastasis, subtype, and progression. Gene ontology enrichments (20) showed MSigDB Oncogenic categories (21) related to KRAS, PTEN, and EGFR signaling (**Table 1**), demonstrating that at least some of the somatic variants in this population are in the same pathways as other ER<sup>+</sup> tumors in other populations (22, 23).

Given that these were ER<sup>+</sup> tumor samples, it was not surprising that we uncovered several genes implicated in ER signaling. We identified mutations in tumor suppressor gene, NBL1, which has been shown to be upregulated in response to an estrogen mimic (24). Additionally, we found mutations in multiple members of the protein tyrosine phosphatase (PTP) family of kinases, of which several members have been implicated in hormone therapy resistance. An additional gene of note is the oncogene VAV3, which is activated downstream of ER signaling via PI3K phosphorylation (25). Together these suggest that the higher incidence of ER<sup>+</sup> tumors in Ethiopian women should focus future studies to examine links to hormone-specific mechanisms of transformation and progression.

### Overlap between mutated genes in Ethiopian samples and the TCGA.

In our preliminary analyses of our Ethiopian whole exome sequencing (WXS) and data from the TCGA we see a larger overlap (**Figure 2**) in mutation in the genes also identified as mutated in samples from self-identified white women, as compared to the overlap with TCGA Black or African American mutations (83 vs. 27 genes, **Figure 2, Table S3, S4**). Our analysis included only



**Figure 1: Age of Ethiopian women with breast cancer and hormone status.** a) Data from world health organization on mortality data across populations from Ethiopia (black), Ghana (red), Nigeria (yellow) and US (blue). **Graphs outline the death rate amongst 100,000 individuals from the four countries over 80+ years.** b) Data adapted from Jemal and Fedewa on age of diagnosis of breast cancer across relevant subpopulations (1). **Profiles the age range at which patients within the subpopulations being studied are diagnosed with the disease with the US subpopulation split to capture data from US born black and US born white populations.** c) Proportion of ER<sup>+</sup> breast cancer patients from the same reference.

white women showing ER+ status by IHC. These are mutations in genes that are not seen in our normal adjacent tissue samples. Our list of genes with mutations was also much smaller compared to the extensive data from the TCGA (300 as compared to 11,813 in the white patients, and 8,588 in African Americans). Preliminary analysis from a small number of Ethiopian tumors suggests that, on a genetic level, the mutational spectrum of tumors from ER+ Ethiopian tumors are more similar to that of White women in the US than to African Americans.

Gene Set Name	# Genes in Overlap	p-value
GO_CENTRAL_NERVOUS_SYSTEM_DEVELOPMENT	12	1.59E-06
GO_ADP_BIOSYNTHETIC_PROCESS	2	4.22E-05
GO_HEAD_DEVELOPMENT	9	4.36E-05
PIGF_UP.V1_UP	5	4.89E-05
VEGF_A_UP.V1_DN	5	5.27E-05
GO_RESPONSE_TO_INTERFERON_GAMMA	5	6.39E-05
GO_PEPTIDYL_AMINO_ACID_MODIFICATION	11	7.14E-05
GO_RAS_PROTEIN_SIGNAL_TRANSDUCTION	6	8.56E-05
GO_RIBONUCLEOSIDE_DIPHOSPHATE_BIOSYNTHETIC_PROCESS	2	8.84E-05
GO_SMALL_GTPASE_MEDIATED_SIGNAL_TRANSDUCTION	7	9.25E-05

**Table 1.** GSEA enrichments of the SNPs found across the Ethiopian patient tumors. Enrichments included Gene Ontology and oncogenic signature gene sets.

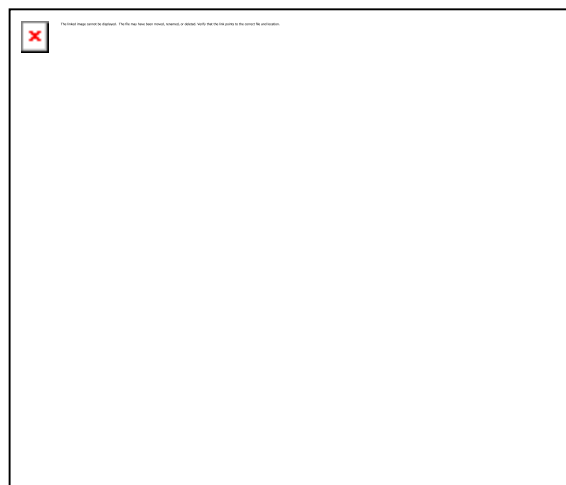
Gene ontology enrichment analysis of the 83 overlapping shows two ontogenetic signature categories “Genes down-regulated by treatment with VEGFA” and “Genes up-regulated by treatment with PIGF”. GO ontology enrichments show that these are enriched for categories in “GTPase mediated signal transduction”, “Ras protein signal transduction”, and “Response to interferon gamma” (Table S3). An enrichment analysis of the 153 genes shared across all three groups showed GO Biological Process categories all related to “Microtubule\_ based process” and “Microtubule cytoskeleton”.

## Discussion

### Need for sequencing information on tumors from Ethiopian patients

The vast majority of breast cancer patients in Ethiopia are treated with radiotherapy and broad-spectrum chemotherapy, resulting in many toxicity-related deaths. Additional immunohistochemical (IHC) and genetic analyses could reveal improved treatment strategies for these patients. Genetic information, such as that from DNA exome sequencing could detect genetic risk factors, and high penetrance mutations in understudied populations could identify new therapeutic targets. This has previously been done with an activating mutation in ER+ breast cancer (26), but focused on populations primarily of European descent. Exome analysis of other small cohorts with non-BRCA familial history of breast and ovarian cancer identified mutations in several genes involved in DNA repair, cell signaling, and apoptosis that were possible predictors for heritable cancer risk (27,

28). Here, we report genetic mutations found in 3 breast tumors and matching normal tissues from Ethiopian women with the intention of motivating future, more rigorous mechanistic studies.



**Figure 2. Overlapping and Distinct Genes Carrying Mutations in Breast Cancer.** The number of genes containing mutations was analyzed from our data on 3 Ethiopian patients, and TCGA data from white or Black patients.

In the US, whole exome sequencing has been used to identify single nucleotide polymorphisms (SNPs) in exome regions associated with risk for cancers in a patient-specific manner (28). Identification of prevalent mutations will lay the groundwork for identifying risk factors or targeted drugs. Additionally, we and others can use this mutation database to develop new exome sequencing panels for breast cancer in women of East African descent. One interesting finding we had here is the ontogenetic signature for genes up-regulated in response to PIGF (Phosphatidylinositol-glycan biosynthesis class F protein). PIGF is more often upregulated in triple negative breast cancer, so it is striking that we observed an increase in this pathway in these ER+ breast cancers. PIGF has been noted as a biomarker in South African pancreatic ductal adenocarcinoma (29). PIGF signaling affects both stemness of cancer cells (30), as well as macrophage polarization (31), which could increase the aggressiveness of the disease.

### Somatic mutations and penetrance in sequenced tumors

All three of these patients harbored somatic mutations in multiple “risk factor genes” denoted by these panels. At the same time, all the mutations we identified were 1) *specific* to the tumor tissue, and 2) *not found* in the matched healthy tissue we analyzed. This points toward these mutations, at least for this small initial patient cohort, to not be reliable risk factor mutations. We

focused here on ECM and ECM-related proteins because of the known role of the ECM environment in driving breast cancer growth, invasion, and metastasis (32-40). Unfortunately, we cannot test directly whether mutations in these proteins are directly responsible for the aggressive nature of the disease in these women. Regardless, the overlap with these shared mutations in the Ethiopian samples and those from ER+ samples in White women from the TCGA data set is striking. It is interesting to note that none of the mutations we saw in all three of our tumor samples were highlighted in a recent review of mutations characteristic of tumors in African women (17). This perhaps points to a mutational spectrum unique to tumors in Ethiopia, but we can only speculate as to whether these mutations are relevant to the aggressive phenotypes observed.

Breast cancer in African American women has an aggressive phenotype, and it disproportionately affects younger women (41). Within the subgroup of these patients that have inherited disease, many of them have mutations in BRCA1, BRCA2, PTEN, and TP53. A recent study of about 300 African American patients with invasive disease showed that about a third of these patients were of the triple negative breast cancer (TNBC) subtype, with another third ER+. However, the limited data indicate that this population is predominately of West African descent. Yet, multiple lines of evidence lead one to the hypothesis that Ethiopian breast cancer is unlike the African American disease. First, a substantial amount of patients at the Black Lion hospital are presenting with cancer at a far younger age than typical for African American disease (nearly 80% of patients are under 50 (2)), and women are disproportionately presenting with advanced disease (greater than 70% are stage 3-4, (2)). Second, a recent study from Jemal and Fedewa demonstrated that breast cancer cases in East Africa (74% of these are from Ethiopia) are more likely to be ER+ compared to West African or African American cases (Figure 1) (1). Third, aggressive ER+ breast cancer in the US is relatively rare, even for African American patients (42), so it is highly likely that the driving factors, genetic or otherwise, for this disease in Ethiopia is distinct from that of African Americans. Outside of our study, it would be valuable to compare the genomic data we have here to White women with ER+ breast cancer in the US, to White women in the US with TNBC, and to African American women with TNBC.

Genetic mutations that contribute to the development of cancer are characterized by their penetrance, which is the proportion of individuals carrying a mutation that also show the associated phenotype. Current studies suggest that any non-BRCA familial mutations are not high-penetrance, which would explain why no other variants have been found and explored as extensively (43). The lack of exploration is primarily due to the over-

representation of women of European ancestry in genetic studies. Additionally, in contrast to earlier reports of a higher prevalence of ER- and TNBC in broadly defined "African" populations, more recent data show >50% ER+ breast cancer in some African populations (44), showing the critical need for more research into the prevalence and variation between populations in receptor status in breast cancers in African populations (45). We hope this data can help elucidate similarly powerful genetic predictors for women of East African descent. Only about 30% of heritable breast cancer can be attributed to known variants (7), and the majority of these are derived from patients with access to the best health care (8, 46).

#### **Future directions: mutations in ECM and ECM-related proteins uncovered**

We found 615 genes that had mutations across all three patients (but were not mutated in the paired healthy tissues for any of the women, Table S1). We were particularly interested in 5 of these genes that code for proteins in the extracellular matrix (ECM): LAMA5, LAMC3, COL6A5, MUC12, or ECM-downstream signaling proteins: MAP2K3, because of the known role of the ECM in tumor growth and metastasis (32, 39, 47-49). We have highlighted those mutations here as potential nucleation points for future studies.

LAMA5 is the gene that encodes for the alpha-5 subunit of laminin-10 (laminin-511), laminin-11 (laminin-521), and laminin-15 (laminin-523). LAMA5 is normally highly expressed in the skin, placenta, and lung, and it has been studied primarily for its role in stem cell maintenance. The few LAMA5 papers possibly related to our observation include reports on how the secreted protein can support breast cancer cell survival (50), adhesion of epithelial cells (51), melanoma cell motility (52), and chemotaxis of macrophages (53). LAMC3 is laminin subunit 3, which is highly expressed in the placenta and testes, and it was recently identified through another GWAS study as linked to oral and pharyngeal cancer (54). COL6A5 is a member of the collagen superfamily, and it is highly expressed in the skin and lung. Mutations in this gene are significantly associated with itch, eczema, dermatitis, etc. Although not directly related to breast cancer, clearly it is involved in epithelial cell detachment, and so its mutation here could be related to primary tumor cell detachment and ultimately metastasis.

Finally, MUC12 encodes an integral membrane glycoprotein (mucin). Mucins are O-glycosylated proteins that form protective mucous barriers on epithelial surfaces. MUC12 is specifically expressed in the colon, and it is linked to colitis and aggressive colon cancer, so its appearance in our study is both novel and puzzling. Future studies with a larger patient cohort are critical to determining the link between mutations in the intestine, colon, and breast.

## Materials and Methods

Age of onset and population mortality rate analysis: Mortality rates for individuals from the age of 1-85+ years from a set of 100,000 women were downloaded from the World Health Organization ([www.who.int](http://www.who.int)). It is important to note that the mortality data from the American subset did not include any information about self-identified genetic background. These data were combined with breast cancer age of onset data from (2).

Sample collection: We obtained tumor and matched normal adjacent tissue collected from 4 patients. From eligible participants, breast tissue specimens were collected within 10 minutes after surgery. The samples were soaked and preserved with phosphate buffered saline (PBS) pH 7 and subsequently transported within a cold chain to Tikur Anbessa Specialized Hospital and stored at -80 °C. All samples were deidentified by the Black Lion Hospital in Addis Ababa, Ethiopia, and no identifying information was shared with the authors of this study.

Subtyping: Formalin fixed tissue samples were processed and paraffin-embedded, then subjected to immunohistochemistry for estrogen receptor at the Biorepository and Tissue Research core facility at The University of Virginia. The stains were interpreted by a Board-certified pathologist (C.A.M.). We pursued a subset of tumors that were ER<sup>+</sup> for sequencing.

Whole Exome Sequencing: DNA was extracted from patient samples with DNeasy Blood & Tissue Kit (Qiagen) and quantified with a Qubit Fluorometer. Next-generation sequencing libraries were prepared using the Illumina TruSeq Exome kit with barcodes. Sequencing was done at the Genomics Core Facility at UMass Amherst on a NextSeq500. We used the TruSeq Exome kit (Illumina) to sequence matched normal and tumor

tissue from 3 patients. After sequencing, data quality was established with FASTQC analysis, which confirmed consistently high per based sequence quality across the entire read. Additionally, data maps to human reference genome build hg38 at > 96%. The data are available on the SRA (PRJNA704855). Fastq files were mapped to hg38 using Bowtie (55), and variants identified using mpileup in Samtools (56), and filtered using bcftools (57). Variants were called between the tumor-normal pairs using the VarScan2. Biological enrichments were performed using GSEA (58). Data were then compared with data generated by the TCGA Research Network: <https://www.cancer.gov/tcga>. In total, genes with SNPs data from 922 patients from the TCGA.

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