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Research Article

The Dynamics Of Breast Cancer Among Patients Attending A Teaching Hospital In Nigeria.

Agu Charles M[1], Yibala Ibor Oboma[2]*, Beredugo, Sylvanus[3].

1. Histopathology Unit, Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Health Sciences. Niger Delta University Wilberforce Island, Bayelsa State, Nigeria. PMB 71, Yenagoa Bayelsa State.
2. Pathology Department, Faculty of Medicine and Allied Health Sciences Mwanza University Mbugani Street, Kishili Ward, P.O.BOX 3068 Mwanza Tanzania.
3. Histopathology Unit, Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. PMB 71, Yenagoa Bayelsa State.

Abstract

Breast cancer is a type of cancer with heterogeneous biology and is the leading cause of cancer-related mortality in women worldwide. This study aimed to establish breast cancer dynamics among breast cancer patients attending Niger Delta University Teaching Hospital Okolobiri. Archived Formalin fixed paraffin embedded breast tissues were retrieved and studied using hematoxylin and eosin staining, immunohistochemistry, and molecular methods. Genotype analysis of the P1K3Ca –rs2699887 SNP (single nucleotide polymorphism) was performed using polymerase chain reaction (PCR) and direct sequencing using a Big-Dye Terminator kit on a 3510ABI sequencer. Avidin biotin method was used for immunohistochemistry. Of 187 breast tumor samples received, 37.9% were malignant tumors and 62.1% benign tumors. Fibroadenoma was the most common form of benign tumor (54.3%). The prevalence of triple-negative, Her2+, estrogen receptor (ER)+, progesterone receptor (PR)+, ER+/PR+, and ER+/PR+ breast cancers were 37.6%. 13.0%, 50.0%, 31.0%, 31.0%, and 19.0%, respectively. The prevalence of triple-negative breast cancer is high in the present study, suggesting aggressiveness and low Her2+. In the present study, 88% of the breast cancer expression was as dominant homozygous (GG), 12% were heterozygous (GA), and 0% expression for recessive homozygous (AA). Individuals harboring GG/GA were prone to triple-negative breast cancer risk across age groups in the present study, indicating aggressive tumors. A relationship exists between P1K3Ca rs2699887 and triple-negative breast cancer. The effect of the GG/GA allele on disease risk was observed and linked to tumor aggressiveness.

Keywords : breast , cancer, dynamics, P1K3Ca –rs2699887.

INTRODUCTION

Breast cancer is the most prevalent cancer type in women and the leading cause of cancer mortality in women worldwide, with a peak incidence at 45 and 65 years [1]. Breast cancer can also occur in males, representing less than 1% of all cancers in the male population [2]. The risk factors for breast cancer include Age, family history, nulliparity, hormonal factors (early menarche or late menopause), lifestyle, alcohol consumption, obesity, and physical inactivity [3]. Histopathologically, ductal carcinoma in situ is the most commonly diagnosed type of breast cancer, followed by lobular carcinoma in situ [4]. Immunohistochemistry techniques further divide breast cancer into subtypes based on the presence or absence of the estrogen receptor (ER), progesterone receptor (PR), HER2 receptor, and Ki-67 [5]. Her2-, ER/PR- and Her2-/ER-/PR- subtypes, and triple-negative indicate a lack of all these receptors [6–10]. The progression and development of breast cancer pathways are mediated by ER and human epidermal growth factor type-2 receptors (HER2/Neu) [11]. The activity of HER2 receptors, in turn, promotes the signaling of other pathways such as mitogen-activated protein kinase (MAPKs) or cell components such as glycogen synthase kinase-3 (GSK-3) and Phosphoinositide 3 kinase (PI3K)/AKT/mTOR pathways denoting the importance of signal integration and transduction processes in the progression and development of breast cancer [12–14].

The Human Genome Project (HGP) reported single nucleotide polymorphisms (SNPs) to be an essential factor in the development of different cancers [15–17]. Each SNP that has been known to date only has a small relative risk. They provide an accurate assessment of the breast cancer risk in

***Corresponding Author:** Agu Charles M, Histopathology Unit, Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, College of Health Sciences. Niger Delta University Wilberforce Island, Bayelsa State, Nigeria. PMB 71, Yenagoa Bayelsa State, **E-mail:** yoboma78@gmail.com
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the general population. It has been reported that at least 94 common breast risk SNPs are associated with breast cancer, with mutations in several genes such as BRCA1, BRCA2, CDH1, PIK3CA, and TP53 [18, 19]. PI3K is an important group of lipid kinases that regulates vital cellular functions such as survival, proliferation, cell growth, motility, differentiation, and intracellular trafficking [20]. Mutations in PIK3CA affect downstream pathways that cause dysregulation of the PI3K/AKT/mTOR signaling pathway and have been reported in various human cancers such as breast cancer [21]. About 27% of breast cancer patients have mutations in their genes. Mutations in PI3KCA are more common in luminal A subtype cancers, where they are detected in 45% of tumors, followed by HER2+ mutations with a frequency of 39%, luminal B represents 30% of cancers, and TNBC alterations appear in 9% of cases [22, 23].

METHODOLOGY

Study area

This study was conducted at the Histopathology Department, Niger Delta University Teaching Hospital (NDUTH), Okolobiri, Bayelsa State, Nigeria. Suburban community in the Yenagoa Local Government Area of Bayelsa State, Niger Delta region of Nigeria. NDUTH is a tertiary hospital that serves the entire State of Bayelsa and its neighboring communities. It is bounded by the Atlantic Ocean to the south of Nigeria. The state is the second largest producer of crude oil in Nigeria and has large gas reserves and oil wells.

Sample size determination and sample selection

The formula by Naing et al. (2006) was used to determine the sample size of this study. Therefore, by applying the formula, $N = Z^2pq/d2$, where N = the calculated sample size (for a population greater than 10,000), Z = the standard (alpha) normal deviate usually set 1.96, which corresponds to 95% confidence, p = disease prevalence in the population study; q = 1.0 - p, d = degree of accuracy (precision) desired, usually set at 0.05, and N = 187, based on a reported prevalence of 14.7%.

Study Design, ethical approval, inclusion and exclusion criteria Ethical approval with protocol number NDUTH/REC/2021/8132 was obtained from the ethics and research committee of Niger Delta University Teaching Hospital (NDUTH) Okolobiri Bayelsa State. A cross-sectional retrospective study design was adopted. Convenience sampling was used to select formalin-fixed paraffin-embedded breast tumor tissue blocks (2010–2020) from the histopathology archives of the center. All previously diagnosed breast tumor tissue blocks with adequate information such as patient age, clinical details, and available tissue masses or blocks were selected.

Laboratory procedure

Preparation of breast cancer tissues

The method of Bancroft and Gamble (2008) was adopted to prepare the breast tissues. The selected tissue blocks were sectioned into four (4) microns using a rotary microtome. Serial sections were placed in a water bath at temperature of 55°C for 1 minute. Floating sections were selected using labeled grease-free frosted end slides. Slides were subjected to immunohistochemistry, Ehrlich's hematoxylin and eosin staining, DNA extraction using extraction kits, and DNA amplification using a restriction fragment length Polymorphism Polymerase chain reaction.

DNA extraction from formalin fixed paraffin embedded tissues blocks

The sections were deparaffinized using two changes in xylene. The samples were scraped and transferred into 1.5 ml micro centrifuge tubes. Xylene (1 ml of xylene was added to the sample tube, vortexed, incubated at room temperature for 1 h with gentle rocking, and centrifuged at 10,000 × g for 1 minute. The supernatant was discarded and washed twice with descending grades of alcohol (absolute, 95%, and 75%) for 5 minutes, each with gentle rocking. The samples were washed with double-distilled water for 5 minute with gentle rocking and as much water as possible was removed using a micropipette. To the deparaffinized tissue samples in the microcentrifuge tube, 45 µL of water, 45 µL of 2x digestion buffers (pH 9.0) and 10 µL of proteinase K were added and incubated at 55°C for 4 hours. The microcentrifuge tubes were then transferred to an incubator at 94°C and incubated for 20 minutes. Then 5 µL of RNase A was added and incubated for an additional 5 minutes at room temperature, followed by the addition of 350 µL of genomic lysis buffer (pH 9.5) and thorough mixing by vortexing. The mixture was centrifuged at 10,000 × g for 1 minute to remove insoluble debris and the supernatant was transferred to a zymo-spinTM 11c column and centrifuged at 14,000 × g for 1 minute. The zymo-spinTM l llc was transferred to a clean microcentrifuge tube. After 50 µL DNA g dilution buffer was added to the tube, it was incubated for 3 minutes at room temperature, and centrifuged at 14,000 × g for 30 seconds to elude the DNA. The eluted DNA was stored at ≤ -20°Cc for use during molecular analysis.

Upstream and downstream flanking regions of rs2230461 were obtained from the National Center for Biotechnology Information (NCBI). Restriction fragment length polymorphism (RFLP) polymerase chain reaction (PCR) primers were designed using Oligo7 software. To avoid the formation of stable primer-dimers, the primers were accurately screened using NCBI/Primer-BLAST online software. 5′ ATAACCTTACCACCCCTT 3′ and 5′ AGCGGTATAATCAGGAGT 3′ were the forward and reverse primers, respectively. RFLP was performed using approximately 40 ng of genomic DNA, 2 pmol of each primer

and 7.5 µL of Ampliqon Taq DNA Polymerase Master Mix in a total volume of 15 µL. After performing the gradient temperature, the best-optimized condition was as follow: the initial denaturation step at 95°C for 5 minutes, 30 cycles of denaturation in 95°C for 30 seconds, annealing at 56°C for 30 seconds, elongation at 72°C for 30 seconds, followed by a final elongation step at 72°C for 5 minutes. The length of PCR product was 451 bp and separated by 2% agarose gel electrophoresis and stain staining. The 451 bp PCR products were digested using one unit of the BsrG1 restriction enzyme (Thermo Scientific, USA) at 37°C for 2 hours. The wild-type allele (Ile), which has no BsrG1 restriction enzyme site, was revealed as a single fragment of 451 bp and is indicative of the homozygous wild-type (AA) genotype, whereas the homozygous mutant genotype (GG) generated two fragments of 313 and 138 bp, and the heterozygous genotype (AG) contained all three fragments of 451, 313, and 138 bp. To confirm the accuracy of the genotype results, 20 samples, including 15 homozygotes and five heterozygotes, were randomly selected and re-genotyped using the same method.

Single Nucleotide Polymorphism (SNP) detection

Sequencing was performed using the Big Dye Terminator kit on a 3510ABI sequencer (Inqaba Biotechnological, Pretoria, South Africa). Sequencing was performed at a final volume of 10 µL, the components include 0.25 µL Big Dye Terminator v1.1/v3.1, 2.25 µL of 5 × Big Dye sequencing buffer, 10 µM Primer PCR, and 10 ng PCR template per 100 bp. The sequencing conditions were as follows: 32 cycles of 96°C for 10 seconds, 55°C for 5 seconds and 60°C for 4 minutes.

Hematoxylin and Eosin staining (Breast Tissues)

Ehrlich's hematoxylin and eosin staining was used for histological studies according to the method described by Bancroft and Gamble (2008). The slides were stained and analyzed to confirm the diagnosis. Histopathological evaluation was made on 4 µm thick sections stained with hematoxylin–eosin. The classification was based on the WHO criteria.

Immunohistochemistry staining for selected antibodies

The method of Oboma et al. [5] was used to prepare breast tissues, which were sectioned into four (4) microns using a rotary microtome, and the antigen on the FFPE tissue blocks was identified using the selected antibody. The antigen and antibody complexes were visualized using an enzyme (HRP)-coupled secondary antibody with specific binding to the primary antibody, which was visualized by enzymatic activation of the chromogen, resulting in visible reaction at the antigenic site. Each step involved precise timing and optimal temperature, and the results were interpreted using a light microscope. Heat retrieval under steam pressure for 15 minutes using EDTA (pH 8.0) for optimal epitope retrieval allowed the solution to cool to RT.. Endogenous peroxidase was blocked using 3% hydrogen peroxide for 15 minutes. The slides were incubated with the primary antibody at room temperature, followed by incubation with the secondary antibody. Color was developed using DAB chromogen for 10 minutes and counterstained with hematoxylin for 3 minutes. Brownish coloration was indicative of overexpression and was regarded as a positive result when compared to the control.

Statistical Analysis

The generated data were analyzed using the statistical software package GraphPad Prism 5 (SSPG) (GraphPad Software Inc., 2014). Data are presented as percentages.

RESULTS AND DISCUSSION

Breast cancer is the most frequently diagnosed cancer in females in developed countries, affecting one in eight women in the United States [25]. In total, 240 samples were collected during the study period. One hundred and eighty seven breast tissue blocks had proper documentation and preservation, while fifty eight (58), representing 24.2 % did not meet the standard and were excluded. Of the 187, 71 (37.9%) presented with evidence of malignancy and 116 (62.1%) were benign tumors.

Histopathological characteristic

The mean age (years) at presentation was 48 and 59.1% of the cases retrieved were aged < 50 years. Younger age at presentation was observed in the present study compared to the Western population, similar to previous studies in India and other countries [33–36]. Male patients accounted for 2.2% of total cases and 97.8% females, corroborating with previous studies [27–29]. Reports on outcomes in male gender breast cancer compared to female are conflicting, with some reporting similar or even better survival in male patients with breast cancer [30–32]. Ductal carcinoma was the most common histological type, accounting for 95.8% of all malignancies, whereas invasive lobular carcinoma (ILC) was less common (4.2%) of all invasive breast cancers, in accordance with previous reports [38]. The right breast lesions stood at 33.5%, left breast (34,5%), and bilateral was 26.7%. From an anatomical point of view, the left breast was more commonly involved than the right breast, which is in agreement with a previous study [37]. Fibroadenoma was the most common benign lesion in the present study, which is in agreement with a previous study [39], followed by fibrocystic, and incomplete patient documentation (see **Tables 1 and 2**).

Table 1. Descriptive statistics of breast lesions studied.

Variables	No. Observed	Percentage
Tumor type		
Malignant	71	37.9%
Benign	116	62.1%
Gender		
Male cases	4	2.2%
Female cases	183	97.8%
Histologic type		
Ductal carcinoma	68	95.8%
Lobular carcinoma	3	4.2%
Anatomical position		
Right breast	61	33.5%
Left breast	63	34.5%
Bilateral	5	26.7%
No documentation	58	31.0%

Table 2. Distribution of breast lesions based on age (years).

Age	Malignant(%)	Benign			
		Fibroadenoma	Fibrocystic	Phylloid	Others
≤15–29	7 (9.8%)	49 (77.7)	5 (25.0)	1 (50.0)	7 (21.8)
30–39	18 (25.3)	8 (12.6)	8 (40.0)	1 (50.0)	6 (18.7)
40–49	14 (19.7)	3 (4.7)	4 (20.0)	0 (0.0)	8 (25.0)
50–59	20 (28.2)	1 (1.5)	2 (10.0)	0 (0.0)	5 (15.6)
60–69	5 (7.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (12.5)
70–79	4 (5.6)	1 (1.5)	0 (0.0)	0 (0.0)	1 (3.1)
≥80	1 (1.4)	(0.0)	0 (0.0)	0 (0.0)	(0.0)
No Age	2 (2.8)	1 (1.5)	1 (5.0)	0 (0.0)	1 (3.1)
Total	71 (37.9)	63 (54.3)	20 (17.2)	2 (1.7)	32 (27.5)

Immunohistochemical characteristic

The immunohistochemical pattern of malignant breast lesions studied, as presented in **Table 3** and **Figure 1**, shows triple-negative breast cancer (37.6%)and epidermal growth factor 2 positive (13%); estrogen receptor positive breast cancer accounted for 50% of the cases studied, while progesterone receptor positivity was 31%. Receptors rich breast cancer accounted for 31%, and receptor poor breast cancer was 19% (see **Table 3**).

Table 3: Immunohistochemical patterns of breast cancer makers studied by age (years).

Age (years)	Triple-Neg	Her2+/Neu	ER+Receptor	PR+ Receptor	ER-	PR-
<30	0 (00)		0 (00)	0 (00)	0 (00)	0 (00)
31–40	4 (5.6%)	2(2.8)	11 (15.5%)	9 (12.7%)	4 (5.6%)	6
(8.5%)						
41–50	4 (5.6%)	0 (00)	2(2.8)	0 (00)	6 (8.5%)	8(11.3%)
51–60	2 (2.8%)	2 (2.8%)	0 (00)	0 (00)	4 (5.6%)	4 (5.6%)
>61	0 (00)	0 (00)	2 (2.8%)	0 (00)	0 (00)	1(1.3)
Total	10 (14%)	4 (2.8%)	14 (21.1%)	9 (12.7%)	14(19.7%)	19 (25.4%)

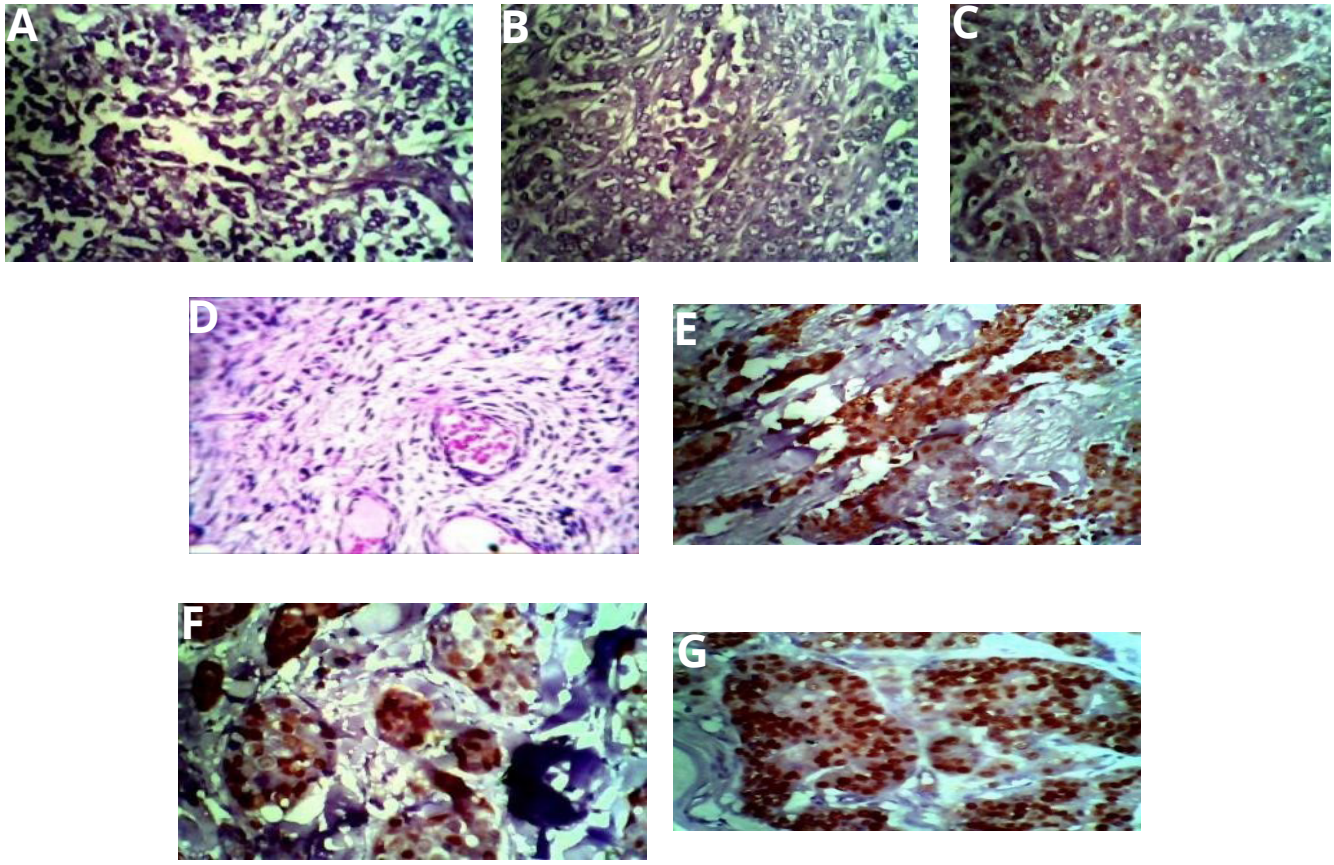

Figure 1. Immunohistochemical staining of breast tissue x400 magnification. (A) HER2-, (B) PR-, (C) ER-, (D) HA/EOSIN, (E) ER+, (F) PR+, (G) Neu2+

Triple-negative breast cancer occurrence among black and white populations has been reported [41]. The prevalence in the present study was low compared with that reported in other studies. A hospital-based study reported a higher prevalence of TNBC among Ghanaian women (79%) compared with African-American (32%) and White American women (10%) [42]. Differences in prevalence have been observed worldwide: 10–13% in Caucasian patients [43], 23–30% in African-American patients, 82% in Ghana [44], 39% in Saudi Arabia [45], 19.3% in Chinese mainland [46], and 15.9% in Taiwan, with 10–19.2% being Hispanic, which is much similar to the Japanese series (8–14%) and 31.7% in Abuja-Nigeria [47]. Variations in the incidence and prevalence of TNBC in women of African ancestry can be linked to differences in the methods of case ascertainment, population age structure, genetic and lifestyle risk factor distribution, access to mammography screening, overestimation of ER and PR negativity, poor or unreliable laboratory standards for tissue handling, type of fixation used, and initiation and duration of fixation. A Her-2/neu positivity rate of 12.5% was observed. Reports from other parts of Nigeria have shown values of 30.8% , 22.0%, 11.4%, and 20.8% for Lagos [48], Maiduguri [49], Nnewi [50], and Benin [51], respectively. The differences in prevalence may be due to variations in protocols, pre-analytical variables, and use of automated equipment and tissue fixation. It is noteworthy that, Seshie et al. reported 25.5% in a retrospective analysis of breast cancer subtypes performed at Korle Bu Teaching Hospital, Ghana, West Africa [52]. A study by Yau et al. [53] also reported Her-2/neu expression in 21.0% of breast cancer cases seen in Hong Kong, while Mahyari et al. [54] observed 38% of Iranian women had early stage breast cancer. A comparative multicenter study that ensures a uniform protocol and minimal analytical variation is necessary to explain these observed variations. A positive ER/PR status has been associated with decreased breast cancer mortality, independent of various demographic factors and clinical tumor characteristics [55]. The predictive value of PR positivity in the absence of ER is controversial, with some reports suggesting that positive PR breast cancer, even in the absence of ER, is more responsive to hormonal therapy but lacks universal findings [56].

In our study, information on receptor status gave 35 (50%) ER positive cases, 22 (31.3%) cases were PR positive, 22 (31.3%) cases were both ER- and PR positive, 31 (44%) cases were both ER- and PR negative, 13 (19%) cases were ER positive and PR negative, and three (2.9%) cases were ER- and PR positive. Therefore, our patients showed much better receptor positivity as compared with studies conducted in the rest of Asia [57–59], where positivity for ER and PR ranges from as little as 28% to a maximum of 75% .

Table 4. P:1K3Ca rs2699887 genotype and breast cancer population studied.

Age (years)	GG	GA	AA
<30	1 (4%)	0 (00)	0 (00)
31–40	12 (48%)	0 (00)	0 (00)
41–50	4 (16%)	2 (8%)	0 (00)
51–60	3 (12%)	0 (00)	0 (00)
: >60	2 (8%)	1 (4%)	0 (00)
Total	22 (88%)	3 (12%)	0 (00)

Molecular characteristic

The Pik3Ca rs2699887 genotype distribution in breast cancer varied slightly across studies, but the general trend was consistent. The GG genotype was the most common, followed by GA and AA genotypes. The current reported 88% GG, 12% GA, and 0% AA in all age groups studied. The results of this study are in agreement with previous studies [60–62]. All reported GG genotypes were the predominant genotypes among the breast cancer populations studied.

CONCLUSION

The study observed that there was high prevalence of triple negative breast cancer; and individuals harboring GG/GA genotype were prone to triple-negative breast cancer risk across age groups. A relationship exists between P1K3Ca rs2699887 and triple-negative breast cancer. Also,the effect of the GG/GA allele on disease risk was observed and linked to tumor aggressiveness.The limitation of the study is that the number of breast tumor samples used for the study were few due to lack of funding; we therefore recommend a larger sample size with adequate funding.

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