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Mutational and expressional association of the *PIK3CA* gene with the risk of breast cancer in the Pakistani population

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ABSTRACT

PI3K pathway is a very important pathway that is reported to be involved in breast cancer. Mutation of PI3K and p110 alpha-catalytic subunit of phosphatidylinositol 3-kinase (PIK3CA) is of high predictive and prognostic values in breast cancer. The purpose of the current study was to screen the hotspot mutations of the PIK3CA gene i.e. rs2677760, rs3806685, rs121913273 & rs121913279 along with expressional analysis of PI3K and PIK3CA genes in breast cancer female patients. For mutational analysis, TaqMan assay & Sanger sequencing were performed while for expressional analysis real-time PCR was carried out. Mutant allele C of rs2677760 was observed to be high in postmenopausal patients. The frequency of mutant allele G of rs3806685 was significantly high in breast cancer patients. All diseased and control samples were of wild type for the hotspot rs121913273 and rs121913279 with allele G for rs121913273 and A for rs121913279. Expression of the PI3K was high in breast cancer tissue samples as compared to the adjacent controls. While the expression of the thePIK3CA gene was significantly high in premenopausal breast cancer patients. It was concluded that the mutant allele C of rs2677760 might have some sort of association with the menopausal status and it could be used as a diagnostic marker in post-menopausal women if studied further. Mutant allele G of rs3806685 was also found to be associated with breast cancer. While multiallelic rs121913273 and rs121913279 showed a different trend for the studied population. For expressional analysis, PI3K showed over-expression in the cases while PIK3CA gene expression was observed to be significantly associated with pre-menopausal status.

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Introduction

The PI3K/AKT/mTOR pathway is very important in cell transcription, migration, translation, proliferation, metabolism, and survival. Studies have confirmed that this pathway plays a critical role in the progression of human tumors and is a key factor to regulate angiogenesis and metabolism in the tumor cell. Abnormal activation of the pathway is detected in a variety of tumors including breast cancer, endometrial carcinoma, colorectal cancer, glioblastoma, and lung cancer (1). This pathway can be altered by several diverse mechanisms including genetic mutation or due to the amplification of key components of the pathway, for instance, amplification or mutation of the PI3K catalytic subunit p110α (encoded by PIK3CA gene) (2). Phosphoinositide 3-kinase (PI3K) activity is enhanced by various oncogenes & growth factor receptors, and high PI3K signaling is considered as a feature of cancer (3). Mutation in the catalytic subunit (p110α) of phosphatidylinositol 3-kinase (PI3K) is also the most common type of PI3K alteration that occurs with a frequency of 18–40% in breast cancer (1). Investigating the clinical effectiveness of PIK3CA mutation as a potential biomarker has aroused great interest and also due to the predictive and prognostic significance of PIK3CA mutations in breast cancer, genotyping of PIK3CA is of great importance (4). PIK3CA gene is located on chromosome 3q26.3 and comprised of 20 exons that code for 124 kDa protein and is consisting of 1068 amino acids. Deletions, insertions, and somatic missense mutations in this gene have been reported in various human cancers, like breast, brain, colon, stomach, lung, and liver cancers (5). PIK3CA gene is mostly mutated at 'hotspots' in exons 9 and 20, corresponding to the helical (E542K and E545K) and kinase (H1047R) domains, respectively. Mutations in the p110α subunit show oncogenic activity and it can cause tumors in in-vitro studies (6). Besides, gain-of-function mutations of PIK3CA are found in many cancers, including breast cancer, where they are observed in 20–25% of cases (2). Studies have also reported that the expression of PIK3CA mRNA is amplified in breast carcinoma tissue when compared to normal breast tissue (7). Similarly, a study reported that the expression of the PIK3CA gene was high in breast cancer tissue when compared with adjacent normal tissue samples (8). All these studies have reported the mutational and expressional importance of the PI3K and PIK3CA gene; therefore, the purpose of the current study was to investigate the hotspot mutations of the PIK3CA gene (rs2677760, rs3806685, rs121913273, rs121913279) along with the expressional alterations of the PI3K and PIK3CA gene in the studied cohort.

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Materials and Methods

The present case-control study comprised pathologically confirmed breast cancer cases. Two study groups were used in this study. The cohort I was used for mutational analysis and consisted of 250 blood samples along with age and gender-matched normal healthy individuals as controls. While cohort II was used for expressional analysis at mRNA level and comprised 50 breast tumor tissue samples along with normal adjacent healthy tissues as control.

Blood samples collection

The study was performed after approval from the ethical committees of universities and Hospitals. Informed written consent was signed by all the patients and participants. Hospital-based study subjects include a total of 500 Pakistani subjects 250 patients and 250 genders and age-matched healthy individuals as control. 5ml of venous blood sample was collected from patients as well as from control by a trained person in an EDTA-coated tube after signing informed consent. Data were collected by a questionnaire and blood samples were stored at low temperatures for further processing.

Tissue Samples collection

Fifty tissue samples along with adjacent normal healthy tissues used as control were collected from the female breast cancer patients for the expressional analysis of the PI3K and PIK3CA genes. Informed written consent was taken from all the participants and the cancer patients were recruited after the confirmed diagnosis by cancer clinicians. Data regarding various parameters were collected from the patients by using a questionnaire. Fresh tissue samples were collected in RNAlater and were stored at -20º C for further processing.

Demographic and clinical data of blood samples

Studied data showed that the average age was 48.46±15 years with 4.91% of patients 20-30 years of age, 20.49% at 31-40 years, 33.23% at 41-50 years, 27.86% at 51-60 years, 11.06% in the 61-70 years and only 2.45% above 70 years of age. The majority of the patients were in the category of 41-50 years age group. The mean age at menarche was 13.16 years. The current study reported that 83.95% of the patients were married, 4.54% were single, 9.87% were widowed, and 1.64% were divorced. Current findings reported that 51% of the patients were premenopausal and 49% were postmenopausal. Ductal carcinoma was observed in 81% of the patients followed by lobular carcinoma in 19%. It was observed that in the available data, 2.50% belong to stage I, 20% belong to stage II, 25% belong to stage III and 52.50% belong to stage IV of breast cancer means the majority fall in stage IV of breast cancer. Data showed that 23% of the cases were metastatic (Table 1a).

Demographic and clinical data of tissue samples

Confirmed breast cancer patients from different hospitals in Rawalpindi and Islamabad were recruited for this study. Demographic data observed, showed that the majority of the patients were between 40-60 years of age and the minimum number of patients belonged to 20-30 years of age. The mean age of menarche was 13 years in 38% of the patients, followed by both 12 & 16 years (21.42 %), 17 years (14.28 %), and 9 years (7.14 %). Marital status showed that 89% of the patients were married. The frequency of the premenopausal patients was higher (64%) as compared to postmenopausal patients i.e. 36%. Invasive ductal carcinoma was the most prevalent (75%) type of breast cancer followed by invasive lobular carcinoma (25%). The majority of patients were of grade II (50%) when compared to grade III (25 %) and grade I (25 %). Most of the patients have their right breast infected (53%) followed by a tumor in the left breast (47%). Nipple discharge was noticed in 38 % of the patients and 83 % of the patients had undergone mastectomy at some stage of their life while most of the patients (53%) were not suffering from metastasis (Table 1b).

DNA extraction from blood samples

DNA extraction was carried out from blood samples by using the Phenol chloroform method (9, 10, 11).

Mutational analysis via TaqMan assay & Sanger Sequencing

Four hotspot SNPs of the PIK3CA gene rs2677760, rs3806685, rs121913273 & rs121913279 were selected for

Table 1a. Demographic and clinical data of the patients (cohort I).

Variables	Frequency of patients (n=250)
Mean Age	48.46±15
Stage	
I	2.50%
II	20%
III	25%
IV	52.50%
Metastasis	
No	77%
Yes	23%
Menopause status	
Pre-menopausal	51%
Post-menopausal	49%
Type of Cancer	
Ductal carcinoma	81%
Lobular carcinoma	19%

Table 1b. Demographic and clinical data of the patients (cohort II).

Variables	Frequency of patients (n=50)
Mean Age	49.73±15
Grade	
I	25%
II	50%
III	25 %
Metastasis	
No	53%
Yes	32%
Undetermined	15%
Location	
Right breast	53%
Left breast	47%
Menopause status	
Pre-menopausal	64%
Post-menopausal	36%
Type of Cancer	
Ductal carcinoma	75%
Lobular carcinoma	25%

mutational analysis. rs2677760 & rs3806685 were screened via TaqMan assay and rs121913273 & rs121913279 by using Sanger Sequencing. Predesigned primers and probes were used for the TaqMan assay and the assay was carried out with a total reaction volume of 5µl. 2µl of purified genomic patient's DNA sample or control was added in a concentration of 2.5ng/µl. Three positive controls for wild type, heterozygous and mutant genotype, and two no-template controls were used along with samples each time. The plate was centrifuged for 30 seconds at 1400 rpm to mix all the content and remove air bubbles. A quant studio machine was used to run the reaction.

Multiallelic rs121913273 (G>A, G>C Ancestral: G) and rs121913279 (A>G, A>T Ancestral: A) were screened through Sanger Sequencing in the studied cohort to check their association with the risk of breast cancer. For this purpose an amplicon size of 261bp for rs121913273 forward 5'-ATCCAGAGGGGAAAAATATG-3' (20bp) and reverse primer 3'-ATTTGGCTGATCTCAGCAT-5' (19bp) with tm 54.3°C and 53°C and amplicon size of 241bp was finalized including both forward 5'-CTCAATGATGCTTGGCTCTG-3' (20bp) and reverse primer 3-'GAAAGCTCACTCTGGATTCCA -5' (21bp) with tm 58.4°C and 59.4°C for rs121913279. PCR reaction was set by mixing the 9.4µl of PCR water, 0.5µl of each forward and reverse primer, 2µl of NH4(SO4)2, 1µl of dNTPs, 1.2µl of Mgcl2, 0.4µl of Taq polymerase, and 5µl genomic DNA per sample. The total reaction volume was 20µl. Conditions used were initial denaturation at 94°C for 5 minutes, followed by 35 cycles of 94°C for 45 seconds, 52°C & 61°C respectively for 10 seconds, and 72°C for 45 seconds and final extension for 10 min at 72°C. PCR product size was confirmed by 2.5% agarose gel. Amplified PCR product was purified manually and Sanger sequencing was carried out by using forward primers.

RNA extraction from tissue samples

RNA extraction was carried out by the Trizol method and RNA quantification was performed via Nanodrop to check the concentration of RNA (12).

Expressional analysis via Real-time PCR

cDNA was synthesized from the RNA samples by using the Superscript-IV kit (Thermo Fisher Scientific) and GAPDH was used as a housekeeping gene. Real-time quantitative PCR (RT-qPCR) was carried out to check the expression of the gene in the disease as well as in adjacent normal healthy tissues used as control. To check the expression of the gene each reaction contains 0.5µl of 100pmol/µl of each of the forward and reverse primers of the gene, 2 µl cDNA template RT+ / RT- / water /Patient sample /control, 13µl SYBR Green PCR Master Mix and 9.5µl PCR water. The total reaction volume was set at 25 µl. Conditions were initial denaturation at 95ºC for 10 minutes, followed by 40 cycles of 95ºC for 15 sec, 60ºC for 60 sec, and 95ºC for 15 sec. Following the completion of the reaction, the results were analyzed.

Statistical and Data analysis

SPSS 20 software was used for the analysis of results. For mutational analysis via TaqMan assay, Hardy-Weinberg calculations were carried out. Frequencies were calculated for various genotypes, and forest plots were constructed to check the alleles associated with the risk of disease in the studied population. The Sanger Sequencing chromatograms of the diseased, as well as control samples, were studied by using Chromas software. Alamut software was used to check whether the genotype change in the sequence is associated with any phenotypic change. Expression of the gene of interest as well as the housekeeping gene was accessed in both diseased and control tissue samples. The difference in expression with age, metastasis, and menopausal status was also observed. The association of the gene expression with breast cancer was analyzed for the above-mentioned factors and fold change was noticed.

Results

Mutational analysis

In the present study, it was observed that the frequency of the mutant allele C of rs2677760 was higher in postmenopausal women as compared to premenopausal women.

Table 2. Genotype frequency distributions of PIK3CA rs2677760 and rs3806685 polymorphisms along with their correlation by OR between patients and controls. **Significant, Non-Significant.*

Genotypes	Patients observed frequency %	Expected H-W frequency %	Control Observed frequency %	Expected H-W frequency %	*p*-value for the risk assessment between patients & controls	Correlation by OR (95% CI) between patient & control	*p*-value
rs2677760	-	-	-	-	0.91	-	-
TT	30.66	29.88	33.06	30.96		0.89 (0.49-1.62)	0.71
TC	48	49.55	45.16	49.35		1.12 (0.64-1.95)	0.68
CC	21.33	20.55	21.77	19.67		0.97(0.49-1.91)	0.92
	p-value 0.95		*p*-value 0.69			-	-
rs3806685	-	-	-	-	0.08		
AA	70.92	66.05	73.6	73.96		0.87(0.47-1.62)	0.67
AG	20.70	30.43	24.8	24.08		0.79 (0.40-1.53)	0.48
GG	8.37	3.50	1.6	1.96		4.53 (0.98-20.95)	0.03*
	p-value 0.00		*p*-value 0.95				

Table 3. Risk assessment of PIK3CA polymorphism, rs2677760 and rs3806685 with combined genotype frequency calculation and OR correlation of combined genotype affects its association with risk of breast cancer. **Significant, Non-Significant.*

Genotypes	Patients observed frequency %	Control Observed frequency %	*p*-value for the risk assessment between patients & controls	Correlation by OR (95% CI) between patient & control	*p*-value
rs2677760			0.71		
TT	30.66	33.06	-	0.96 (0.55-1.67)	0.88
TC/CC	69.33	66.93	-	0.99 (0.57-1.74)	1.00
rs3806685			0.67		
AA	70.92	73.60	-	0.70 (0.33-1.50)	0.36
AG/GG	29.07	26.40	-	1.41 (0.66-3.01)	0.36

While the mutant allele G of rs3806685 was found to be significantly associated (0.03) with the risk of breast cancer in the studied population (Tables 2 and 3). The analysis for rs121913273 showed that all the samples were having a wild-type allele (G) for the SNP rs121913273, (G>A/C |Ancestral: G) (Figure 1). Similarly, all the samples were wild-type (A) for the SNP rs121913279 (A>G/T Ancestral: A) (Figure 2). No mutation was found in any of the samples for respective SNPs in the Pakistani population. The same trend was observed for both SNPs in control samples (Figures 3 and 4).

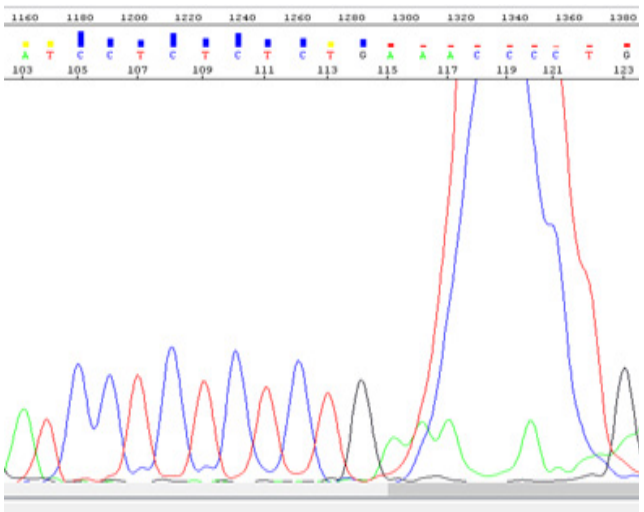

Figure 1. Sequencing results of rs121913273.

Expressional analysis

Expression of PI3K, PIK3CA, and housekeeping genes was analyzed in normal and tumor samples. PI3K gene was observed to be over-expressed in 37.50% of the patients (Figure 5a). Expression of the PI3K and PIK3CA genes was also analyzed with respect to i.e., age, metastasis, and menopausal status. The expression of the PI3K gene was high in patients above 50 years of age, and all the patients were non-metastatic with the majority (66.66%) belonging to the postmenopausal group (Figure 5b, 5c, 5d). No expressional differences were seen for the PIK3CA gene between cancerous and normal samples (Figure 6a). Similarly, no difference in expression was noticed for the PIK-3CA gene according to age and metastasis (Figure 6b, 6c). But, when analyzed according to menopausal status, it was noted that expression of the gene was high in premenopausal patients (66.66%) (Figure 6d Expression of *PIK3CA*

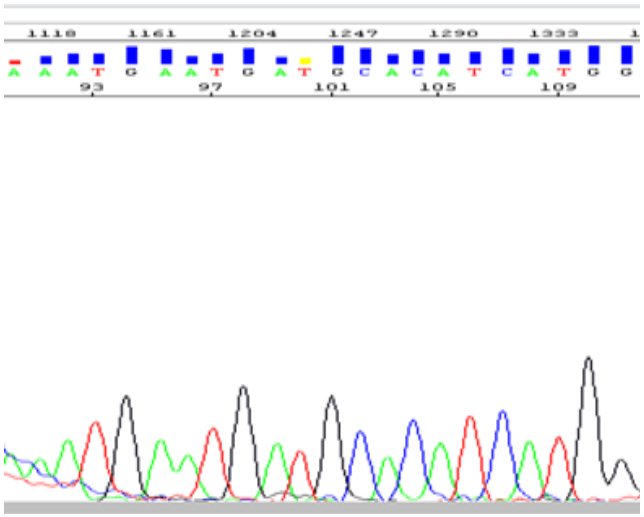

Figure 2. Sequencing results of rs121913279.

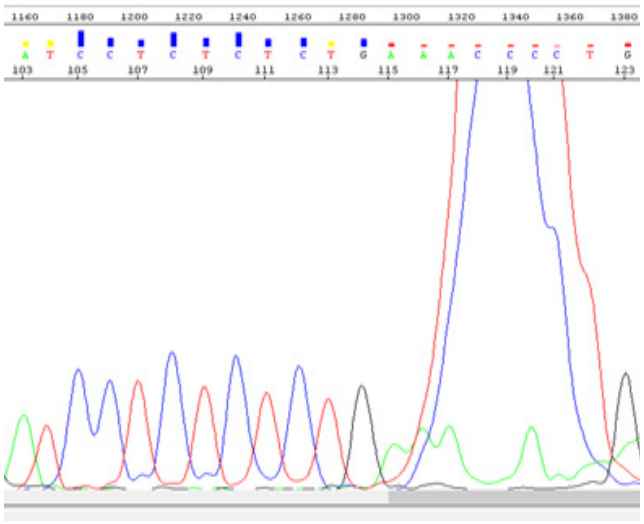

Figure 3. Sequencing results of the control sample for rs121913273.

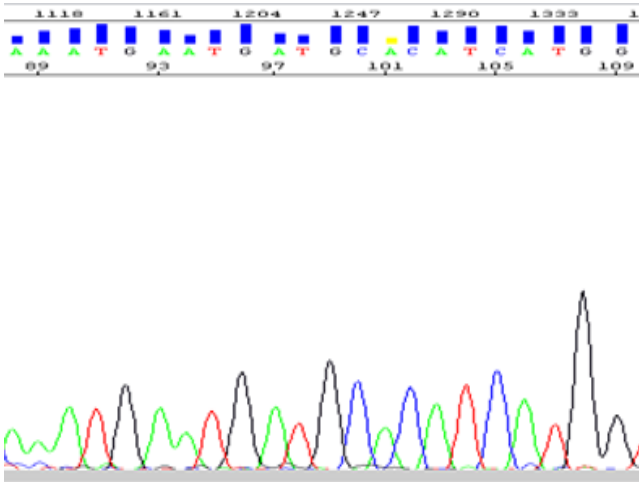

Figure 4. Sequencing results of control sample of rs121913279.

gene and menopausal status).

Discussion

Breast cancer is the most widespread cancer with more than 2.2 million cases diagnosed in 2020. Nearly 1 in 12 women has the risk of developing breast cancer in their lifetime. It is the main cause of death from cancer in women causing 685, 000 women deaths in 2020 (13). It is responsible for thirty percent of all cancers in women, making it the most commonly diagnosed cancer in women around the world and it is among the top 20 death causes in Pakistan (14). Cancer prevalence is higher in the Pakistani population when compared to the Western population; one in nine females is suffering from breast cancer in Pakistan which is the highest rate of incidence in Asia (15).In the current study average age of the patients was 48.46 years, earlier studies reported that the mean age of breast cancer in Pakistan was 48 (16), 49.5 (±13) years (17), 44 (18), 50 (19), 47.5+11.02 (20), 48 (21). The current study reported that the mean age at menarche was 13.16 years. Age at menarche in one study was 13.4±1.4 years (22), and 13.2 ±1.2 years in another study (23). Current findings reported that 51 % of the patients were premenopausal and 49% were postmenopausal. Similarly, a study conducted at Nuclear Medicine, Oncology and Radiotherapy Institute (NORI), Islamabad, reported that the frequency of postmenopau-

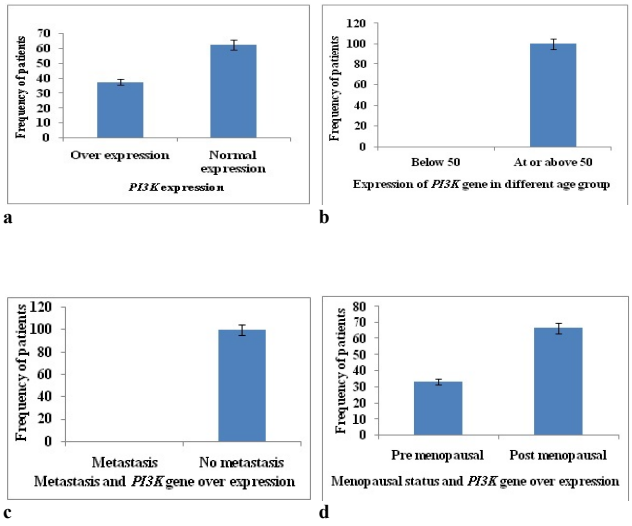

Figure 5. a. Over-expression of PI3K gene**. b.** Over-expression of the PI3K gene in the different age groups. **c.** Overexpression of PI3K gene in metastasis and non-metastasis group. **d.** Overexpression of PI3K gene and Menopausal status.

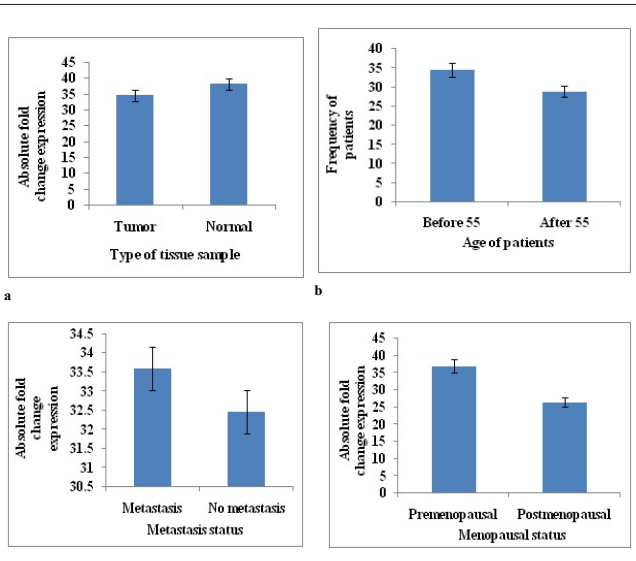

Figure 6. a. Expression of *PIK3CA* gene**. b.** *PIK3CA* gene expression in the different age groups. **c.** Expression of *PIK3CA* gene in metastasis and non-metastasis group. **d.** Expression of *PIK3CA* gene and menopausal status.

sal patients was 49.50% (24). A study conducted in China reported that 65.42% patients were premenopausal and 34.58% were postmenopausal patients (25). In the current study ductal carcinoma was the most prevalent type (81 %) followed by lobular carcinoma (19%). Similarly, another study reported that the frequency of ductal carcinoma was high (95.50 %) as compared to lobular carcinoma (26). It was observed that in the available data majority patients fall into stage IV of breast cancer as 2.5 % patients were noted to be of stage I, 20 % of stage II, 25 % of stage III while 52.50 % were in stage IV of breast cancer. Similar findings were reported in another study revealing that 33% patients were in the early stages and 67% were with late-stage breast cancer (27).

PI3K/Akt/mTOR is a cell signaling pathway that plays important role in growth, survival, proliferation, motility, metabolism, and immune response regulation. This pathway has also been noted to be involved in various diseases including cancer. Changes to this pathway have been found in practically all human tumors, including breast cancer, where up to 60% of the tumors present different variations that hyper-activate this pathway. Deregulation of this pathway has been linked to a wide variety of cancer hallmarks, including uncontrolled proliferation, genomic instability, and metabolic reprogramming in tumor cells. Activating mutations in the PIK3CA gene, which encodes the p110α catalytic subunit, have been identified as potent oncogenic mechanisms involved in the hyperactivation of this pathway. These mutations are especially notable in breast cancer, where up to 27% of patients have mutations in this gene (28). It is indicated that changes in PIK3CA gene sequence and expression play a vital role in breast cancer. But a comprehensive knowledge and study of PIK3CA mutations in breast cancer are so far insufficient in the Pakistani population. In this context screening of some hotspot SNPs of this gene along with expressional analysis of the PI3K and PIK3CA gene was carried out to check their association and involvement in breast cancer in the studied population. Pande et al. (29) reported that rs2677760 was associated with worse survival of breast cancer. Association noted that the study was statistically significant in the overweight and obese group. Similarly, another study reported that rs2677760 had the strongest association with colon cancer (30). The Association of rs2677760 of PIK3CA in the current study, when analyzed with the risk of breast cancer, indicated that the association of various genotypes of rs2677760 with the disease was statistically non-significant. However, it was noticed that the frequency of the mutant genotype CC was higher in postmenopausal patients. Probably there is some sort of association between the risk allele C and the hormonal status of patients. No study has reported the link of this SNP with hormonal status yet.

A previous study conducted to check the association of rs3806685 showed that the p-value for the Hardy-Weinberg calculation was 0.95, 0.11, and 0.93 for stages I, II, and III respectively. While the combined frequency of the minor allele in these cases was 0.171 and the stage-wise minor allele frequency in cases is 0.15 (stage I), 0.16 (stage (II), and 0.18 (stage III). The combined minor allele frequency in healthy people was 0.17, and stage-wise minor allele frequency in controls was 0.18 (stage I), 0.17 (stage II), and 0.16 (stage III) (31). A Korean population indicated that a combined odds ratio for homozygous dominant AA was 0.69 (0.28-1.70), for heterozygous genotype AG 0.97 (0.78-1.21), and homozygous mutant GG, it was recorded as 0.94 (0.74-1.18) with p-value 5.9 ×10-1. The odds ratio for heterozygous genotype AG was 0.90 (0.79-1.03), for homozygous dominant AA it was 0.25 (0.15-0.41) and for mutant GG the odds ratio was 0.78 (0.69-0.88) in stage I with a p-value 3.4 ×10-5. While the odds ratio for homozygous dominant was 1.04 (0.72-1.52), heterozygous genotype 0.84 (0.73-0.97) and the odds ratio per allele was 0.90 (0.80-1.01) for stage II with p-value 9.7 ×10-1. The odds ratio for wild type genotype in stage III was 1.24 (0.83-1.83), for heterozygous genotype 1.22 (1.05-1.42), and for per allele odds ratio was 1.18 (1.04-1.34) at 95% confidence interval with p-value 1.8 ×10-2 (16). Another study evaluated the association of rs3806685 with estrogen status and show that the association was the same in ER-positive and ER-negative cases (32). In the current study, it was observed that the frequency of the homozygous dominant AA was 70.92% and 73.60% in patients and controls with the odds ratio of 0.87 (0.47-1.62) and a p-value of 0.67 (>0.05), frequency of the heterozygous AG was 20.70% and 24.80% with an odds ratio of 0.79 (0.40-1.53) and a p-value of 0.48 while the frequency of homozygous recessive GG was 8.37% in patients and 1.60% in controls with an OR of 4.53(0.98-20.95). Current findings reported that minor allele G was significantly associated with the risk of breast cancer in the studied population.

Mutational analysis of PIK3CA gene multiallelic SNPs indicated that, although rs121913273 & rs121913279 are very hotspot mutations, already reported in different populations interestingly all the samples were wild type (G) for the PIK3CA SNP (rs121913273) and wild type (A) for the PIK3CA SNP (rs121913279) in the current population. None of the mutant alleles A or C, and G or T were found in the population for rs121913273 and rs121913279 i.e. not a single sample was harboring the mutation. While a study conducted in China showed that the range of overall prevalence of the mutation in the PIK3CA gene was 7.5-38.8% in the Chinese population with an overall mutation frequency of 2.2% for rs121913273 while 16.6 % for rs121913279 A>G and 4.8% for rs121913279 A>T) confirming their important role in breast carcinogenesis (33). A study conducted in Australia reported 40% of patients having PIK3CA mutations with 7.14 percent having rs121913273 mutation and 7.14% for rs121913279 A>G and 5.71% for rs121913279 A>T. This establishes that these mutations occur in a high percentage of breast cancers (34). Similarly, a study conducted in a Japanese population identified 35.10% PIK3CA mutations in exon 9 of the PIK3CA gene (35).

A study conducted in Spain revealed that the frequency of rs121913273 was 11% while the frequency of rs121913279 A>G was 35% & rs121913279 A>T was 4% in the population (36). A study from Brazil also reported the significant association of rs121913273 and rs121913279 (p.H1047R A>G) 14 % with the risk of breast cancer (37). Another study conducted in the neighboring country (India) indicated that the prevalence of Exon 9 was 5.6 % among the reported PIK3CA mutations while the frequency of PIK3CA rs121913279 A>G was 14.01% and rs121913279 A>T was 5.60% (38). A study from Greece showed that the prevalence of PIK3CA gene mutation was 81.30% among them the frequency of rs121913279 A>G was 68.80% (39). Another study

in China reported a 27.60% prevalence of rs121913273 while the frequency of rs121913279 A>G was 23.90% and rs121913279 A>T was 37.50% (40). All studies reported a significant association of PIK3CA SNP rs121913273 & rs121913279 with breast cancer risk in various European & Asian populations. However, in current findings, none of the single patients, as well as the control, had the mutation and all samples were of wild type.

This is a different trend that was seen in current results, where none of the single patients was found with mutation. All samples either patients or controls were carrying wild-type genotypes, this condition has not been reported before in any study. These results revealed that PIK-3CA rs121913273 & rs121913279 might be not acting as breast cancer risk factors in the Pakistani population. The PI3K pathway is a very complicated intracellular network that plays a significant role in breast cancer cell growth and proliferation. A study reported that the overexpression of PI3K occurred in 24 percent of all tumor samples in breast carcinomas (41). The current study reported that expression of the PI3K gene was high in 37.50 % of breast cancer patients. When analyzed the overexpression of the PI3K was observed in old age, non-metastatic, and post-menopausal patients. Likewise, in another study has been observed that over-expression of PIK3CA or deletion of this gene via somatic cell knockouts has played its oncogenic role (42). Several studies have already reported that PIK3CA is involved in the risk of the development of breast cancer in many populations. Also, many other studies observed the over-expression of genomic regions containing AKT, PDPK1, or PIK3CA genes in different cancer types (5). Another study reported that PIK3CA expression was significantly high in breast carcinoma tissue compared to normal breast tissue (7). In the current study, although there was no considerable difference in the expression of PIK3CA between tumor and normal samples, it was found that expression was significantly high in premenopausal patients (66.66%) when compared with postmenopausal cases (33.33%). Based on current observations, it was concluded that PI3K is over-expressed in the breast cancer patients of the studied population. While the PIK3CA gene is playing its role in breast carcinogenesis in young age groups in the studied population.

In conclusion, this study is the first time to evidence of rs2677760, rs3806685, rs121913273 & rs121913279 mutations of PIK3CA, in the breast cancer patients of the studied population. The frequency of mutant allele C of rs2677760 was found to be associated with postmenopausal breast cancer patients which can be a predictive observation of hormonal role. While the association of mutant allele (G) of rs3806685 was found in breast cancer patients as compared to controls that are projecting towards its diagnostic value. Although, rs121913273 & rs121913279 are very hotspot mutations of the PIK3CA gene reported in other studies, interestingly, it was noted that the current population was wild type for both hotspot SNPs. The whole studied population showed the reverse trend for the respective SNPs. Surprisingly no mutation of rs121913273 & rs121913279 was reported in the studied population so there is a need for large-scale genetic studies because the genes that are playing important role in breast cancer development in other populations might not be the risk factor for breast cancer in the current population. Moreover, when studied at the expressional level this pathway is also contributing to breast carcinogenesis. Studies like the recent one help make future diagnostic and prognostic along with therapeutic strategies as in addition to already observed SNPs, novel genetic trends were also observed.

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Interest conflict
The authors declare no conflict of interest.

Author's contribution
All of the authors have participated in the design, execution, and analysis of the study, and they have approved the final version.

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