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Genetic Susceptibility of *BRCA Gene rs1799950* in Breast Cancer Development in Different Ethnic Groups in South South, Nigeria

[a]Esionye Fedorah Nkiru, [a]Chukwuma Prince Nnamdi, [a]Clove Okatuaton Donald, [a]Friday Ayebaye, [a]Olubunmi O. Ezomoh, [b]Echendu Chibuzor Emmanuel,[c]Tawari Erebi Patricia,[d]Pughikumo Dibo Tabot,[e]Biyi-Olutunde Olugun, [e]Okere Enyinna Slyva,[a]Madukosiri Helen Chinyere, [b]Tatfeng Mirabeau Yotchou

[a]Department of Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Bayelsa State, Nigeria
[b]Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Health Sciences, Nigeri Delta University, Bayelsa State, Nigeria
[c]Department of Chemical Pathology, Faculty of Basic Clinical Sciences, College of Health Sciences, Nigeri Delta University, Bayelsa State, Nigeria
[d]Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Nigeri Delta University, Bayelsa State, Nigeria
[e]Department of Radiation and Clinical Oncology, University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria

A B S T R A C T

Cancer is the uncontrolled growth of abnormal cells anywhere in the body. These abnormal cells are termed cancer cells, malignant cells, or tumor cells. Nigerian breast cancer patients have a high frequency of BRCA1 and BRCA2 mutations (7.1 and 3.9 %, respectively). This study was conducted to detect *BRCA* SNP rs1799950 in patients from South-south Nigeria. Restriction Fragments Length Polymorphism (RFLP) was used for the detection of point mutation in the structural region (single nucleotide polymorphism). The amplicons (product of PCR) was digested by EcoR1-HF restriction enzyme and analyzed using agarose gel electrophoresis and viewed using the blue lights UV-trans-illuminator. A total of 50 subjects participated in this study, out of which 35 were Patients with different cancers, and the remaining 15 are control Patients. Among the 35 Cancer Patients (22 females and 13 males), 8 were Breast Cancer Patients. Restriction Fragment length Polymorphism (RFLP) detected the expression of BRCA 1 SNPs rs1799950 in 2 of the breast cancer patients, while the Control samples did not show the BRCA 1 SNPs. According to the distribution of Cancer by sex and age, Breast Cancer in females was widespread in each gender at 28%, while other cancer diseases was shown to be of lesser percentage. In association to genotyping, the TA heterozygous allele has significances in patients between the ages of 31-40 Years, to 50 years and above for BRCA 1 gene. However this study is regarded as the first of its kind in this population, therefore, larger sample size would be required to confirm the data necessary to reproduce the methodology adopted to clarify the findings.

Keywords:*BRCA* SNP rs1799950, Breast cancer, Genetic, Southern Nigeria, Susceptibility

Introduction

Cancer is a malignant disease condition arising from uncontrolled division of cells in the body forming mass of tissues (Eggert, 2017). Cancer is the most common human genetic disease which occurs at a transition from a normal cell to a malignant cancer driven by changes to cell's DNA also called mutation (Ferguson *et al.*, 2015). Breast cancer is defined as a disease that is characterized by the abnormal growth of cells in the breast. It is the most common malignant disorder affecting women and the leading cause of death among them (Santos *et al*., 2015). The incidence of breast cancer is higher in developed than in developing countries (Bray *et al*., 2018; Saibu *et al*., 2017), however, the incidence is increasing in developing countries especially in Africa.

* *Corresponding author.*
E-mail address: *perebi8@yahoo.com*

Breast cancer was also the 5th leading cause of cancer deaths worldwide in 2020, with 685,000 deaths attributed to it (WHO, 2020).

The incidence of cancer and cancer types are influenced by many factors such as age, gender, race, local environmental factors, diet and most importantly genetics (Siegel *et al*., 2020). Consequently the incidence of cancer and cancer types vary depending on these variable factors. Combinations of mutations in important genes can lead to the development of cancer. Mutations are not only a hallmark of cancer but may be central to how cancers evolve. Cancer cells divide where normal cells do not; they invade, metastasize and kill the host of origin. The fact that cancer is inheritable at the cellular level and that cancer cells contain multiple mutations, suggest that tumour progression is driven by mutagenesis. These mutations either arise from copying unrepaired DNA damage or from errors committed during DNA synthesis (Keith and Lawrence, 2000).

If all the potentially modifiable risk factors could be controlled, this would only reduce the risk of developing breast cancer by at most 30% (Desantis et al 2015). Certain inherited "high penetrance" gene mutation greatly increases breast cancer risk, the most dominant being mutations in the genes BRCA 1, BRCA 2 and PALB-2. Women found to have mutations in these major genes could consider risk reduction strategies such as surgical removal of both breast (Desantis et al 2015).
The gene most common to breast cancer is Breast Cancer 1 (BRCA1) and Breast Cancer 2 (BRCA2) genes, which normally confers protection against cancer. BRCA1 and BRCA2 (Breast-Cancer susceptibility gene 1 and 2) are tumor suppressor genes, the mutant phenotypes of which predispose to breast and ovarian cancers. However, not everyone who inherits a BRCA1 or BRCA2 mutations will get breast cancer or ovarian cancer (Weitzel, 2007). Recent studies suggest that BRCA proteins are required for maintenance of chromosomal stability, thereby protecting the genome from damage (Jianlin *et al*., 2020). New data also show that BRCAs transcriptionally regulate some genes involved in DNA repair, the cell cycle, and apoptosis. Many of these functions are mediated by a large number of cellular proteins that interact with BRCAs. The functions of BRCA proteins are also linked to distinct and specific phosphorylation events; however, the extent to which phosphorylation-activated molecular pathways contribute to tumor suppressor activity remains unclear (Irminger-Finger *et al.,* 2016). The major aim of this research was to detect, amplify the BRCA rs1799950 using polymerase chain reaction (PCR) and to determine the point of mutation on the SNPs rs1799950.

METHODOLOGY

Study Area
Blood samples were taken from patients in Niger Delta University Teaching Hospital (NDUTH), University Port Harcourt Teaching Hospital (UPTH), Federal Medical Centre (F.M.C) Yenegoa, and the controls were taken from final year biochemistry students of Niger Delta University and some cancer patients living within Bayelsa states and other South South states such as Rivers State.

Subjects
The subjects used in this study are male and female volunteer cancer patients from Niger Delta University Teaching Hospital (NDUTH), Federal Medical Centre (F. M. C) Yenegoa, and University of Port Harcourt Teaching Hospital (UPTH). The control subjects constitute of volunteers from the final year biochemistry students of Niger Delta University. The entire patients were diagnosed by laboratory evaluation. A standard question was carried out in order information regarding their demographic and lifestyle characteristics of subjects informed. Consent was obtained from subjects prior to participate in the study. The ethical committee of Basic Medical Sciences in Niger Delta University authorised and granted the performance of this study. The subjects were included in the study after been clinically examined and certified fit for the consultation.

Ethical Committee
A letter of introduction and proposal from the department of biochemistry, Niger Delta University was presented to the research ethical committee of the college of health sciences, Niger Delta University Teaching Hospital, Okolobim, University of Port Harcout Teaching Hospital, Rivers State, Federal Medical Centre, Yenegoa and an approved letter from these constitutional authorities were obtained enabled the collection of samples from the subjects based on the ethical rules and regulations of Basic Medical Sciences, Niger Delta University

Sample Collection
Blood samples were collected under aseptic condition using proper venepuncture technique. 5ml of blood was collected from various subjects with a sterile hypodermic needle and dispensed into ethylenediamine tetra acetic acid (EDTA) Anticoagulated bottle containers and stored at 20 until utilization.

DNA Extraction

Extraction was carried out using quick-DNA™ miniprep kit. 200ml of blood was pipetted into a properly labelled 1.5 Eppendorf tubes, 400ml of genomic lysis added to the blood and mixed completely by vertexing for 4-6 seconds to lyse the cell. The mixture was transferred to a zymo-spin™ IIc column in a collection tube and centrifuged at 10,000xg for one minute. The collection tube was discarded with the flow through. The zymo-spin column was transferred to a new collection tube and 200 of DNA pre-wash buffer was added to the spin column and centrifuged for one minute at 10,000xg. 500 of the g-DNA wash buffer was be added to the spin column and centrifuged at 10,000xg for one minute. The spin was transferred to a clean micro centrifuge tube and incubated for 2-5 minutes in room temperature followed by centrifugation at top speed of 30 seconds to elute the DNA. The eluted DNA can be used immediately for molecular based application or stored at 20 for future use.

DNA Quantification

After DNA extraction, the amount of DNA present in extract was quantified and checked for purity using pre-installed nanodrop software (Nanodrop 1000), before carrying out the measurement, the nanodrop software was blocked using TBE buffer and calibrated micropipette exactly 1 of each extract and was placed on the low pedestal and then closed and measured.

BRCA SNPS RS1799950 Amplification.

The BRCA1 rs1799950 region of the BRCA gene of the isolates were amplified using the BRCA1 rs1799950F: 5^{i} TTGCCAAACGAAAATTATGG-3^{i} and BRCA1799950R: 5^{i} AGATTTTCCACTTGCTGTGC-3^{I} primers on an ABI 9700 applied biosystem thermal cycler at a final volume of 50µl for 35 cycles. The PCR mix include the X2 dream Taq master Mix supplied by inquaba south adfrica (taq polymerase, DNTPs Mgcl), the primers at a concentration of 0.4M and the extracted DNA as template. The PCR conditions were as follows: initial denaturation, 95^{o}c for 5minutes, denaturation 95oc for 30 seconds; annealing 52^{o}c for 30 seconds; extension, 72^{o}c for 30 seconds for 35 cycles and final extension, 72^{o}c for 5 minutes. The product was resolved on a 1% agarose gel at 120v for 15mins and visualized on a uv transilluminator.

Restriction Fragment Length Polymorphism (RFLP)

Restriction Fragment Length Polymorphism (RFLP), several methods have been described for the detection of mutations in the structural region, such as PCR followed by Restriction Fragment Length Polymorphism (RFLP) analysis. This is simple and gives unambiguous results, PCR was carried out in the final volume of 50 l (30-100mg) of genomic DNA, 2.5mm $MgCl_2$ (100-500m deoxynucleotides triphosphate Forward primer TTGCCAAACGAAAATTATGG, 25M Reverse primer AGATTTTCCACTTGCTGTGC) and 1l of AmpliTaq Gold (applied Bio-system) in a buffer containing 100nM. The polymerases chain reaction (thermocycler) were carried out and initiated by a 5mindenaturation and enzyme activation step at 94and final denaturation 94 for 30min, then the PCR will Annealing at 51 for 5mins and a final extension step of 72 for 10mins. The temperature cycles were as follows: 30 cycles of 94 for 5mins, 51 for 30s and 72 for 30s for 10mins. The amplification product with a length of 152bp, was digested by Rsal (cutsmartenzyme) according to the manufacture instruction (New England Biolab Inc.). The PCR product was analysed using 3% agarose gel electrophoresis to identify the purity integrity, and the results were confirmed Blue light Trans illuminator (Embitech Pi-1002-prepone™ filter) the one enzyme digested fragment was observed for the TT genotype (153bp).

Preparation of Agarose Gel Electrophoresis.

TBE buffer solution 10x concentration were prepared (usually 1x TBE) that is 10% in 90% distilled water, add 900ml of distilled water in a measuring cylinder and 100ml of TBE buffer solution to the cylinder using the weighing balance to measure 3g of agarose powder. 3g of agarose powder was added to 200ml of TBE buffer solution and poured into a conical flask and mixed thoroughly, the solution was heated in a microwave oven until the agarose dissolve. The edges of the casting tray was sealed and combs were put in. When the molten gel has cooled down and solidified, a gel slab with a row of wells at the top were created, 0.5g/ml of ethidium bromide of added. Gel solution was mixed thoroughly by gentle swirling. The warm agarose solution was poured into the casting tray and allowed to set completely (30-45 minutes at a room temperature), a small amount of electrophoresis buffer was poured on the top of the gel and the comb carefully removed. The electrophoresis buffer was poured off. The gel was mounted the gel in the electrophoresis tank and electrophoresis buffers was added to cover the gel. The PCR products, alongside the Quick-load DNA molecular ladder were resolved on 1.5% agarose gel electrophoresis tinted with ethidium bromide in 1x TBE buffer. The electrophoretic machine ran at 12volts for 30 minutes and thereafter visualized with a blue light illuminator.

RESULTS

A total of 50 subjects participated in the study, out of which 35 were Patients with different cancers, and the remaining 15 are control Patients. Among the 35 Cancer Patients (13 males and 22 females), 8 are Breast Cancer Patients. Only 2 Breast Cancer patients showed the presence of BRCA 1 SNPs rs1799950 while the Control samples didn't show the BRCA 1 SNPs. When

running RFLP for every gene in each sample, SNPs was expressed only in BRCA 1 and ALL gene. This result shows the association of the allele frequency of SNPs (rs1799950) in BRCA 1 gene in association to breast cancer in the south-south region of Nigeria.

In determining the prevalence of Cancer in South-South, 12 different types of cancers were used from different sex and age. A cummulation of these were measured and represented in a bar chart.The distribution of these cancers by age and sex is represented below in Figure 1. According to the distribution of Cancer by sex and age, Breast Cancer in Females is prevalent than other cancers in each gender at 28 percent. Fibrosarcoma in male is prevalent at 14 percent, while Leukemia in male is prevalent at 11 percent. The other cancers are prevalent between 6 percent and 3 percent. In a 100 base pair DNA or Molecular ladder using a representative gel, the bands shown in Figure 2 below is positioned at a length of 500bp. The bands shown on the representative gel indicates that each and every of the band has a BRCA 1 gene. Therefore, BRCA 1 gene is present in both positive and negative samples.

In association to genotyping, the TA heterozygous allele has two significances in patients between the ages of 31-40 Years, 50 years and above for BRCA 1 gene. Table 1 showed different alleles which includes; TT, TA, and AA, and their various age ranging from 20 to 50 and above. For homozygous alleles, TT and AA, no subject falls in AA. As for TT, one subject falls between ages 31- 40 years, while three subjects fall in TT between ages 41 – 50 years of age.

Table 1. Genotyping of BRCA 1 gene by age

BRCA 1	TT	TA	AA
20 – 30 yrs	0	0	0
31 – 40 yrs	1	1	0
41 – 50 yrs	3	0	0
50 >	0	1	0

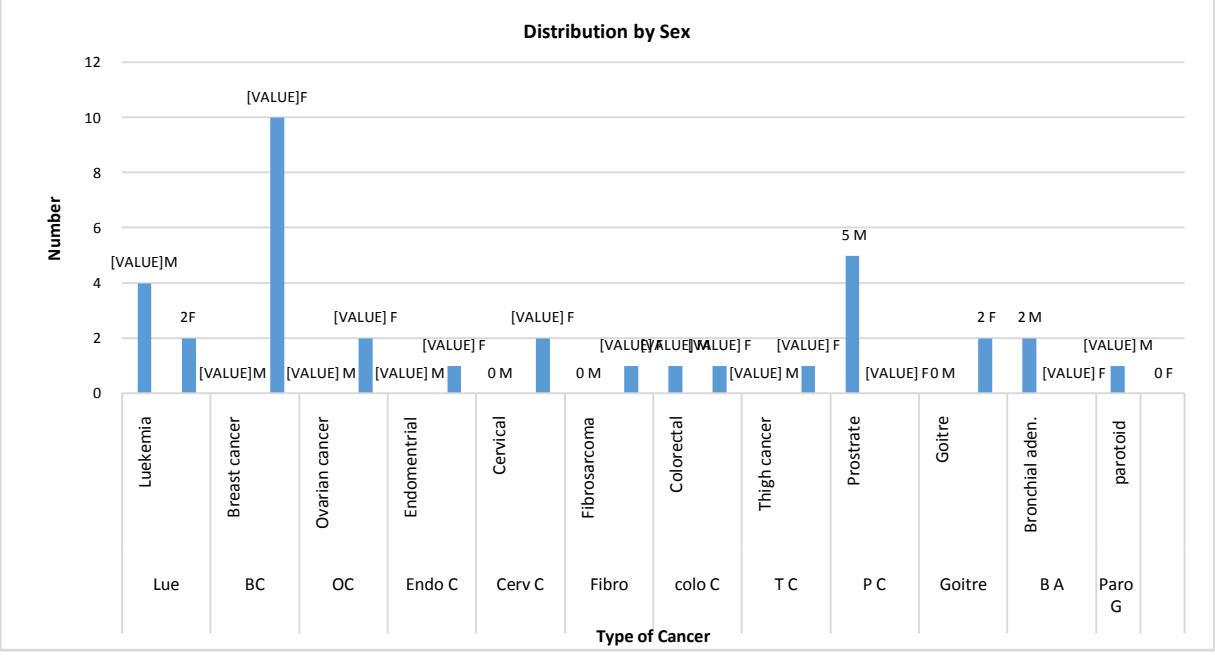
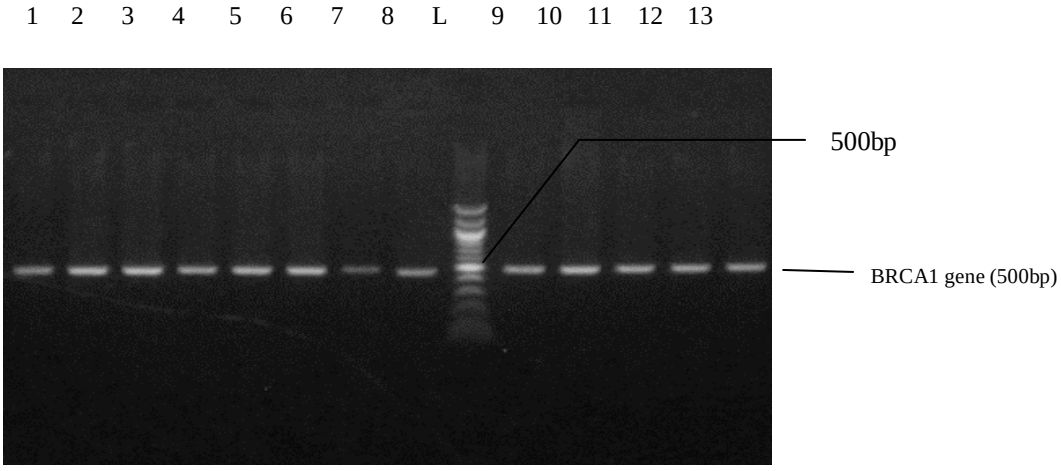


Figure 1. Distribution of Cancer by Sex



Agarose gel electrophoresis of the BRCA1 gene of the subjects. Lanes 1-13 showing the amplified BRCA1 gene at 500bp while L represents the 100bp ladder

Figure 2. Representative gel showing the base pair of BRCA 1 gene

DISCUSSION

The BRCA 1 gene provides instructions for making protein that acts as a tumor suppressor. Tumor suppressor proteins help prevent cells from growing and dividing too rapidly or in an uncontrolled way. The BRCA 1 protein is involved in repairing damaged DNA (Wu Q *et al*, 2017). Results from this study showed the association of BRACA SNPs (rs1799950) polymorphism with breast cancer and other cancer developments in the different ethnic group in river state, Nigeria. A total of 50 subjects participated in the study, out of which 35 were patients with twelve different cancers and the remaining 15 are control patients. Both the 35 Cancer patients, (13males and 22 females) and the 15 control samples showed the presence of BRACA SNPs rs1799950. When running RFLP for every gene in each sample, ALL SNPs was expressed in BRCA 1 gene, and ALL gene. As for JAK2 genes, only one bands or sample didn't show a single SNP.

Figure 1 showed the distribution of Cancer by sex and age, breast cancer is more prevalent in females (28%) than males (6%). It also affected females more than any other cancer. This in line with previous findings that breast cancer is most common among women, which can be as a result of some predisposition factors. One of which is smoking. Smoking plays a huge risk in breast cancer development. Women who are active smokers, especially post-menopausal and prenatal women who smoke, have an increased risk of developing breast cancer (Hanahan and Weinberg 2011). Exposure to second-hand smoke has also shown to be a risk factor for breast cancer.Fibrosarcoma is more prevalent in males (14%) than females (6%), while Leukemia is also more prevalent in males (11%) compared to females (5%). The other cancers were found to be between 6 percent and 3 percent.

The genotyping result (Table 1) showed that the TA heterozygous allele has two significances in patients between the ages of 31-40 Years and 50 years and above for BRCA 1 gene. Data showed different alleles which includes; TT, TA, and AA, and their various age ranging from 20 to 50 and above. For homozygous alleles, TT and AA, no subject falls in AA. As for TT, one subject falls between ages 31- 40 years, while three subjects fall in TT between ages 41 – 50 years of age.
In a 100 base pair DNA or Molecular ladder using a representative gel, the bands shown in Figure 2 is positioned at a length of 500bp. The bands shown on the representative gel indicates that each and every one of the band has a BRCA 1 gene. Therefore, BRCA 1 gene is present in both positive and negative samples.

CONCLUSION

This study has showed the association of BRCA 1 gene rs1799950 with different cancers in cancer patients resident in River State, Nigeria. This is of utmost importance as this has broadened our knowledge of the implication of BRCA 1 gene in various cancers other than Breast cancer. The study, however, is the first of its kind in this population. It is therefore important that more work that would involve larger sample size be carried out to further increase our understanding and awareness of this subject matter

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