

A rare *BRCA1* alternative missense mutation identified in African American women with breast cancer

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Abstract

Introduction: Hereditary breast cancer is most commonly caused by inherited mutations in the *BRCA1* or *BRCA2* genes, which significantly increase the risk of breast and ovarian cancers.

Methods: Denaturing High-Performance Liquid Chromatography (DHPLC) and DNA sequencing were used to analyze *BRCA1* mutations, missense variants, polymorphisms, haplotypes, and large genomic rearrangements in ninety-nine African American (AA) patients from high-risk families. Sorting Intolerant From Tolerant (SIFT) analysis was applied to predict the functional impact of amino acid substitutions. Potential effects on splicing were evaluated by examining disruption of conserved exonic splicing enhancer (ESE) elements or canonical splice site sequences.

Results: DHPLC and sequencing analysis identified a deleterious protein-truncating *BRCA1* mutation, 5296delGAAA, in two unrelated patients diagnosed with bilateral and early-onset breast cancer, both with extensive family histories of breast and ovarian cancers. A second pathogenic variant, IVS16+6T>C, was detected in two additional patients with early-onset or bilateral breast cancer and a strong familial history. Three missense variants (Ile379Met, Glu1210Lys, Glu1794Asp), predicted to be deleterious by SIFT, were identified, including two exon 22 variants (Gln1785His and Glu1794Asp) likely to affect splicing. A rare silent variant, 884G>A (Glu255), located in an alternative exon 11 ESE, was identified in one patient and is predicted to abolish tissue-specific splicing. Multiple coding and non-coding polymorphisms were detected, and haplotype analysis revealed two predominant *BRCA1* haplotypes among 64% of AA patients, suggesting population-specific allele distributions. MLPA analysis of 26 patients heterozygous for the *BRCA1* exon 11 Pro871Leu polymorphism revealed a novel large deletion encompassing exons 1a, 1b, and 2, extending into the promoter region. This deletion, supported by linkage disequilibrium analysis, represents the first report of such a mutation in an AA family. Preliminary MLPA screening of an additional 40 patients and 2 cancer-free controls identified a potential exon 17 duplication in one case, warranting further investigation.

Conclusion: These findings highlight the complexity and diversity of *BRCA1* alterations in AA breast cancer patients and underscore the importance of comprehensive genetic screening in this population to enhance risk assessment and guide clinical management.

Keywords: *BRCA1*, Breast Cancer, African American, Missense mutation

Introduction

Globally, breast cancer affects more than one in ten women and has become the most frequently diagnosed cancer worldwide. It is the most prevalent malignancy among women of all ethnicities, accounting for approximately 30% of all newly diagnosed cancers in women. In the United States, an estimated one in eight women (about 13%) will be diagnosed with breast cancer in her lifetime [1,2] and an estimated 42,170 U.S. women will die from breast cancer in 2025 [3].

Significant disparities exist between African American (AA) and European American (EA) women in both incidence and mortality rates [4,5]. Although EA women have higher age-adjusted incidence rates (143 vs. 119 per 100,000), AA women are more often diagnosed at younger ages and with more aggressive tumor subtypes, typically characterized by higher histological grade and negative estrogen/progesterone receptor status. Notably, AA women have a 41% higher mortality rate from breast cancer compared to EA women. Among women under 50, the incidence of aggressive subtypes such as triple-negative breast cancer (TNBC) is nearly twice as high in AA women [5]. Thus, while EA women of European descent are slightly more likely to be diagnosed with breast cancer, women of African descent are more likely to die from this disease.

Hereditary breast cancer is predominantly attributed to mutations in the *BRCA1* and *BRCA2* genes, which are associated with a markedly increased lifetime risk of breast and ovarian cancers. These mutations account for approximately 5% of all breast cancer cases and over 80% of hereditary cases [4,6,7]. The lifetime risk of breast cancer in *BRCA1* and *BRCA2* mutation carriers ranges from 35% to 85% respectively [8,9], with *BRCA1* mutations conferring an estimated risk of 55%–72% and *BRCA2* mutations 45%–69% [1,5]. Despite these well-established risks, most genetic studies on hereditary breast cancer have been conducted in non-Hispanic white populations, with limited representation of AA women [10]. Consequently, the mutation spectrum within AA populations remains underexplored.

Previous research suggests that the frequency and spectrum of germline mutations in *BRCA1*, *BRCA2*, and other susceptibility genes such as *CHEK2* may differ substantially between AA and EA populations [10–12]. At the Howard University Cancer Center, we have assembled one of the largest cohorts of AA families at substantial risk for hereditary breast cancer. We hypothesize that AA patients possess a distinct and potentially broader spectrum of *BRCA1* germline mutations compared to those observed in other populations.

To investigate this hypothesis, we employed denaturing high-performance liquid chromatography (DHPLC) and DNA sequencing to analyze *BRCA1* mutations, including missense variants, polymorphisms, haplotypes, and large genomic rearrangements, in 99 AA patients from high-risk hereditary breast cancer families. We used Sorting Intolerant From Tolerant (SIFT) analysis to predict the functional consequences of detected amino acid substitutions. In addition, we assessed potential splicing effects by examining disruptions of conserved exonic splicing enhancer (ESE) motifs and canonical splice site sequences.

Materials and Methods

Human subjects

Ninety-nine AA patients from families at substantial risk for hereditary breast cancer with histologically confirmed breast cancer were recruited at Howard University Hospital in Washington, D.C. The study was approved by the Howard University Institutional Review Board (IRB-09-MED-86, IRB-04-MED-04, IRB-97-MED-35), and written informed consent was obtained from all participants. Inclusion criteria for the high-risk cohort were based on one or more of the following: 1) two or more first- or second-degree relatives with breast cancer, or with both breast and ovarian cancer; 2) diagnosis of early-onset breast cancer (≤ 40 years); 3) bilateral breast cancer; 4) diagnosis of both breast and ovarian cancer in the same individual; 5) or a family history of male breast cancer. Among the 99 high-risk patients: 71% reported a family history of multiple breast cancer cases; 14% had families with both breast and ovarian cancer; 8% were diagnosed with early-onset breast cancer (≤ 40 years); 3% had bilateral breast cancer; 2% had a family history of male breast cancer; and 1% had bilateral breast cancer without any reported family history (Table 1).

A control group of 122 disease-free individuals, with no personal or family history of breast or ovarian cancer, was also recruited through the free screening mammography program at the Howard University Cancer Center (HUCC). Controls were frequency-matched to cases by age.

DNA extraction and polymerase chain reaction (PCR)

To identify genomic rearrangements and germline mutations in the coding regions (exons), intron-flanking sequences, promoter, and untranslated regions of the *BRCA1* gene, genomic DNA was extracted from cryopreserved peripheral blood lymphocytes of both cases and controls using the QIAamp DNA Blood Maxi Kit (Qiagen Inc., Valencia, CA) following the manufacturer’s protocol.

PCR amplification of *BRCA1* target regions was performed using sequence-specific primers as recommended by Teresa Wagner *et al.* [13,14]. Reactions were conducted in an AmpGene 9700 thermal cycler (Perkin-Elmer 600, Foster City, CA).

PCR reactions were performed in a total volume of 50 μ L containing 50 ng genomic DNA, 15 mM Tris-Cl (pH 8.0), 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM of each deoxynucleotide triphosphate (dNTP), 0.4 μ M of each forward and reverse primer, 1 unit of AmpliTaq Gold® DNA polymerase (Applied Biosystems, Foster City, CA), and 1X Gold PCR buffer. The thermal cycling conditions

Table 1. Characteristics of selected high-risk African American breast cancer patients.

Category for selection	Mean age at diagnosis (years)	Number of cases
Multiple cases of breast cancer ($\geq 2^a$)	50	71
Breast and ovarian cancers ^b	45	14
Early onset (≤ 40 years)	36	8
Bilateral breast cancer	48	3
Bilateral breast cancer (no family history)	37	1
Male breast cancer	52	2
Total cases		99

were followed according to the protocol described by Wagner *et al.* [13,14].

Denaturing high-performance liquid chromatography (DHPLC) and mutation analysis

In a blinded comparison, DHPLC has been reported as one of the most reliable methods for *detecting BRCA1 mutations*, ranking second only to DNA sequencing [13]. DHPLC was performed using the WAVE® DNA Fragment Analysis System (Transgenomic Inc., Omaha, NE) equipped with a DNASep® column (Transgenomic Inc., San Jose, CA), which contains C18-alkylated, polystyrene-divinylbenzene beads optimized for nucleotide fragment separation.

Optimal temperatures for resolving heteroduplex and homoduplex DNA were determined using the DHPLC melting algorithm [13]. Gradient parameters were set based on PCR product size using WAVEMaker™ software (v1.5.4). DHPLC was conducted under preset conditions with an initial gradient of 45% buffer A (0.1 M TEAA, pH 7.0) and 55% buffer B (0.1 M TEAA + 25% acetonitrile, pH 7.0), followed by a final gradient of 36% buffer A and 64% buffer B. PCR products (25 µL) were injected and eluted at 0.9 mL/min with a total acquisition time of 8.7 minutes.

All putative mutations and sequence variants identified by DHPLC were confirmed by direct DNA sequencing.

DNA sequencing and mutation analysis

Samples exhibiting heteroduplex peaks on DHPLC were further analyzed. PCR products were purified using the QIAquick PCR Purification Kit (Qiagen) and sequenced by ACGT Inc. (Wheeling, IL). Sequence data were analyzed with Sequencher™ v3.1 (Gene Codes, Ann Arbor, MI). Nucleotide positions were assigned according to the *BRCA1* reference sequence (GenBank U14680) based on cDNA numbering and compared to the published *BRCA1* mRNA sequence (GenBank HSU43746).

SIFT analysis

SIFT is a computational tool used to predict whether an amino acid substitution affects protein function. The *BRCA1* protein sequence (GenBank accession no. HSU43746) was submitted to the SIFT server (<http://blocks.fhcrc.org/~pauline/SIFT.html>). Substitutions with normalized probabilities below 0.05 were classified as intolerant (potentially damaging), while those with probabilities ≥ 0.05 were considered tolerated, following established thresholds.

BRCA1 ESE analysis

To assess whether *BRCA1* variants disrupted ESE motifs, wild-type and mutant sequences were analyzed using ESEfinder (<http://rulai.cshl.edu/tools/ESE/>). Four position weight matrices (PWMs) for SR proteins were selected for analysis. Scores exceeding the program's predefined thresholds were considered high and the position of each predicted ESE within exons was also evaluated to estimate its potential functional impact on splicing.

Multiplex Ligation-dependent Probe Amplification (MLPA)

MLPA [15] was performed to detect *BRCA1* copy number variants. Twenty-six patients were screened using the *BRCA1*-MLPA kit P002 (MRC-Holland, Amsterdam, Netherlands) which includes FAM-labeled primers for all *BRCA1* exons and nine control probes.

Amplified, fluorescently labeled PCR products were separated by size using an ABI 3100 capillary sequencer (Applied Biosystems), with ROX-500 as the internal size standard. Data analysis was performed with GeneScan and Genotyper software (Applied Biosystems). Peak heights were normalized and compared to controls to calculate dosage quotients (DQs): approximately 1.0 indicates two copies of the target sequence (2n), ~ 0.5 indicated deletions (n), while ~ 1.5 indicate duplications (3n).

Preliminary MLPA screening of 40 patient samples was conducted and suspected rearrangements were confirmed by sequencing breakpoints.

Amplification Refractory Mutation System (ARMS)

ARMS, a sensitive PCR-based assay, was employed to detect *BRCA1* mutations. This method effectively differentiates heterozygous individuals from homozygotes at target loci. Samples exhibiting multiple DHPLC peaks, such as patient BC70, and representative samples harboring the *BRCA1* β -promoter 1802 C/C, C/G, or G/G alleles were analyzed. Primers were designed at a sufficient distance from the polymorphic site to prevent interference [16].

Results

BRCA1 pathogenic mutations

DHPLC analysis, followed by DNA sequencing, identified a deleterious protein-truncating *BRCA1* mutation, 5296delGAAA, in two unrelated patients (BC69 and BC88) (Table 2). Patient BC69 was diagnosed with bilateral breast cancer at ages 32 and 48 (ER/PR status unknown) and had a family history including 12 cases of breast cancer and 3 cases of ovarian cancer. Patient BC88 was diagnosed at age 30 with ER-negative/PR-negative breast cancer and had a family history of 3 breast cancer cases, 1 ovarian cancer case, and 2 prostate cancer cases.

A second pathogenic *BRCA1* mutation, IVS16 + 6T→C, was detected in two unrelated patients (BC86 and BC90) with a family history of multiple breast cancer cases [17]. Patient BC86 was diagnosed with bilateral breast cancer at ages 35 and 48 (ER/PR status unknown) and had a family history of 2 breast cancer cases. Patient BC90 was diagnosed at age 27 with ER-negative/PR-negative breast cancer and had a family history of 4 breast cancer cases.

BRCA1 missense variations and ESE Sequences

Three missense variations, Ile379Met, Glu1210Lys, and Glu1794Asp, were detected in patients with multiple cases of breast cancer and are predicted to affect *BRCA1* function (Table 3). The Ile379Met variation, previously reported in both AA and Caucasian populations, was found in patient BC48 who was diagnosed with bilateral ER-negative/PR-negative, p53-positive breast cancer at ages 41 and 46; and her mother was diagnosed with breast cancer at age 49.

The Glu1210Lys variation, not previously reported in the ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>) or The BRCA Exchange (<https://brcaexchange.org>), is predicted by SIFT analysis to impact protein function. The patient carrying this variant was diagnosed at age 79 with ER-positive/PR-positive breast cancer and has two affected sisters and a brother with prostate cancer.

Table 2. Deleterious, protein-truncating *BRCA1* mutations.

Patient ID	Age at diagnosis	ER/PR ^b status	Mutation nucleotide no. ^a	Effect	Number of affected individuals per family		
					Br ^c	Ov ^d	PCa ^e
BC69	32/48	unknown	5296delGAAA	frameshift	12	3	0
BC88	30	ER-ve, PR-ve	5296delGAAA	frameshift	3	1	2
BC86	35/48	unknown	IVS16+6T/C ^f	cryptic splicing yielding frameshift ^g	2	0	0
BC90	27	ER-ve, PR-ve	IVS16+6T/C	cryptic splicing yielding frameshift ^g	4	0	0

^aNumbering starts 120 nucleotides upstream of the ATG start codon.

^bER: Estrogen Receptor; PR: Progesterone Receptor; -ve: negative; +ve: positive.

^cBr: Breast Cancer; ^dOv: Ovarian Cancer. ^ePCa: Prostate Cancer; ^fIVS: Intervening Sequence (intron); ^g‘+’ denotes the number of nucleotides downstream from the exon-intron boundary within the intron.

^gScholl *et al.*, 1999 [19]

Table 3. ^aRare *BRCA1* gene missense mutations as well as variations in exonic splicing enhancers (ESE) that may affect RNA splicing and protein function.

Patient ID	Age at Diagnosis	ER/PR ^a status	Nucleotide Change ^b	Amino Acid Change (codon)	Evidence for Protein Function or Splicing Effect	Number of Breast Cancer Cases in family
BC48	41, 46	ER-, PR- p53-positive	T1256G	Ile379Met	SIFT ^d , Conserved in human, ancestral eutherian, marsupial proteins	2
BC103	79	ER+, PR+	G3747A	Glu1210Lys	SIFT	2
BC40	42	Unknown	G5501T ^e	Glu1794Asp	SIFT, Conserved in mouse, dog, rat proteins	2
BC25	56	ER+, PR+	G884A	Glu255Glu	ESE decreased 18-fold	3
BC01	33	Unknown	G5474T ^e	Gln1785His aa substitution	ESE decreased 6-fold	1

^aRare variants are defined as those occurring at an allelic frequency of less than 1 in 100 chromosomes (<1%).

^bNumbering starts 120 nucleotides upstream of the ATG start codon.

^cER: Estrogen Receptor; PR: Progesterone Receptor; -ve: negative; +ve: positive.

^dSorting Intolerant From Tolerant, change expected to affect function.

^ePanguluri *et al.*, 1999 [11].

Glu1794Asp, detected in patient BC40 diagnosed at age 42, is also predicted by SIFT to affect *BRCA1* function. Additionally, two *BRCA1* exon 22 missense variants, Gln1785His and Glu1794Asp, are predicted by ESE analysis (<http://exon.cshl.edu/ESE/>) to disrupt binding of serine/arginine-rich (SR) proteins, potentially affecting splicing. The Gln1785His variant, found in BC01 diagnosed at age 33, is similarly predicted by SIFT to impact protein function.

The rare *BRCA1* 884G>A (Glu255Glu), located within a known alternative exon 11 splicing enhancer (ESE) was identified in patient BC25. The wild-type alternative splice site typically results in the deletion of most of exon 11. This variant is predicted to abolish the alternative splicing of *BRCA1*, which may have functional consequences due to its tissue specificity and conservation. Patient BC25 was diagnosed at age 56 with ER-positive/PR-positive breast cancer and had a family history of three breast cancer cases. This variant has not been reported in ClinVar or The BRCA Exchange.

BRCA1 polymorphisms and haplotypes

Numerous coding and non-coding polymorphisms, along with unclassified variations, were detected in the *BRCA1* gene through DHPLC analysis. Variants were further evaluated using SIFT analysis, evolutionary conservation, ESE scores, and their proximity to introns. Several polymorphisms identified have not been previously documented.

Haplotype analysis was conducted on the 10 most frequent polymorphisms identified. **Table 4** presents the *BRCA1* haplotypes of common polymorphisms (frequency ≥ 5% of chromosomes) in AA patients. Haplotype I and II frequencies were 0.60 and 0.041, respectively, representing 64% of AA patients in this cohort. Haplotype I includes the more frequent allele at exons 11K, 11N, 11S, 13, and 16; whereas haplotype II is the least frequent allele at these loci.

Table 4. *BRCA1* mutations and ^ahaplotypes: comparison of the ten most frequent ^bpolymorphisms in African Americans and other populations.

Exon/Intron	Nucleotide Change ^c	Effect ^b	Most allelic frequency	Less allelic frequency	AA patients allelic frequency	Allelic frequency in other populations
In 8	IVS8-58delT	Non-coding	18	15	0.162	0.2 AA pt 0.3 U.S. Cau pt Hispanic-U.S. Cau-Spain Malay, Indian, Chinese
Ex 11K	2201C>T	Silent Ser694Ser	18	15	0.153	0.32 Can pt 0.27 Cancon Europe, China, Malaysia
Ex 11K	2430T>C	Silent Leu771Leu	18	15	0.167	0.19 AA pt 0.32 Can pt/Cau pt U.S.; Global
Ex 11N	2731C>T	Pro871Leu	16	17	0.240	0.42 Can pt 0.23 Can con 0.42 Can pt 0.34 Utah con Global
Ex 11S	3232A>G	Gly1038Gly Sift not tolerated	15	18	0.167	0.19 AA pt 0.32 Cau pt 0.34 Can pt Global
Ex 11TU	3667A>G	Lys1183Arg	19	14	0.150	0.32 Can pt 0.32 U.S., Can Cau pt, con 0.19 AA pt; Eur
Ex 13	4427T>C	Silent Ser1436Ser	14	19	0.199	0.33 U.S. pt 0.4 Spa Cau Pt. Malay, Indian, Chinese, Eur Pt
Ex 16	4956A>G	Ser1613Gly	18	15	0.143	0.30 Can pt 1 A, 0.35 Eur, 0.33 Malay, 0.33 U.S. & 0.31 Can, 0.12 Cau
In 16	IVS16-68G>A	Non-coding	18	15	0.162	0.25 Can pt 0.25 U.S. Euro, Cau
In 18	IVS18+64G>A	Non-coding	15	18	0.164	0.39 BIC

^aHaplotypes are groups of common polymorphisms (occurring in at least 5 out of 100 chromosomes) that are inherited together due to linkage disequilibrium.

^bPolymorphisms are variant present in $\geq 1\%$ of the population (≥ 1 in 100 chromosomes) variations.

^cNumbering begins 120 nucleotides upstream of the *BRCA1* ATG start codon. "IVS" denotes intervening sequences (introns); "+" indicates the number of nucleotides into the intron from the exon-intron boundary; "-" refers to the number of nucleotides from the 3' end of the intron. Intron numbers correspond to the exon they follow (e.g., intron 8 follows exon 8). Deletions and insertions are designated at the 3'-most position of the affected sequence.

^dAllelic frequency: number of alleles divided by the number of chromosomes. Rare variants: allelic frequency of 0.01 (< 1 in 100 chromosomes). Polymorphisms: allelic frequency of ≥ 0.01 (≥ 1 in 100 chromosomes).

^eBreast Cancer Information Core, BIC; Olopade *et al.*, 2003 [10]; pt: patient; con: control; AA: African American (including Caribbean); A: African; Eur: European; Cau: Caucasian; Can: Canadian; Spa: Spanish.

^fBIC, Breast Cancer Information Core; Durocher *et al.*, 1996 [35]; Dunning *et al.*, 1997 [28]; Liu & Barker, 1999. ^gWagner *et al.*, 1999 [13]

***BRCA1* genomic rearrangements**

Twenty-six patients heterozygous for the *BRCA1* exon 11 Pro871Leu polymorphism were analyzed using MLPA to assess the presence of large deletions in *BRCA1* exons 1a, 1b, and 2. Among these, 16 cases (62%) exhibited linkage disequilibrium, being heterozygous for both Pro871Leu and the *BRCA1* β-promoter C/G1802 polymorphism. Five cases (19%) were homozygous for the G allele (G/G), while the remaining five cases (19%), including patient BC70, were homozygous for the C allele (C/C) (**Figure 1, Table 5**).

Heterozygosity at the exon 11 polymorphism paired with apparent homozygosity at the promoter polymorphism suggests a deletion encompassing the promoter region (**Figure 1**). Among the patients sequenced for both Pro871Leu (C/T) and the β-promoter 1802 polymorphisms, 10 were in linkage disequilibrium, whereas 5 (33%) were not. The observed hemizyosity at the *BRCA1*

β-promoter C/G1802 polymorphism supports the presence of a large deletion encompassing exons 1a, 1b, and 2, the first such report in an AA family. DNA sequencing of a subset of samples that displayed multiple DHPLC peaks also confirmed the Pro871Leu polymorphism.

Preliminary MLPA testing was performed on 40 patient samples and two cancer-free subjects. **Figure 2** shows the 9 standards (blue) for copy number normalization and the *BRCA1* exons (green) from a disease-free control lying in the normal dosage quotient range (DQ, 0.85–1.15 = normal). **Figure 3** illustrates the 9 standards (blue) used for copy number normalization alongside the *BRCA1* exons (green) for breast cancer patient BC12. Comparison to these standards, indicated by a dosage quotient (DQ) between 1.35 and 1.65, revealed a duplication of *BRCA1* exon 17 in patient BC12.

Table 5. Linkage disequilibrium between *BRCA1* β promoter C/G 1802 and *BRCA1* Pro871Leu (C/T) in 15 African American individuals.

	Pro871Leu (C/T)	Pro871Leu (T/T)
	Total number of patients (Patient ID)	Total number of patients (Patient ID)
<i>BRCA1</i> promoter C/C 1802	3 (BC24, BC48, BC53)	2 (BC59, BC70)
<i>BRCA1</i> promoter C/G 1802	5 (BC06, BC07, BC16, BC41, BC45)	0
<i>BRCA1</i> promoter G/G 1802	0	5 (BC65, BC73, BC98, BC99, BC106)

16/26 heterozygous C/G
5/26 homozygous G/G
5/26 homozygous C/C including patient# 70

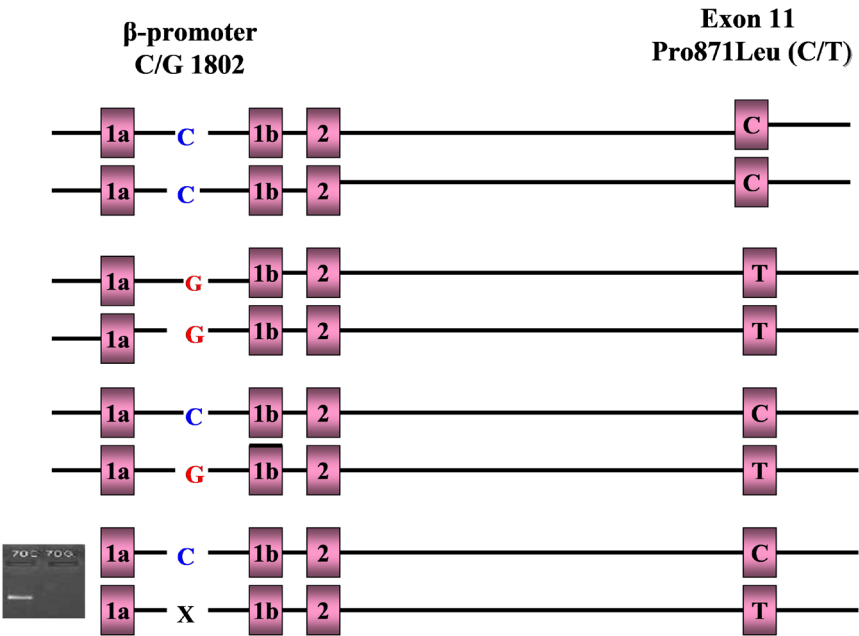


Figure 1. Linkage Disequilibrium. A C/G 1802 polymorphism in the *BRCA1* β promoter and the Pro871Leu polymorphism, situated in exon 11 were found to be in linkage disequilibrium (Catteau *et al.*, 1999 [16]). Linkage disequilibrium means that the 1802 promoter C allele was frequently in association with Pro871 (C), and the promoter G allele is in association with Leu871 (T).

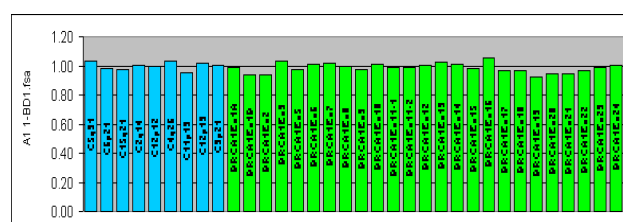


Figure 2. Quantitative Multiplex Ligation-dependent Probe Amplification (MLPA) analysis of the *BRCA1* gene. The 9 standards (blue) for copy number normalization and the *BRCA1* exons (green) from a disease-free subject lying in the normal dosage quotient range (DQ, 0.85-1.15 = normal). The y-axis represents the fluorescent intensity of amplification. For each probe, the X-axis represents the Ratio (A ratio of 1 signifies a normal copy number; a probe ratio of <0.75 indicates a heterozygous deletion; and a ratio of >1.3 stands for duplication).

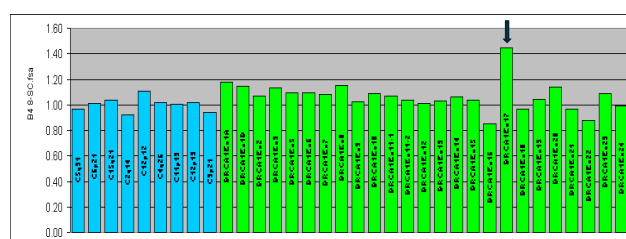


Figure 3. Quantitative Multiplex Ligation-dependent Probe Amplification (MLPA) analysis of *BRCA1* gene. The 9 standards (blue) used for copy number normalization alongside the *BRCA1* exons (green) for breast cancer patient BC12. Comparison to these standards indicates a duplication of *BRCA1* exon 17 (dosage quotient between 1.35 and 1.65) in patient BC12. The y-axis represents the fluorescent intensity of amplification. For each probe, the X-axis represents the Ratio (A ratio of 1 signifies a normal copy number; a probe ratio of <0.75 indicates a heterozygous deletion; and a ratio of >1.3 stands for duplication).

Discussion

We identified a deleterious *BRCA1* mutation (5296delGAAA) in two unrelated patients with breast and ovarian cancer, and a pathogenic *BRCA1* mutation (IVS16+6T→C) in two additional unrelated patients. All four probands, each from different families, were diagnosed before the age of 50 and belonged to families with a history of multiple cases of breast cancer and ovarian cancer, or both cancers in the same individual. Two of the *BRCA1* mutation carriers had ER-negative/PR-negative tumors, while the hormone receptor status of the remaining two probands is unknown. Both early onset of disease and ER-negative/PR-negative tumor phenotype are characteristic features of *BRCA1* mutation carriers [10].

The deleterious mutations (5296delGAAA and IVS16+6T→C) have been previously reported in AA, African, and other diverse populations and may represent founder mutations [18]. Two pathogenic *BRCA1* mutations identified earlier in this cohort were found in women diagnosed with ER-negative/PR-negative breast cancer at ages 48 and 50 [11]. The IVS16+6T→C mutation has been shown to create a cryptic splice site, resulting in a truncated protein [19,20].

We also identified rare missense variants (Ile379Met, Glu1210Lys, and Glu1794Asp) that may impact *BRCA1* function, as suggested by SIFT analysis, evolutionary conservation, early age of onset, and ER-negative/PR-negative tumor status [6]. Notably, the Ile379Met variant was found in a patient who exhibited all of these characteristics.

ESE sequences can be disrupted by single-base substitutions, including missense, nonsense, silent mutations, or single-nucleotide polymorphisms (SNPs) [21], leading to exon skipping in breast cancer patients with *BRCA1* or *BRCA2* mutations [20,21]. Recent studies have shown that some exonic substitutions can abolish ESE motifs.

The Ile379Met variant, previously reported in both AA and Caucasian populations, was identified in patient BC48, who was diagnosed with bilateral, ER-negative/PR-negative, p53-positive breast cancer at ages 41 and 46. Her mother was also diagnosed with breast cancer at age 49. Ile379Met variant was found in a critical domain, the BRCT-associated serine-rich domain [22] and the functional prediction using SIFT analysis [21,23], along with evolutionary comparison of human, ancestral eutherian, and marsupial protein sequences [23,24], suggests that the Ile379Met variant may impact *BRCA1* function.

We also identified a unique silent variant (884G>A; Glu255Glu) within an alternative exon 11 ESE sequence that has not been reported in ClinVar or The BRCA Exchange (<https://brcaexchange.org>). Additionally, two missense variants in exon 22 of *BRCA1* (Gln1785His and Glu1794Asp) are predicted to affect splicing. Two other patients carry rare variants located in ESE sites near intron-exon boundaries, which are expected to reduce splicing efficiency. However, transformed lymphocyte cell lines were not available to assess the biological impact of these putative missense and ESE variants.

Furthermore, numerous coding and non-coding polymorphisms were observed in *BRCA1*, several of which were previously undocumented. In many cases, the same combination of polymorphisms (haplotypes) appeared in different patients. Many patients shared specific combinations of polymorphisms (haplotypes), like those reported in U.S., European, and Asian populations, reflecting linkage disequilibrium [25–27]. For example, in European and Asian control populations, two major haplotypes, haplotype I (frequency: 0.65) and haplotype II (frequency: 0.33), account for approximately 98% of chromosomes [27]. In contrast, our AA cohort exhibited greater haplotype diversity than the European or Asian populations, accounting for 64% of the chromosomes, with haplotype I at a frequency of 0.60 and haplotype II at a frequency of 0.041. Haplotype I corresponds to the more frequent alleles at positions 11K, 11N, Ex13, and Ex16, while haplotype II carries the less frequent alleles at these same sites. Similarly, Dunning *et al.*, reported that two major haplotypes (haplotype I at 0.59 and haplotype II at 0.34) accounted for 93% of chromosomes in Caucasian breast cancer cases [28]. In our AA patients, the same two haplotypes accounted for only 66% of the cases, with haplotype I at 0.60 and haplotype II at 0.06. Notably, the frequency of haplotype II in AA is 6–8 times lower than in Caucasians and Asians, further underscoring the increased haplotype diversity in this population. This greater diversity among AA is consistent with human evolutionary history. As modern humans originated in Africa, there has been more time for genetic variation to accumulate within African and AA populations. Furthermore, the AA population has ancestral roots in diverse regions across Africa and has experienced admixture with EA and Native Americans, contributing to the broader range of haplotype combinations observed [18].

We also identified a large deletion spanning *BRCA1* exons 1a, 1b, and 2, extending into the promoter region in one of the 55 high-risk AA families. This deletion was detected in patients heterozygous at the *BRCA1* exon 11 Pro871Leu polymorphism but apparently homozygous at the β -promoter C/G1802 polymorphism, consistent with hemizygosity from a large deletion. Linkage disequilibrium between these loci was present in some patients but absent in others, supporting the deletion hypothesis. No such deletion was observed in disease-free controls. This is the first report of such a deletion in an AA family.

Similar deletions have been reported in European populations, where homologous recombination between *BRCA1* intron 2 and the highly similar pseudo-*BRCA1* intron 2 generates an approximately 37 kb deletion encompassing the promoter and exons 1a, 1b, and 2, resulting in complete loss of *BRCA1* transcript expression [29–34]. Our findings are consistent with this mechanism and confirm that the deletion extends into the promoter region, as suggested by the absence of linkage disequilibrium between *BRCA1* exon 11 and the β -promoter polymorphisms in several patients.

Confirmation of the *BRCA1* Pro871Leu polymorphism was obtained by sequencing samples with multiple DHPLC peaks. ARMS testing of patient BC70 and others confirmed that the deletion extended into the promoter region. In our cohort, AA displayed less linkage disequilibrium than Caucasians which is consistent with prior studies and the increased haplotype diversity observed. Patient BC70, carrying the deletion, was diagnosed with ER-positive/PR-negative breast cancer at age 63, and her sister was diagnosed at age 42.

ARMS testing confirmed deletion-associated alleles and DHPLC showed characteristic multiple peaks. Although MLPA confirmed the deletion of exons 1a, 1b, and 2, its exact extension into the promoter region requires further investigation.

Conclusion

This study reveals a rare *BRCA1* genetic alteration, a large deletion encompassing exons 1a, 1b, and 2 and extending into the promoter in AA breast cancer patients. This finding suggests that large genomic rearrangements may contribute substantially to hereditary breast cancer risk in this population. Moreover, the diversity of rare missense and ESE-disrupting variants underscores the need for comprehensive genetic screening in AA. These findings should be validated in larger cohorts, and meta-analyses may further clarify the significance of these rare variants.

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Contributions

DB, LJR, and RLC conceived and designed the experiments. DB and YMK performed experiments, analyzed and interpreted the data. DB, YMK, and DAS wrote the manuscript.

MA, AHD, AL, OOK, RW, SN, and BK reviewed and corrected the manuscript. All author(s) reviewed and approved the final manuscript.

Ethics Approval and Consent to Participate

The study was approved by the Howard University Institutional Review Board (IRB-09-MED-86, IRB-04-MED-04, IRB-97-MED-35), and written informed consent was obtained from all participants.

Consent for Publication

Not applicable.

Competing Interests

The authors declare no competing interests.

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