RESEARCH ARTICLE

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Pattern of Somatic Mutations in the *PIK3CA* Oncogene and Their Role as a Potential Prognostic Biomarker in Breast Cancer Patients in Sri Lanka: A Pilot Study

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Abstract

Background: Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit-alpha *(PIK3CA)* oncogene is one of the most frequently mutated oncogenes in breast cancer, with mutations influencing prognosis and therapeutic response. This study aimed to determine the pattern of hotspot *PIK3CA* mutations and assess their association with clinicopathological parameters and relapse-free survival (RFS) among Sri Lankan breast cancer patients. **Materials & Methods:** A qPCR-based genetic analysis was performed on DNA from formalin-fixed, paraffin-embedded (FFPE) tissue samples of 63 clinically diagnosed female Sri Lankan breast cancer patients, using the QClamp® *PIK3CA* Mutation Detection Test to detect hotspot mutations in *PIK3CA* (i.e., H1047R, E545K, E542K), followed by statistical analysis. Patient samples and clinical data were fully anonymized, with no identifying information available to the authors at any point during the study. **Results:** Somatic missense *PIK3CA* mutations H1047R and E542K were detected in 17.46% of the cohort. The E545K mutation was not detected. The observed mutations were associated with an increased risk of lymph node (LN) metastasis (p=0.036, OR 9.60) and reduced recurrence-free survival (RFS) (p<0.001, HR 26.19). Patients with a high Ki67 index (p=0.029, HR 79.69) and LN-positive status (p=0.026, HR 123.94) also showed worse outcomes. In addition, the combination of all three factors- presence of a *PIK3CA* mutation, LN metastasis, and a high Ki67 index- was associated with reduced RFS (p<0.001). **Conclusion:** Despite being a pilot study, the findings indicate that *PIK3CA* mutations are associated with adverse prognostic outcomes in Sri Lankan breast cancer patients. These results demonstrate the potential utility of *PIK3CA* testing and PI3K-targeted therapy in clinical management in Sri Lankan, pending validation in larger cohorts.

Keywords: PI3K Pathway- South Asia- Breast Cancer- Somatic Mutation Profiling

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Introduction

Breast cancer is the most commonly diagnosed cancer in women worldwide [1]. Asian women, particularly those in developing countries, often have a poorer prognosis for breast cancer, with higher cancer grades and stages as well as an earlier onset of the disease [2].

Breast cancer has distinct molecular subtypes that are categorized based on the immunohistochemical (IHC) expression of steroid hormone receptors (SHR), including estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2/*ERBB2* or HER2/*neu*). These subtypes are Luminal A, Luminal B, HER2-positive (HER2+), and triple-negative breast cancer (TNBC) [1].

More than 85% of breast cancers are sporadic, primarily caused by somatic mutations [3]. These mutations in oncogenes (OGs) and tumor suppressor genes (TSGs) often initiate tumorigenesis [4]. Breast cancer tumorigenesis has been linked to mutations in several cell signaling pathways crucial for normal development such as the PI3K/AKT/mTOR pathway, commonly referred to as the PI3K pathway. Genetic hyperactivation of this pathway is one of the most common driver mechanisms in breast cancer, often through alterations to its components like p110α (encoded by the phosphatidylinositol-4,5-

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bisphosphate 3-kinase catalytic subunit alpha gene/ *PIK3CA*; NCBI Gene ID 5290), p110β, and p85α [1, 5, 6].

Approximately 30% of the mechanisms that over-activate PI3K signaling are due to somatic mutations in the *PIK3CA* oncogene [5], making it the most commonly mutated oncogene in breast cancer [6-8]. While *PIK3CA* mutations can occur across the p110α subunit, about 80% are caused by the three hotspot mutations clustered in two domains of p110α: E545K and E542K in the helical domain encoded by exon 9, and H1047R in the kinase domain encoded by exon 20. Among these, H1047R is the most frequently detected *PIK3CA* mutation, while E542K is the least prevalent [9].

Studies have shown that between 10 to 45% of all human breast cancers harbor *PIK3CA* driver mutations, with the exact percentage varying based on the studied population and ancestry [5-10]. Activating mutations of *PIK3CA* are reported in nearly 42% of SHR+/HER2-, 31% of HER2+ and 16% of TNBC tumors [5].

Importantly, the presence of *PIK3CA* mutations has been associated with poorer prognostic outcomes, especially in ER+ cases [11]. Numerous studies illustrate that *PIK3CA* mutations are linked with resistance to various therapies, including endocrine therapy, chemotherapy, radiotherapy, anti-HER2 therapy and immunotherapy. This resistance negatively affects all available treatment modalities for all breast cancer subtypes, with more pronounced effects in advanced and metastatic cases [5, 7-10, 12]. These mutations also promote cell division and inhibit apoptosis in TNBC cells, leading to resistance to their primary treatment option: chemotherapy [12]. In addition to their role in treatment resistance, accumulating evidence suggests a strong association between *PIK3CA* mutations and tumor recurrence, poor relapse/recurrence-free survival (RFS), and/or overall survival (OS), particularly in postoperative Asian patients with metastatic SHR+/HER2- breast cancers [9, 11, 13-15]. Despite extensive research, the overall relationship between *PIK3CA* mutations and the clinicopathological profile of breast cancer patients remains controversial, with conflicting findings across various patient populations, such as observed associations of *PIK3CA* mutations with longer RFS and/or OS [16, 17].

Recent advances in genomic studies done predominantly involving patients from European and African ancestries have provided significant insights into the relationship between genetic ancestry and somatic mutations in genes like *PIK3CA* across cancer types via changes in the germline [18-22]. However, Asian populations, particularly those of South Asian descent, remain highly underrepresented in these studies, limiting a comprehensive understanding of somatic mutations in the region [22].

Sri Lanka is a unique island nation in the Indian Ocean with a rich tapestry of unique ethnic diversity. Over 70% of the population is composed of Sinhalese, descendants of Indo-Aryan ancestry, found exclusively in Sri Lanka [23]. Other major ethnic groups include Tamils and Moors, who trace their roots to Dravidian ancestry, and Burghers, descendants of European colonists in Sri Lanka, who also share Indo-Aryan ancestry [23, 24]. This distinct combination of Indo-Aryan and Dravidian ancestries, together with its centralized geographical location, particularly during the maritime silk route, makes Sri Lanka a melting pot of genetic diversity.

Breast cancer is the most frequently diagnosed cancer among Sri Lankan women [25]. As a developing, lower-middle income island nation in the Indian Ocean, Sri Lanka has limited research on breast cancer trends and genetic markers, despite the aforementioned genetic and geographic distinctiveness as well as a significant rise in incidence and mortality rates over time [26, 25]. To the best of our knowledge, no studies have been conducted on the prevalence and prognostic outcomes of *PIK3CA* mutations in breast cancer patients of Sri Lanka. Given the mutational spectrum of *PIK3CA* and its impact on the clinicopathological profile and prognosis of breast cancer, which varies significantly depending on the population, it is important to elucidate the status of Sri Lankan breast cancer patients in this context - an area that remains largely unexplored.

This study was therefore designed to achieve the overarching aim of cataloguing hotspot mutations in the *PIK3CA* oncogene within a cross-section of Sri Lankan breast cancer patients. Using DNA extracted from tumor tissues obtained at the point of diagnosis, we aimed to correlate the detected mutations with specific clinicopathological characteristics and ultimately assess the prognostic potential of *PIK3CA* mutations using RFS as a key metric. Given that the Sinhalese population is found nowhere else in the world (except for few migrant individuals), results from this study offer a rare opportunity to explore how the genetic makeup in an island nation intersects with somatic mutations, such as those in the *PIK3CA* oncogene. This study managed to successfully catalog *PIK3CA* mutations in our cohort of Sri Lankan breast cancer patients and deduced their impacts on disease prognosis in a purely South Asian cohort of breast cancer patients, for the first time.

Materials and Methods

Patient samples and data acquisition

Ethical approval for the analysis of an existing set of formalin-fixed paraffin-embedded (FFPE) breast tumor biopsy samples and fully anonymized medical records of the relevant patients was obtained from the Ethics Review Committee of the Institute of Biology, Sri Lanka (ERC IOBSL 303 07 2023, Figure S1). This retrospective cohort study was conducted in compliance with the REMARK (Reporting Recommendations for Tumor Marker Prognostic Studies) and ICMJE (International Committee of Medical Journal Editors) guidelines. As no direct patient contact or intervention was involved and all data were anonymized prior to analysis, the requirement for individual informed consent was waived by the Ethics Review Committee. All procedures were conducted in accordance with the ethical standards of the institutional and national research committees and with the principles of the 1964 Declaration of Helsinki and its later amendments.

The fully anonymized database of breast cancer patient

records from the Diagnostics Laboratory at the University Hospital of General Sir John Kotelawala Defense University was accessed on the 27th of August 2023 where patient records from 2019 to 2023 were screened. Inclusion criteria comprised adult female patients with histologically confirmed primary invasive breast carcinoma, with adequate FFPE tumour tissue available for DNA extraction and molecular testing, and complete clinicopathological information. Patients were excluded if they had a history of recurrent or metastatic cancer at diagnosis, if the available tissue was insufficient or yielded poor-quality/degraded DNA, if essential clinical data were missing, or if they had received neoadjuvant chemotherapy or radiotherapy prior to tissue collection. The selection workflow to select eligible cases for both the profiling and survival study- showing screening, exclusions, and the final cohort size for each analysis, is shown in Figure 1.

As illustrated therein, data from sixty-six (n = 66) patients meeting the clinical inclusion criteria were used for the analysis of clinicopathological features and demographic characteristics. After DNA extraction and quality assessment, sixty-three (n = 63) samples yielded amplifiable DNA and were therefore included in the *PIK3CA* mutation profiling. Of these, forty-two (n = 42) patients had available follow-up data of up to five years post-diagnosis and were consequently included in the relapse-free survival (RFS) analysis. The corresponding FFPE blocks were retrieved from the hospital's diagnostics laboratory and stored at room temperature until further analysis. Clinicopathological data at diagnosis and follow-up information collected every 3-6 months for 1 to 5 years post-diagnosis were collected from the hospital database.

To ensure patient anonymity, medical and follow-up data of individual patients were recorded, and the samples were identified exclusively using identification numbers assigned to each sample for the purpose of research. Therefore, as authors did not have access to information that could identify individual participants during or after data collection, total anonymity of patient identity was maintained throughout the study.

DNA extraction and hotspot mutation assay

Genomic DNA was extracted from FFPE samples using the QIAamp DNA FFPE Tissue Kit according to the manufacturer's instructions (Cat. No. 56404; Qiagen, Hilden, Germany) and assessed for quality and quantity using the Thermo ScientificTM NanoDropTM One Spectrophotometer. The three hotspot mutations of the *PIK3CA* oncogene- H1047R, E545K, and E542K- were genotyped in 63 of the selected samples using the QClamp® *PIK3CA* Mutation Detection Test Kit (Cat. No. DC-10-1072R; DiaCarta, CA 94588, USA), which utilized a Xeno-nucleic acid (XNA)-based mutation-specific quantitative real-time polymerase chain reaction (qPCR) method.

Endpoint and statistical analysis

The prevalence of *PIK3CA* mutations in the study cohort and their associations with clinicopathological features were determined using the $\chi 2$ test or Fisher's exact test as appropriate. For the clinicopathological parameters, patients were stratified into two groups for each variable based on clinically meaningful and widely accepted cut-off values in oncology (Table S1). For parameters with missing data, thresholds were adjusted to account for the available sample size within each category while maintaining clinical relevance, thereby ensuring statistical robustness and minimizing the risk of Type I errors. In terms of ancestry, patients were grouped into Indo-Aryan ancestry (Sinhalese & Burghers) and Dravidian ancestry (Tamils & Moors). Depending on data normality, either a t-test or Mann-Whitney Test was performed to compare the mean or median of populations for the relevant variables. For specific variables showing significant associations, binary logistic regression was used to obtain the odds ratio (OR) and the 95% confidence interval (CI). This binary logistic regression model assumed that there were no significant multicollinearity issues, and all independent variables were linearly related to the log-odds of the dependent variable.

As all patients with survival data (n=42) were alive at the last follow-up, RFS was used as the survival metric instead of OS, and was defined as the time from the date of curative surgery to the clinical diagnosis of recurrence. Events considered in RFS included distant and local invasive recurrence. Patients without survival data were excluded from the survival analysis. The median follow-

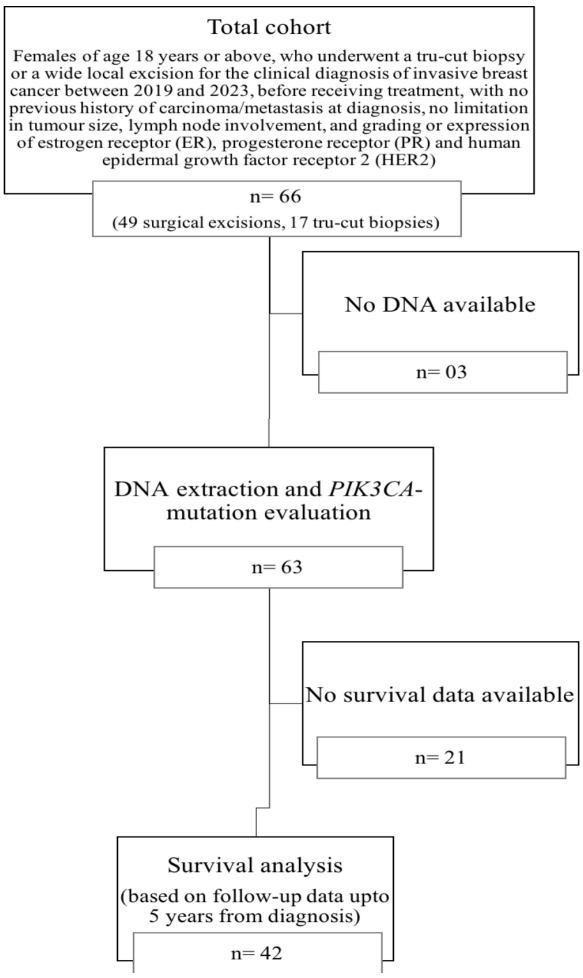

Figure 1. Flowchart of Patient Enrollment and Study Workflow

up time after diagnosis was 37.00 months (0.00–81.19).

The Kaplan-Meier method was used to plot RFS, while the Mantel-Cox log-rank test was applied to compare different subgroups of the variables. Significant parameters identified by the log-rank test ($p<0.05$) were further analyzed using a univariate Cox proportional hazard regression model to estimate hazard ratios and 95% confidence intervals. Finally, a multivariate model was used to analyze the combined effect of all significant associations on RFS. However, for parameters which could not generate a statistically robust Cox proportional hazard regression model due to limited data on survival, only the p-value was reported.

Samples with missing data for specific variables were excluded from corresponding analyses. All statistical tests were two-sided and a p-value below 0.05 was considered statistically significant. Analyses were performed using SPSS 25 (Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) and R (version 4.0.2, R Foundation for Statistical Computing, Vienna, Austria).

Results

Analysis of the clinicopathological features

The total studied cohort consisted of 66 patients clinically diagnosed with breast cancer between 2019 and 2023. The median age at the time of diagnosis was 58.00 years, with a range of 39 to 86 years (Figure S2).

Table 1 presents several key characteristics of the cohort. Many of the patients (nearly 60%) were diagnosed between 46 and 65 years of age. IHC analysis revealed that over 80% of the tumours were steroid hormone receptor (SHR)-positive and HER2-negative luminal subtypes, with Luminal A and Luminal B subtypes occurring in equal proportions. Over 95% of the cohort comprised of Sinhalese patients while the remaining 5% included two Tamil patients, one Moor, and one Burgher patient. This represents only a rough approximation of the actual ethnic distribution in Sri Lanka, where approximately 72% of the population are Sinhalese [23].

Most tumours were invasive breast carcinomas of no special type (IBC-NST; 86.4%) and were moderately differentiated (Grade 2; 56.1%), while 33.3% were well differentiated (Grade 1). Regarding tumour size, over 55% measured > 2 cm (Figure S3), and the majority were staged as pT2 at diagnosis, consistent with Stage II disease. Lymph-node (LN) metastasis was present in 31.8% of patients, whereas 43.9% were LN-negative. In terms of proliferative activity, 42.4% exhibited a high Ki67 index (≥ 20%).

Prevalence of PIK3CA mutations in the cohort

Sixty three (n=63) patients (95.5% of the total cohort) were successfully analyzed for their *PIK3CA* mutation status, where missense mutations were detected in 17.46% (n=11) of the cohort. The mutations observed included H1047R (c.3140A>G) in 81.81% (n=9) and E542K (c.1624G>A) in 18.18% (n=2) of the mutated samples. Notably, both H1047R and E542K mutations were detected in one sample (co-mutation prevalence: 9.09% of the mutated and 1.58% of the total samples). No E545K mutations (c.1633G>A) were detected in the cohort.

Association of PIK3CA mutations with clinicopathological parameters

All *PIK3CA* mutations identified were found in SHR+/HER2- luminal tumors (Table 2). Importantly, as shown in Table 2, a significant association was observed between *PIK3CA* mutational status and lymph node (LN) metastasis, with patients harboring a *PIK3CA* mutation exhibiting 9.60 times higher odds of LN metastasis compared to those without a *PIK3CA* mutation ($p=0.036$, OR 9.60, 95% CI 1.05-87.78). An association was also observed between the ancestry of the patients and the presence of a *PIK3CA* mutation ($p=0.028$). Other parameters showed no significant association with the mutational status.

Associations among other clinical and histopathological parameters

Table S2 outlines the associations between the Ki67 proliferation index and other clinicopathological features. As shown in Table S2, a significant association was identified between Ki67 status and tumor grade, with higher Ki67 proliferative indices being linked to a 4.29 times higher likelihood of developing moderately or poorly differentiated breast cancers of higher tumor grades, compared to those with proliferative indices < 20% ($p=0.024$, OR 4.29, 95% CI 1.28-14.41).

No statistically significant associations ($p>0.05$) were observed between LN metastasis or patient age group and other clinicopathological parameters (Table S3, S4).

Impact of PIK3CA mutational status on RFS

Survival data was available only for 42 (63.6% of the total cohort) patients. Among them, 9.5% (n=4) experienced a relapse in a different organ (e.g., pelvic bone and lungs) following curative surgery. Three of these patients (75.0%) had *PIK3CA* mutations, with two harboring the H1047R mutation (50%) and one carrying the E542K mutation (25%).

The presence of a *PIK3CA* mutation was thereby significantly associated with reduced RFS ($p<0.001$), as shown in the Kaplan-Meier curve (Figure 2A and Table 3). Patients with a *PIK3CA* mutation had 26.19 times higher odds of relapse compared to the those without a mutation (HR 26.19, 95% CI 2.67-256.47). The mean RFS for patients with *PIK3CA* mutations was 26.5 months (Table 3).

However, no significant association ($p>0.05$) was found between individual *PIK3CA* hotspot mutations (H1047R or E542K) and RFS.

Impact of selected clinicopathological features on RFS

As shown in Table 4, out of the 42 patients with available survival data, LN metastasis information was available for 34 (81.0%). Among them, LN metastasis was significantly associated with RFS. Patients with LN-positive status had 123.94 times higher hazard of relapse compared to LN-negative patients ($p=0.026$, HR 123.94; Figure 2B). Additionally, a significant association was

Table 1. Clinicopathological and Population Characteristics of the Cohort

Age at diagnosis	No. of patients	%
<35	0	0.00
36-45	5	7.58
46-55	20	30.30
56-65	19	28.79
66-75	15	22.73
76-85	6	9.09
>85	1	1.52
Side	No. of patients	%
L/S	30	45.45
R/S	36	54.55
Site of the tumor	No. of patients	%
Upper outer quadrant	2	3.03
Upper inner quadrant	14	21.21
Lower inner quadrant	0	0.00
Lower outer quadrant	3	4.55
Central	5	7.58
Clock Positions	22	33.33
Two quadrants	2	3.03
Unknown	18	27.27
Histological type	No. of patients	%
IBC-NST	57	86.36
Invasive mucinous carcinoma	4	6.06
Invasive lobular carcinoma	2	3.03
Invasive papillary carcinoma	1	1.52
Invasive tubular carcinoma	1	1.52
DCIS	1	1.52
Tumor grade/ differentiation	No. of patients	%
Grade 1/ well-differentiated	22	33.34
Grade 2/ moderately differentiated	37	56.06
Grade 3/ poorly differentiated	5	7.57
Unknown	2	3.03
Tumor size	No. of patients	%
≤2 cm	12	18.18
>2 cm	37	56.06
Unknown	17	25.76
Pathological tumor stage	No. of patients	%
Tis	1	1.52
pT1	10	15.15
pT2	31	46.97
pT3	6	9.09
Unknown	18	27.27
Pathological nodal stage	No. of patients	%
pN0	29	43.94
pN1	13	19.70
pN2	6	9.09
pN3	2	3.03
Unknown	16	24.24
Lymph node metastasis	No. of patients	%
Positive	21	31.82
Negative	29	43.94
Unknown	16	24.24

Table 1. Continued

ER status	No. of patients	%
ER+	60	90.91
ER-	6	9.09
PR status	No. of patients	%
PR+	46	69.70
PR-	20	30.30
SHR status	No. of patients	%
SHR+	60	90.91
SHR-	6	9.09
HER2 status	No. of patients	%
HER2+	6	9.09
HER2-	59	89.39
Unknown	1	1.52
Receptor based subtype	No. of patients	%
SHR+/HER2-	55	83.34
SHR+/HER2+	4	6.06
SHR-/HER2+	2	3.03
SHR-/HER2-	4	6.06
Unknown	1	1.51
Ki67 Proliferation index	No. of patients	%
<20% (low)	30	45.45
≥20% (high)	28	42.42
Unknown	8	12.12
IHC-based molecular subtype	No. of patients	%
Luminal A	24	36.36
Luminal B	24	36.36
Luminal (undetermined for A/B as Ki67 data unavailable)	7	10.60
HER2 enriched	6	9.09
TNBC	4	6.06
HER2 unknown	1	1.51
Ethnicity	No. of patients	%
Sinhalese	63	95.45
Tamil	1	1.51
Moor	1	1.51
Burgher	1	1.51
Ancestry	No. of patients	%
Indo-Aryan	64	96.96
Dravidian	2	3.03

DCIS, ductal carcinoma in-situ; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; IBC-NST, invasive breast carcinoma of no special type; IHC, immunohistochemical; L/S, left side; pN, pathological nodal stage; PR, progesterone receptor; pT, pathological tumor stage; R/S, Right side; SHR, steroid hormone receptor; Tis, carcinoma in-situ stage; TNBC, triple-negative breast cancer

observed between Ki67 proliferative status and RFS (N=38, 90.5%), with patients having higher proliferative indices facing a 79.69 times higher hazard of relapse compared to those with Ki67 indices <20% (p=0.029, HR 79.69; Figure 2C). No other clinicopathological characteristics showed significant associations with RFS.

Finally, the multivariate Cox proportional hazard model (Figure 2D) demonstrated a significant association

Table 2. Distribution of *PIK3CA* Mutational Status According to Age and Clinicopathological Characteristics

Parameter	Mutations absent (n)	PIK3CA Mutated (n)	Total (n)	p-value
All	52	11	63	
Age at diagnosis (N=63; 100%)				
≤50 years	14	2	16	
>50 years	38	9	47	0.714
Histological type (N=63; 100%)				
IBC-NST	45	10	55	
Other	7	1	8	1.000
Tumor grade/ differentiation (N=61; 96.8%)				
G1/ well-differentiated	17	4	21	
G2 or G3/ moderately or poorly differentiated	33	7	40	1.000
Tumor size (N=46; 73.0%)				
≤2 cm	9	2	11	
>2 cm	30	5	35	1.000
Mean (cm) (95% CI)	3.5 (2.8-4.2)	3.2 (1.7-4.7)		
Median (cm) (Range)	3.2 (0.7-12.0)	3 (1.1-6.0)		0.994
Pathological tumor stage (N=45; 71.4%)				
Early stage (pT1/pT2)	32	6	38	
Late stage (pT3/pT4)	6	1	7	1.000
Lymph node Metastasis (N=46; 73.0%)				
Negative	24	1	25	
Positive	15	6	21	*0.036
ER status (N=63; 100%)				
ER+	47	11	58	
ER-	5	0	5	0.576
PR status (N=63; 100%)				
PR+	37	7	44	
PR-	15	4	19	0.721
SHR status (N=63; 100%)				
SHR+	47	11	58	
SHR-	5	0	5	0.576
HER2 status (N=62; 98.4%)				
HER2+	6	0	6	
HER2-	45	11	56	0.580
Receptor based subtype (N=63; 100%)				
SHR+/HER2-	42	11	53	
Other	10	0	10	0.187
Ki67 Proliferation index (N=55; 87.3%)				
<20%	23	5	28	
≥20%	22	5	27	1.000
IHC-based molecular subtype (N=62; 98.4%)				
Luminal	42	11	53	
HER2/TNBC	9	0	9	0.341
Ancestry (N=63; 100%)				
Indo-Aryan	52	9	61	
Dravidian	0	2	2	*0.028

*, association is significant at 5% level; CI, confidence interval; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; IBC-NST, invasive breast carcinoma of no special type; IHC, immunohistochemical; LN, Lymph Node; N, number of samples analysed; a percentage of the total samples in the mutation analysis; OR, odds ratio; PR, progesterone receptor; pT, pathological tumor stage; SHR, steroid hormone receptor; TNBC, triple-negative breast cancer

Table 3. Impact of the Overall and Specific *PIK3CA* Mutation Status on Relapse-Free Survival (RFS)

	p-value	Hazard Ratio Exp (B)	95% CI	
Association between *PIK3CA* mutation & RFS	**<0.001	26.187	2.674 - 256.475	
	PIK3CA mutated	*PIK3CA* not mutated		
Mean RFS (months)	26.5	54.5		
95% CI of mean	13.99 - 39.01	51.12 - 57.84		
Median RFS in months (95% CI)	37 (0.00-81.91)			
PIK3CA Mutation	Relapse (n)	No relapse (n)	Total (n)	p-value
H1047R	2	2	4	
Other	1	1	2	
Total	3	3	6	0.281

**, Association is significant at 1% level; CI, confidence interval; RFS, Relapse-free survival; "Other" includes the E542K mutation and the H1047R/E542K co-mutation

between the presence of a *PIK3CA* mutation, LN metastasis and a high Ki67 index with RFS (p<0.001).

Discussion

This study, to our knowledge, is the first to profile

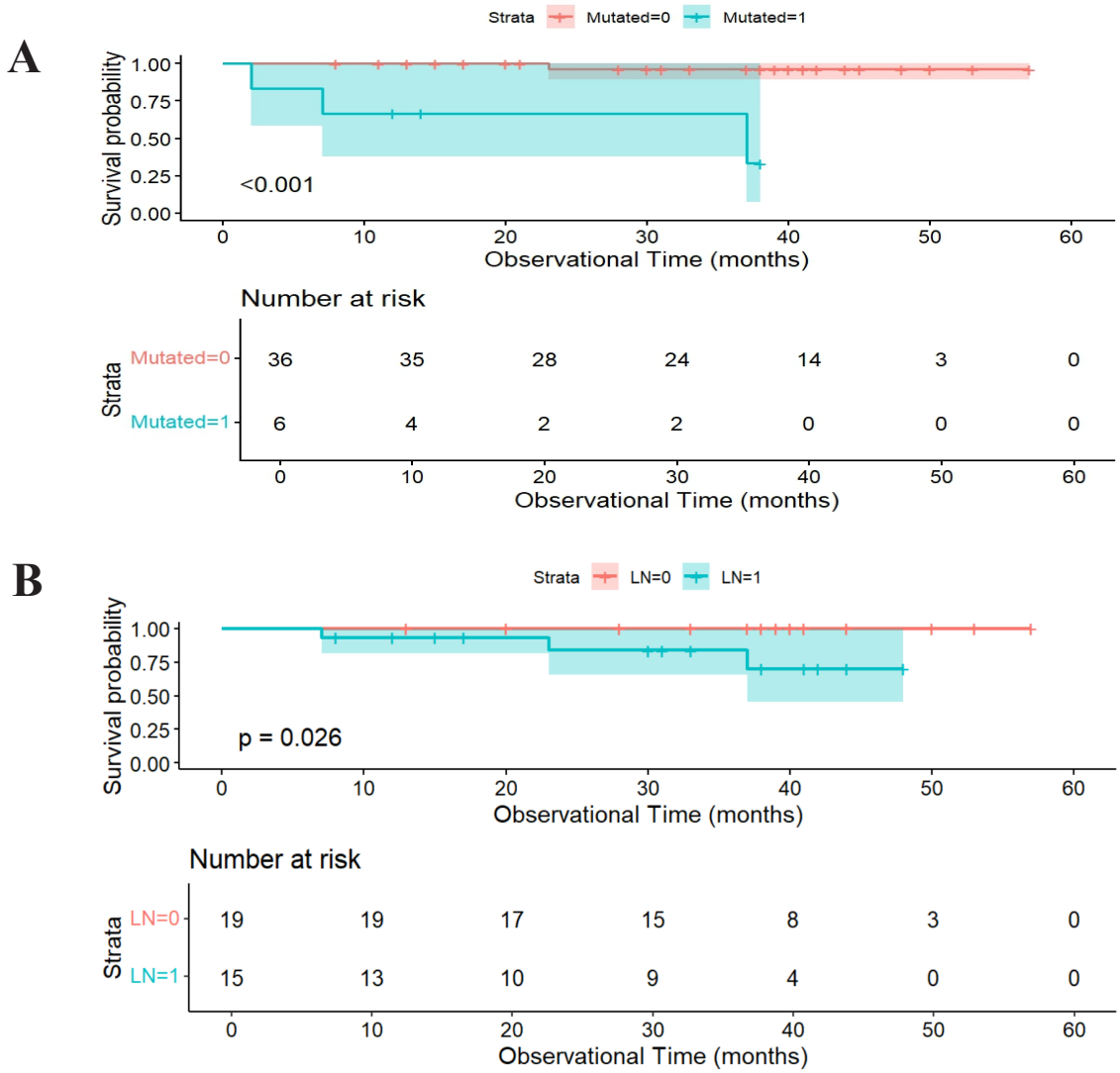

Figure 2. Kaplan-Meier Survival Estimates for Relapse-Free Survival (RFS) Stratified by *PIK3CA* Mutation Status (A), status of lymph node (LN) metastasis (B) and Ki67 proliferative status (C) while (D) shows the multivariate impact of the above three factors - PIK3CA mutation, LN metastasis and Ki67 index on RFS. A: p-value<0.001, HR 26.187, 95% CI 2.674 - 256.475. B: p-value=0.026, HR 123.939, 95% CI could not be generated with statistical robustness due to small sample size. HR: hazard ratio, CI: confidence interval

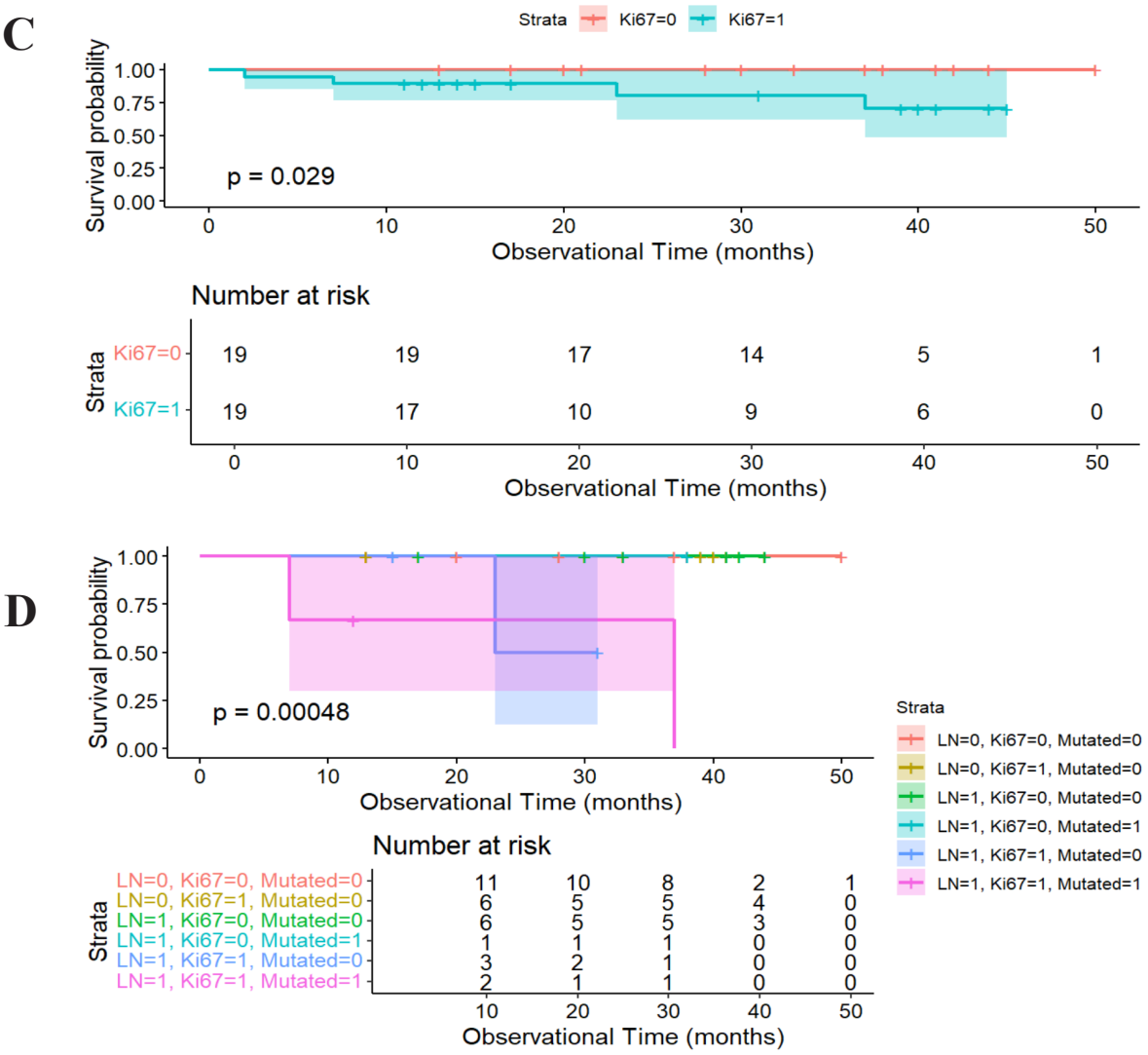

Figure 2. Continued

PIK3CA mutations and to assess their prognostic value in Sri Lankan breast cancer patients. Despite being a pilot study conducted in an underrepresented island population that is genetically and geographically distinct, our findings lay the groundwork for future research, including meta-analyses in larger cohorts.

The analysis of the clinicopathological parameters showed a median age of 58 years at breast cancer diagnosis, higher than the median age reported in a Sri Lankan study done over a decade ago, and higher than those reported from several Southeast Asian countries and Arab countries [26-29]. This could be due to Sri Lanka having one of the fastest ageing populations in the world [26]. The exponentially growing population of older women [26] would thus raise the incidence of breast cancer among this demographic, making it a key factor in the observed age shift in diagnosis. However, the fact that most patients were already at stage 2 at the time of diagnosis, even with slow-growing cancers (i.e., luminal A and B), suggests a much earlier onset of the disease. This trend, consistent with other Asian countries, could probably be attributed to similar genetic and sociocultural factors and contrasts with developed Western countries, where median age of diagnosis occurs much later [30].

Like reports by previous studies conducted in Sri Lanka [31, 32], it is noticeable that many of the tumors in our cohort were invasive breast carcinomas (IBC-NST) of tumor grade 2 and/or stage II at diagnosis. This could be likely due to the absence of an established screening program aimed at early detection in Sri Lanka, thus reducing the likelihood of the cancer being detected at stage I and worsening disease outcomes [31]. These observations therefore highlight the need for the implementation of an effective screening system for breast cancer in Sri Lanka, to facilitate early diagnosis and ultimately reduce the overall burden of breast cancer in the country. Consistent with both local and global statistics [31-33], most tumors were ER/PR+ and HER2-, confirming that SHR+/HER2- luminal subtypes are the most common in Sri Lanka.

One of the study's primary objectives was to catalog *PIK3CA* mutations in the cohort of patients and to detect associations with clinicopathological features. The detection of H1047R and E542K mutations in 17.46% of cases falls near the prevalence range of *PIK3CA* mutations reported in many global studies on breast cancer from

Table 4. Univariate Analysis of Relapse-Free Survival (RFS) with Selected Clinicopathological Parameters

Parameter	Relapse (n)	No relapse (n)	Total	p-value
All	4	38	42	
Age at diagnosis (N=42; 100%)				
≤50 years	1	10	11	
>50 years	3	28	31	0.772
Histological type (N=42; 100%)				
IBC-NST	3	31	34	
Others	1	7	8	0.701
Tumor grade/ differentiation (N=40; 95.2%)				
G1/ well-differentiated	1	13	14	
G2 or G3/ moderately or poorly differentiated	3	23	26	0.717
Tumor size (N=33; 78.6%)				
≤ 2 cm	1	9	10	
>2 cm	2	21	23	0.941
Mean (cm)	3.164			0.830
Pathological tumor stage (N=32; 76.2%)				
Early stage (pT1/pT2)	3	26	29	
Late stage (pT3/pT4)	0	3	3	0.585
Lymph node metastasis (N=34; 81.0%)				
Negative	0	19	19	
Positive	3	12	15	*0.026
ER status (N=42; 100%)				
ER+	4	37	41	
ER-	0	1	1	0.769
PR status (N=42; 100%)				
PR+	3	30	33	
PR-	1	8	9	0.805
SHR status (N=42; 100%)				
SHR+	4	37	41	
SHR-	0	1	1	0.769
HER2 status (N=41; 97.6%)				
HER2+	0	3	3	
HER2-	4	34	38	0.529
Receptor based subtype (N=42; 100%)				
SHR+/HER2-	4	33	37	
Other	0	5	5	0.417
Ki67 Proliferation index (N=38; 90.5%)				
<20%	0	19	19	
≥20%	4	15	19	▲ 0.029
IHC-based molecular subtype (N=41; 97.6%)				
Luminal A/B	4	33	37	
HER2/TNBC	0	4	4	0.478

*, association is significant at 5% level, HR 123.929, A statistically robust 95% CI not generated due to small sample number; ▲, association is significant at 5% level, HR 79.688, A statistically robust 95% CI not generated due to small sample number; CI, confidence interval; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; IBC-NST, invasive breast carcinoma of no special type; IHC, immunohistochemical; LN, Lymph Node; N, number of samples analysed; a percentage of the total samples in the survival analysis; PR, progesterone receptor; pT, pathological tumor stage; SHR, steroid hormone receptor; TNBC, triple-negative breast cancer

USA, Spain, Germany, China, etc. [5-10]. Notably, this study also provides the first evidence of *PIK3CA* mutations in Sri Lankan breast cancer patients. India, the closest nation to Sri Lanka both geographically and genetically, reports a *PIK3CA* mutation prevalence of 23.2% [34], closely aligned with our findings. This similarity likely reflects common hereditary factors shaped by historical migrations and cultural exchanges, which may have

influenced the prevalence of *PIK3CA* mutations in the South Asian region.

Although the predominance of H1047R as the most frequently observed *PIK3CA* mutation mirrors observations in other studies [35], our study reports a much a higher frequency of 81.81% for H1047R among the total *PIK3CA* mutations, compared to the frequency (≈58%) reported in other Western and Asian populations such as in Brazil and Taiwan [35, 36]. A particularly rare and unexpected observation was the absence of the E545K mutation in our cohort, which is commonly reported as a moderately prevalent *PIK3CA* mutation (≈10%) in many other global populations [37, 38]. This observation aligns with findings from an Iranian study, where the E545K mutation was similarly absent [39]. Given that over 96% of our cohort comprises Sri Lankan Sinhalese and Sri Lankan Burghers (both of Indo-Aryan ancestry), this shared absence is likely to be stemming from a common ancestral link with Iranians, as both Iranians and Indo-Aryans are descendants of the Indo-Iranian branch of the Indo-European language family [23, 24, 40]. The absence of the E545K mutation, may thus be tied to ancestral roots, and consequent genetic backgrounds, among other possible factors. This provides valuable preliminary data from Sri Lanka, contributing to the global effort of cataloging *PIK3CA* mutations, which are highly heterogeneous across populations with different ancestral origins [10].

The observed association between patient ancestry and the presence of a *PIK3CA* mutation ($p = 0.028$) emerged as a secondary finding of this study and was not a predefined objective. While this preliminary association- suggesting potential differences in *PIK3CA* mutation frequencies between individuals of Indo-Aryan and Dravidian descent- echoes global evidence that links somatic mutations to genetic ancestry [19-22], it must be interpreted with caution. Notably, the study cohort was not stratified based on ancestry during participant recruitment, and certain ethnic groups, such as those of Dravidian origin, were markedly underrepresented (with only one Tamil and one Moor included). As such, the statistical power to detect robust ancestry-based associations is limited, and the observed relationship cannot be regarded as a definitive scientific conclusion. Nonetheless, the signal warrants further investigation. Sri Lanka's genetically distinct population from other highly studied Caucasian populations, shaped by both Indo-Aryan and Dravidian lineages, offers a valuable context for future studies focused explicitly on ancestry-driven mutational patterns. Larger, purposefully designed studies with comprehensive documentation of genetic ancestry and adequate representation across ethnic groups are needed to validate and expand on this finding. Such research could ultimately inform more ancestry-specific interpretations of mutation profiles, with implications for personalized cancer diagnostics and therapeutic strategies in South Asian populations.

Another notable observation was that the *PIK3CA* mutations in the cohort were exclusively seen in SHR+/HER2- luminal tumors, consistent with a similar subtype-specific dominance of the mutation in previously mentioned populations [9]. The molecular mechanisms, including cross talk between the PI3K/AKT/mTOR and ER signaling pathways are postulated to be mediating this, with *PIK3CA* mutations driving oncogenesis via ER signaling, thereby predominating in SHR+ breast cancer subtypes [17].

Like observations in some Western populations [41], the significant association between *PIK3CA* mutations and increased likelihood of axillary LN metastasis ($p=0.036$, OR 9.60) highlights the potential prognostic value of *PIK3CA* mutations in predicting breast cancer progression in the Sri Lankan context. LN metastasis typically indicates a more advanced disease stage, a higher risk of distant metastases and worse outcomes [42]. Therefore, *PIK3CA* mutation testing could guide therapeutic strategies, particularly in decisions related to axillary management and risk assessment, with more intensive therapeutic approaches needed by patients with tumor-positive axillary LNs [43]. Divergent findings such as an association between *PIK3CA* mutations and lymph node negativity in an American cohort [44], may reflect differences in genetic makeup and lifestyle factors, further emphasizing the need for nuanced molecular investigations in underrepresented populations like Sri Lanka.

The absence of associations between clinicopathological features and age group may indicate that other parameters, such as histological grade and Ki67 index, hold greater prognostic value than age, particularly in early-stage breast cancer cohorts [45], like ours. This is supported by the significant association between higher Ki67 indices and an increased risk of developing higher-grade tumors ($p=0.024$, OR 4.29). These findings further support the use of Ki67 index as a valuable prognostic marker [46] in breast cancer patients and its importance in clinical decision-making.

The striking association between *PIK3CA* mutations and reduced RFS in Sri Lankan breast cancer patients, with high statistical significance ($p<0.001$, HR 26.19, 95% CI 2.67 – 256.47) suggests that these mutations have a considerable impact on disease prognosis. The broad confidence interval reflects the small sample size, but even the lower bound presents a significantly increased risk of relapse, nearly three times greater than patients without a *PIK3CA* mutation. This implies a significant impact of *PIK3CA* mutations on disease progression and brings out its potential clinical relevance as a prognostic biomarker in Sri Lankan breast cancer patients. As the first study to analyze *PIK3CA* mutations in Sri Lankan breast cancer patients, our findings are consistent with results from other Asian studies as well as studies on other malignancies, such as colorectal cancer [13, 47, 48].

However, our results did not show mutation-specific associations with RFS, which may be attributed to the smaller cohort size, necessitating further investigation with larger studies. Nevertheless, the predominance of the H1047R mutation- with an observed frequency of 0.81 within the total mutations, suggests that it is likely to be responsible for reducing RFS [35] in our cohort, mirroring findings of the aforementioned Iranian study [39]. Given the shared Indo-Iranian ancestry of the

Sinhalese and Iranians, this parallel may hint at ancestral genetic traits influencing both mutation occurrence and its clinical impact. This further emphasizes the need for nuanced investigations in populations with specific ancestral backgrounds.

Our analysis of clinicopathological characteristics also revealed that LN metastasis and Ki67 proliferative index are key determinants of relapse risk. Patients with either tumor-positive LNs or a higher Ki67 index (≥20%), exhibited a significantly greater hazard of relapse compared to their counterparts without tumor deposits in LNs (p=0.026, HR 123.93) and with lower proliferative indices (p=0.029, HR 79.68). These results reinforce the established role of LN metastasis and cellular proliferation rates as reliable indicators of relapse and survival in breast cancer patients [49-51], while also suggesting underlying molecular mechanisms that contribute to cancer recurrence [51]. This further underscores the importance of these factors in staging, prognosis, and therapy of invasive breast cancer in Sri Lankan patients.

One of the most paramount points in our study is the validation of the observed association between LN metastasis and RFS (p=0.026) by the compounded association of *PIK3CA* mutations with both LN metastasis (p=0.036) and significantly reduced RFS (p<0.001). This enables us to bridge all three findings. Additionally, the multivariate model demonstrated the compounded impact of all these factors (p<0.001) on the likelihood of disease relapse, ultimately emphasizing the importance of considering all three factors- *PIK3CA* mutations, LN status, and Ki67 index, in prognosis and treatment planning for breast cancer patients in Sri Lanka and across South Asia. This may also suggest an underlying mechanistic link that contributes to the aggressive behavior of breast cancer. It is plausible that these mutations promote the cancer spread via lymphatic channels, leading to LN metastasis and subsequent recurrence. This progression highlights a crucial step in cancer spread, as LNs function as critical filtering sites within the lymphatic system, where cancer cells infiltrate and establish secondary tumor deposits [52].

The above findings align with Stephen Paget's 'Seed and soil hypothesis,' which proposes that metastasis occurs when certain tumor cells (the "seed") preferentially grow in particular organ microenvironments (the "soil") - and that metastases only develop when the right seed is implanted in the right soil [53]. Our study suggests that *PIK3CA* driver mutations, possibly the predominant H1047R mutation, may act as a genetic change that enhances the metastatic potential of breast cancer cells by promoting survival and proliferation. These genetic alterations could facilitate the invasion of lymphatic vessels, leading to the establishment of metastatic colonies in LNs. In this context, *PIK3CA* mutations may assist in planting the "seed" in the "soil" by promoting the establishment of tumor deposits in axillary LNs. Strikingly, further developments to this hypothesis, elaborate how the above mechanisms could result in the primary breast cancer metastasizing to the lungs and bones [54], the exact two organs of relapse diagnosed in our *PIK3CA*-mutated patient population. Therefore, by elucidating this molecular pathway, our study offers valuable insights into the potential role of *PIK3CA* in the underlying biology of aggressive breast cancers, particularly in the Sri Lankan population.

To our knowledge, PI3K inhibitors are not currently prescribed for breast cancer patients in Sri Lanka. Our study's preliminary observation of a negative prognostic outcome in the predominantly SHR+/HER2-, Sri Lankan breast cancer cohort with *PIK3CA* mutations, serves as a calling out for Sri Lankan clinicians to incorporate *PIK3CA* mutational testing in routine diagnostic protocols. Patients identified with these mutations could benefit from PI3Kα inhibitors such as Alpelisib, which are approved for this clinical scenario and are known to prolong the RFS in patients [55]. Furthermore, this treatment approach could be tailored for high-risk groups within Sri Lanka, should a larger study with a better representation confirm an association of *PIK3CA* mutations with patient ancestry. This personalized approach could extend beyond breast cancer to other malignancies such as colorectal, gastric, ovarian, and cervical cancers, which have also exhibited *PIK3CA* mutations and consequent impacts on prognosis [8, 56].

Despite the significant implications of our study, there are several limitations. The primary limitation is the sample size, which was restricted due to financial and logistical constraints. This limitation impacted the statistical power of several results and affected the ability to perform robust regression and multivariate analyses. Additionally, the retrospective nature of our study also introduced certain constrains. Although an association between ancestry and *PIK3CA* mutation status was observed, this was not an initial objective of the study and instead emerged as a secondary, exploratory finding. Importantly, patients were not recruited based on ancestry-related criteria, and classification was based on self-reported ethnicity in hospital records rather than genetic testing. The underrepresentation of minority ethnic groups- particularly those of Dravidian descent- further limits the reliability of this finding. As such, the ancestry-related observation should be interpreted with caution and considered hypothesis-generating rather than conclusive. Overcoming this limitation might require recruiting patients from a broader array of hospitals and oncology clinics around the country, to ensure adequate representation of all ancestral groups.

Another limitation is the absence of data on other potentially interacting mutations within the *PIK3CA* gene or in other OGs and TSGs that were not tested in the cohort, and their interplay with the tested mutations. Additionally, the follow-up period in our study was relatively short. All patients with survival data (n=42) were alive, and 90.5% remained recurrence-free within 5 years post-diagnosis, which limited the survival analysis to RFS as a proxy. A longer follow-up period would provide a more comprehensive understanding of long-term outcomes. Nevertheless, this pilot study, the first to analyze *PIK3CA* mutations in relation to breast cancer in Sri Lanka, together with its observed prognostic implications, offers sufficient insight and direction to the chosen objectives, so that a larger, more conclusive study can be conducted to confirm

and validate the reported findings. Accordingly, a study on a larger Sri Lankan cohort with a subsequent functional analysis is necessary to address the above limitations, especially to confirm the absence of the E545K mutation in Sri Lankan breast cancer patients and to validate the significance of observed mutations.

In conclusion, this study is the first to report on *PIK3CA* mutations in Sri Lankan breast cancer patients, a population with a distinct genetic background, demonstrating a *PIK3CA* mutation prevalence of 17.46% and significant associations between mutations, LN metastasis and cancer relapse. Our findings also suggest the existence of a possible molecular link contributing to metastasis.

Our findings provide a foundation for prognostic stratification and therapeutic decision-making for breast cancer patients in Sri Lanka - rooted in the presence of *PIK3CA* mutations - for identifying high-risk patient subsets who may benefit from targeted therapies such as PI3K inhibitors, which are not currently prescribed in Sri Lanka. Further large-scale functional studies are necessary to validate these findings and to improve clinical outcomes for breast cancer patients in Sri Lanka.

Author Contribution Statement

TRC: Conceptualization, funding acquisition, Methodology development, Experimental investigation, results analysis and interpretation, Writing the original manuscript, Logistics & coordination. IK: Resources, identifying malignant tissues, clinicopathological data analysis and interpretation, manuscript reviewing & editing. RP: Funding acquisition, sample and data acquisition and management, clinicopathological data analysis and interpretation reviewing & editing of manuscript. JB: Resources, Patient identification, sample and data acquisition and management, clinicopathological data analysis and interpretation, review & editing of manuscript . SV: Data visualization & curation, Statistical analysis, Interpretation & validation of data, review & editing of manuscript . NP: Methodology Design, Experimental troubleshooting, reviewing of manuscript . GHG: Conceptualization, Methodology Design, Logistics & coordination, Experimental troubleshooting, results analysis and interpretation, review & editing of manuscript

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Approval

This study formed a component of the approved and graded undergraduate research thesis of Ms. Tharini Ruwinya Cabraal, submitted in partial fulfillment of the requirements for the Bachelor of Science (Honours) in Immunology and Integrative Molecular Biology, Faculty of Science, University of Colombo.

Data availability statement

The data generated or analyzed in this study are included within the article and its supplementary data files. Raw data were generated and processed by the authors and are available upon reasonable request from the corresponding author.

Conflict of Interest

The authors declare that there are no conflicts of interest relevant to this study. No financial, institutional, or personal relationships have influenced the design, execution, interpretation, or reporting of the data presented in this manuscript.

References

1. Barakeh DH, Aljelaify R, Bashawri Y, Almutairi A, Alqubaishi F, Alnamnakani M, et al. Landscape of somatic mutations in breast cancer: New opportunities for targeted therapies in saudi arabian patients. Oncotarget. 2021;12(7):686-97. https://doi.org/10.18632/oncotarget.27909.
2. Fan L, Goss PE, Strasser-Weippl K. Current status and future projections of breast cancer in asia. Breast Care (Basel). 2015;10(6):372-8. https://doi.org/10.1159/000441818.
3. Zhukova LG AI, Zavalishina LE, Zakiriakhodzhaev AD, Koroleva IA, Nazarenko AV, Paltuev RM, et al. Breast cancer. Journal of Modern Cancer. 2021;23(1):5-40. https://doi.org/10.26442/18151434.2021.1.200823.
4. Stratton MR, Campbell PJ, Futreal PA. The cancer genome. Nature. 2009;458(7239):719-24. https://doi.org/10.1038/nature07943.
5. Miricescu D, Totan A, Stanescu S, II, Badoiu SC, Stefani C, Greabu M. Pi3k/akt/mtor signaling pathway in breast cancer: From molecular landscape to clinical aspects. Int J Mol Sci. 2020;22(1). https://doi.org/10.3390/ijms22010173.
6. Bachman KE, Argani P, Samuels Y, Silliman N, Ptak J, Szabo S, et al. The *PIK3CA* gene is mutated with high frequency in human breast cancers. Cancer Biol Ther. 2004;3(8):772-5. https://doi.org/10.4161/cbt.3.8.994.
7. Samuels Y, Waldman T. Oncogenic mutations of *PIK3CA* in human cancers. Curr Top Microbiol Immunol. 2010;347:21-41. https://doi.org/10.1007/82_2010_68.
8. Karakas B, Bachman KE, Park BH. Mutation of the *PIK3CA* oncogene in human cancers. Br J Cancer. 2006;94(4):455-9. https://doi.org/10.1038/sj.bjc.6602970.
9. Reinhardt K, Stuckrath K, Hartung C, Kaufhold S, Uleer C, Hanf V, et al. *PIK3CA*-mutations in breast cancer. Breast Cancer Res Treat. 2022;196(3):483-93. https://doi.org/10.1007/s10549-022-06637-w.
10. Chen JW, Murugesan K, Newberg JY, Sokol ES, Savage HM, Stout TJ, et al. Comparison of *PIK3CA* mutation prevalence in breast cancer across predicted ancestry populations. JCO Precis Oncol. 2022;6:e2200341. https://doi.org/10.1200/PO.22.00341.
11. Li SY, Rong M, Grieu F, Iacopetta B. *PIK3CA* mutations in breast cancer are associated with poor outcome. Breast Cancer Res Treat. 2006;96(1):91-5. https://doi.org/10.1007/s10549-005-9048-0.
12. Hu H, Zhu J, Zhong Y, Geng R, Ji Y, Guan Q, et al. *PIK3CA* mutation confers resistance to chemotherapy in

triple-negative breast cancer by inhibiting apoptosis and activating the pi3k/akt/mtor signaling pathway. Ann Transl Med. 2021;9(5):410. https://doi.org/10.21037/atm-21-698.
13. Cho YA, Ko SY, Suh YJ, Kim S, Park JH, Park HR, et al. *PIK3CA* mutation as potential poor prognostic marker in asian female breast cancer patients who received adjuvant chemotherapy. Curr Oncol. 2022;29(5):2895-908. https://doi.org/10.3390/curroncol29050236.
14. Fillbrunn M, Signorovitch J, Andre F, Wang I, Lorenzo I, Ridolfi A, et al. *PIK3CA* mutation status, progression and survival in advanced hr + /her2- breast cancer: A meta-analysis of published clinical trials. BMC Cancer. 2022;22(1):1002. https://doi.org/10.1186/s12885-022-10078-5.
15. Zagami P, Fernandez-Martinez A, Rashid NU, Hoadley KA, Spears PA, Curigliano G, et al. Association of *PIK3CA* mutation with pathologic complete response and outcome by hormone receptor status and intrinsic subtype in early-stage *ERBB2*/her2-positive breast cancer. JAMA Netw Open. 2023;6(12):e2348814. https://doi.org/10.1001/jamanetworkopen.2023.48814.
16. Cizkova M, Susini A, Vacher S, Cizeron-Clairac G, Andrieu C, Driouch K, et al. *PIK3CA* mutation impact on survival in breast cancer patients and in eralpha, pr and *ERBB2*-based subgroups. Breast Cancer Res. 2012;14(1):R28. https://doi.org/10.1186/bcr3113.
17. Zardavas D, Te Marvelde L, Milne RL, Fumagalli D, Fountzilas G, Kotoula V, et al. Tumor *PIK3CA* genotype and prognosis in early-stage breast cancer: A pooled analysis of individual patient data. J Clin Oncol. 2018;36(10):981-90. https://doi.org/10.1200/JCO.2017.74.8301.
18. Wang S, Pitt JJ, Zheng Y, Yoshimatsu TF, Gao G, Sanni A, et al. Germline variants and somatic mutation signatures of breast cancer across populations of african and european ancestry in the us and nigeria. Int J Cancer. 2019;145(12):3321-33. https://doi.org/10.1002/ijc.32498.
19. Pitt JJ, Riester M, Zheng Y, Yoshimatsu TF, Sanni A, Oluwasola O, et al. Characterization of nigerian breast cancer reveals prevalent homologous recombination deficiency and aggressive molecular features. Nat Commun. 2018;9(1):4181. https://doi.org/10.1038/s41467-018-06616-0.
20. Keenan T, Moy B, Mroz EA, Ross K, Niemierko A, Rocco JW, et al. Comparison of the genomic landscape between primary breast cancer in african american versus white women and the association of racial differences with tumor recurrence. J Clin Oncol. 2015;33(31):3621-7. https://doi.org/10.1200/JCO.2015.62.2126.
21. Carrot-Zhang J, Chambwe N, Damrauer JS, Knijnenburg TA, Robertson AG, Yau C, et al. Comprehensive analysis of genetic ancestry and its molecular correlates in cancer. Cancer Cell. 2020;37(5):639-54 e6. https://doi.org/10.1016/j.ccell.2020.04.012.
22. Arora K, Tran TN, Kemel Y, Mehine M, Liu YL, Nandakumar S, et al. Genetic ancestry correlates with somatic differences in a real-world clinical cancer sequencing cohort. Cancer Discov. 2022;12(11):2552-65. https://doi.org/10.1158/2159-8290.CD-22-0312.
23. Perera N, Galhena G, Ranawaka G. X-chromosomal str based genetic polymorphisms and demographic history of sri lankan ethnicities and their relationship with global populations. Sci Rep. 2021;11(1):12748. https://doi.org/10.1038/s41598-021-92314-9.
24. Singh PP, Kumar S, Pasupuleti N, Weerasooriya PR, van Driem G, Tennekoon KH, et al. Reconstructing the population history of the sinhalese, the major ethnic group in sri lanka. iScience. 2023;26(10):107797. https://doi.org/10.1016/j.isci.2023.107797.
25. Vithana PVSC DD, Handagiripathira HMI, Alahapperuma S, Nilaweera I, Perera S. Sri lankan patterns of female cancers: Incidence and mortality over 1995-2010.
Asian Pac J Cancer Care. 2021;6(1):27-33. https://doi.org/10.31557/apjcc.2021.6.1.27-33.
26. Fernando A, Jayarajah U, Prabashani S, Fernando EA, Seneviratne SA. Incidence trends and patterns of breast cancer in sri lanka: An analysis of the national cancer database. BMC Cancer. 2018;18(1):482. https://doi.org/10.1186/s12885-018-4408-4.
27. Anwar SL, Raharjo CA, Herviastuti R, Dwianingsih EK, Setyoheriyanto D, Avanti WS, et al. Pathological profiles and clinical management challenges of breast cancer emerging in young women in indonesia: A hospital-based study. BMC Womens Health. 2019;19(1):28. https://doi.org/10.1186/s12905-019-0724-3.
28. Najjar H, Easson A. Age at diagnosis of breast cancer in arab nations. Int J Surg. 2010;8(6):448-52. https://doi.org/10.1016/j.ijsu.2010.05.012.
29. Abdel-Razeq H, Almasri H, Abdel Rahman F, Abdulelah H, Abu Nasser M, Salam M, et al. Clinicopathological characteristics and treatment outcomes of breast cancer among adolescents and young adults in a developing country. Cancer Manag Res. 2019;11:9891-7. https://doi.org/10.2147/CMAR.S229337.
30. Leong SP, Shen ZZ, Liu TJ, Agarwal G, Tajima T, Paik NS, et al. Is breast cancer the same disease in asian and western countries? World J Surg. 2010;34(10):2308-24. https://doi.org/10.1007/s00268-010-0683-1.
31. Balawardena J, Skandarajah T, Rathnayake W, Joseph N. Breast cancer survival in sri lanka. JCO Glob Oncol. 2020;6:589-99. https://doi.org/10.1200/JGO.20.00003.
32. Perera NRP SA, Balawardena J, Malaviarachchi SL, Kumarasinghe HIS, De Silva AP. Clinicopathological analysis of breast cancers, in a single unit of a sri lankan tertiary care hospital. Sri Lanka Journal of Cancer. 2022;3(1):27-35.
33. Howlader N, Altekruse SF, Li CI, Chen VW, Clarke CA, Ries LA, et al. Us incidence of breast cancer subtypes defined by joint hormone receptor and her2 status. J Natl Cancer Inst. 2014;106(5). https://doi.org/10.1093/jnci/dju055.
34. Ahmad F, Badwe A, Verma G, Bhatia S, Das BR. Molecular evaluation of *PIK3CA* gene mutation in breast cancer: Determination of frequency, distribution pattern and its association with clinicopathological findings in indian patients. Med Oncol. 2016;33(7):74. https://doi.org/10.1007/s12032-016-0788-y.
35. Mangone FR, Bobrovnitchaia IG, Salaorni S, Manuli E, Nagai MA. *PIK3CA* exon 20 mutations are associated with poor prognosis in breast cancer patients. Clinics (Sao Paulo). 2012;67(11):1285-90. https://doi.org/10.6061/clinics/2012(11)11.
36. Lai YL, Mau BL, Cheng WH, Chen HM, Chiu HH, Tzen CY. *PIK3CA* exon 20 mutation is independently associated with a poor prognosis in breast cancer patients. Ann Surg Oncol. 2008;15(4):1064-9. https://doi.org/10.1245/s10434-007-9751-7.
37. Martinez-Saez O, Chic N, Pascual T, Adamo B, Vidal M, Gonzalez-Farre B, et al. Frequency and spectrum of *PIK3CA* somatic mutations in breast cancer. Breast Cancer Res. 2020;22(1):45. https://doi.org/10.1186/s13058-020-01284-9.
38. Chao TC, Tsai YF, Liu CY, Lien PJ, Lin YS, Feng CJ, et al. Prevalence of *PIK3CA* mutations in taiwanese patients with breast cancer: A retrospective next-generation sequencing database analysis. Front Oncol. 2023;13:1192946. https://

doi.org/10.3389/fonc.2023.1192946.
39. Nazeri E PM, Olfatbakhsh A, Najafi S, Peyman M, Karami B, Halvaei S, Esmaeili R. Evaluation of *PIK3CA* gene mutations in breast cancer patients treated by trastuzumab. Archives of Breast Cancer. 2023;10(3):248-55. https://doi.org/https://doi.org/10.32768/abc.2023103248-255.
40. Cardona G. Indo-Iranian languages. Encyclopedia Britannica; 2025. Available from: https://www.britannica.com/topic/Indo-Iranian-languages. Accessed October 21 2025.
41. Saal LH, Holm K, Maurer M, Memeo L, Su T, Wang X, et al. *PIK3CA* mutations correlate with hormone receptors, node metastasis, and *ERBB2*, and are mutually exclusive with pten loss in human breast carcinoma. Cancer Res. 2005;65(7):2554-9. https://doi.org/10.1158/0008-5472-CAN-04-3913.
42. Luo M, Lin X, Hao D, Shen KW, Wu W, Wang L, et al. Incidence and risk factors of lymph node metastasis in breast cancer patients without preoperative chemoradiotherapy and neoadjuvant therapy: Analysis of seer data. Gland Surg. 2023;12(11):1508-24. https://doi.org/10.21037/gs-23-258.
43. Rao R. The evolution of axillary staging in breast cancer. Mo Med. 2015;112(5):385-8.
44. Kalinsky K, Jacks LM, Heguy A, Patil S, Drobnjak M, Bhanot UK, et al. *PIK3CA* mutation associates with improved outcome in breast cancer. Clin Cancer Res. 2009;15(16):5049-59. https://doi.org/10.1158/1078-0432.CCR-09-0632.
45. Arikan AE, Kara H, Dulgeroglu O, Erdogan EN, Capkinoglu E, Uras C. Do prognosis and clinicopathological features differ in young early-stage breast cancer? Front Surg. 2022;9:900363. https://doi.org/10.3389/fsurg.2022.900363.
46. Kanyilmaz G, Yavuz BB, Aktan M, Karaagac M, Uyar M, Findik S. Prognostic importance of ki-67 in breast cancer and its relationship with other prognostic factors. Eur J Breast Health. 2019;15(4):256-61. https://doi.org/10.5152/ejbh.2019.4778.
47. Deng L, Zhu X, Sun Y, Wang J, Zhong X, Li J, et al. Prevalence and prognostic role of *PIK3CA*/akt1 mutations in chinese breast cancer patients. Cancer Res Treat. 2019;51(1):128-40. https://doi.org/10.4143/crt.2017.598.
48. Kato S, Iida S, Higuchi T, Ishikawa T, Takagi Y, Yasuno M, et al. *PIK3CA* mutation is predictive of poor survival in patients with colorectal cancer. Int J Cancer. 2007;121(8):1771-8. https://doi.org/10.1002/ijc.22890.
49. Rakha EA, Tan PH, Varga Z, Tse GM, Shaaban AM, Climent F, et al. Prognostic factors in metaplastic carcinoma of the breast: A multi-institutional study. Br J Cancer. 2015;112(2):283-9. https://doi.org/10.1038/bjc.2014.592.
50. Sun X, Kaufman PD. Ki-67: More than a proliferation marker. Chromosoma. 2018;127(2):175-86. https://doi.org/10.1007/s00412-018-0659-8.
51. Liu N, Yang Z, Liu X, Niu Y. Lymph node status in different molecular subtype of breast cancer: Triple negative tumours are more likely lymph node negative. Oncotarget. 2017;8(33):55534-43. https://doi.org/10.18632/oncotarget.15022.
52. Ji H, Hu C, Yang X, Liu Y, Ji G, Ge S, et al. Lymph node metastasis in cancer progression: Molecular mechanisms, clinical significance and therapeutic interventions. Signal Transduct Target Ther. 2023;8(1):367. https://doi.org/10.1038/s41392-023-01576-4.
53. Paget S. The distribution of secondary growths in cancer of the breast. 1889. Cancer Metastasis Rev. 1989;8(2):98-101.
54. Langley RR, Fidler IJ. The seed and soil hypothesis revisited--the role of tumor-stroma interactions in metastasis to different organs. Int J Cancer. 2011;128(11):2527-35. https://doi.org/10.1002/ijc.26031.
55. Andre F, Ciruelos E, Rubovszky G, Campone M, Loibl S, Rugo HS, et al. Alpelisib for *PIK3CA*-mutated, hormone receptor-positive advanced breast cancer. N Engl J Med. 2019;380(20):1929-40. https://doi.org/10.1056/NEJMoa1813904.
56. Ogino S, Nosho K, Kirkner GJ, Shima K, Irahara N, Kure S, et al. *PIK3CA* mutation is associated with poor prognosis among patients with curatively resected colon cancer. J Clin Oncol. 2009;27(9):1477-84. https://doi.org/10.1200/JCO.2008.18.6544.