

Investigating the Exon 5 *PTEN* Gene Mutations in Human Breast Cancer: Hospital-Based Study from Lahore, Pakistan

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ABSTRACT

Breast cancer is the most frequent cancer among women in Pakistan. It contributes to a significant public health burden. The progression of breast cancer involves complex genetic and molecular changes, among which alterations in tumor suppressor genes like *PTEN* are of considerable interest. *PTEN* is a key regulator of PI3K/AKT signaling pathway and plays a vital role in maintaining cellular homeostasis. Mutations in exon 5 of this gene have been reported to impair its tumor suppressive function in distinctive cancers. This study intended to investigate the presence of mutations in exon 5 of the *PTEN* gene in a cohort of 15 breast cancer patients from Pakistan. Tissue samples were used to extract genomic DNA, which was then amplified using PCR and sequenced using Sanger. According to sequencing data, no mutations in exon 5 of *PTEN* gene were found in any of the examined samples. These findings provide useful baseline information for the Pakistani population and emphasize the significance of investigating more extensive genetic and epigenetic landscapes outside of exon-specific mutation analysis. To completely understand the function of *PTEN* and its potential as a prognostic biomarker in the treatment of breast cancer in Pakistan, future research with larger sample sizes and whole-gene or genomic-wide sequencing is necessary.

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UF: Wrote original draft, data curation, investigation, software, writing review and editing. MM: Conceptualization, resources, supervision, validation, review and editing. ZH: Supervision, resources, investigation, formal analysis.

Key words

Breast cancer, *PTEN*, PI3K/AKT signalling pathway, Exon 5, Mutations analysis

INTRODUCTION

Basic biological processes that control healthy growth and development throughout life are cell division and programmed cell death. Pathological conditions such as cancer, neurodegenerative diseases, and autoimmune diseases are caused by dysregulation of these processes (Loftus *et al.*, 2022). Uncontrolled cell proliferation, apoptosis evasion, persistent angiogenesis, and the capacity to penetrate and metastasize are characteristics of cancer that are primarily caused by genetic and epigenetic abnormalities (Lundberg and Phoosuwan, 2022). Normal cellular control mechanisms are disrupted by aberrant mitogenic signaling and mutations in cell cycle-regulatory genes, which promote neoplastic transformation and tumor progression (Blackadar, 2016).

Cancer has been recognised as a complex, multi-step

process involving cumulative molecular changes and interactions with environmental and population-level factors, rather than merely a genetic disease driven by isolated mutations (Aguadé *et al.*, 2022). Multiple driver mutations, sometimes known as “hits,” are responsible for cancer cells’ unchecked growth, loss of differentiation, and invasive behavior (Rane *et al.*, 2023). Cancer is a broad category of disorders that can develop in almost any tissue, and its progression is impacted by a variety of intracellular signaling pathways that either promote or inhibit tumorigenesis (Walter and Arnold, 2019; Mohammadi *et al.*, 2024). Cancer continues to be a leading cause of death worldwide and a significant health burden. According to the World Health Organization, cancer-related deaths and new cases are projected to reach 13.2 million and 21.4 million annually by 2030 (Bray *et al.*, 2018). Globally, there were 9.7 million fatalities due to cancer and almost 20 million new instances of cancer in 2022 alone, with rising incidence largely linked to population growth, ageing, and lifestyle-associated contributing factors (Cao *et al.*, 2024).

Breast cancer arises from the malignant transformation of epithelial cells within the breast ducts or lobules. Its molecular features and clinical behavior vary greatly (Dalla *et al.*, 2025). Breast cancer is frequently classified into four groups based on immunohistochemistry profiling: triple-negative breast cancer (TNBC), progesterone receptor-positive (PR+), estrogen receptor-positive (ER+),

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and human epidermal growth factor receptor 2-positive (HER2+) (Shaath *et al.*, 2021). ER expression is reported in approximately 70% to 75% of invasive breast cancers and serves as a critical diagnostic and prognostic marker (Miah *et al.*, 2019; Nicolini *et al.*, 2018). 15 to 25% of breast tumors have HER2 overexpression, which is linked to advanced disease and specific treatment choices (Iqbal *et al.*, 2014). TNBC, accounting for nearly 20% of cases, is highly aggressive and lacks targeted treatment options (Loibl and Gianni, 2017).

Pakistan has one of Asia's highest age-standardized incidences of breast cancer, which is concerning. According to GLOBOCAN 2022 estimates, there were over 30,000 new cases of breast cancer and over 15,000 deaths, which highlights the disease's substantial impact on public health (Siddiqui *et al.*, 2024). Breast cancer in Pakistani women tends to present at a younger age compared to western populations, often between 47 and 49 years, and is frequently diagnosed at advanced stages due to limited awareness, socioeconomic barriers, and restricted access to healthcare facilities (Kamal *et al.*, 2024). Risk factors include hormonal influences, obesity, reproductive history, genetic predisposition, and lifestyle factors (Azim *et al.*, 2021; Ahmad *et al.*, 2023).

The development of breast cancer is caused by cumulative changes in tumor suppressor and oncogenes at the molecular level. Phosphatase and Tensin homolog (PTEN), which is found on chromosome 10q23, is crucial tumor suppressor gene (Masson and Williams, 2020). PTEN encodes a 403-amino acid dual-specificity phosphatase that negatively regulates the phosphatidylinositol 3-kinase (PI3K)/AKT/mTOR signaling pathway, which is central to cellular proliferation, survival, and metabolism (Sansal and Sellers, 2004; Papa and Pandolfi, 2019). PTEN loss or inactivation causes prolonged PI3K/AKT signaling, which promotes unchecked cell division, survival, and tumor growth (Abadi *et al.*, 2021). Breast and other cancers have frequently been linked to PTEN alterations, omissions, promoter methylation and post-translational modifications (Leslie *et al.*, 2010). A crucial portion of the phosphatase domain is encoded by exon 5 of the *PTEN* gene, which is regarded as a mutational hotspot. Changes in this exon have been demonstrated to reduce PTEN's lipid phosphatase activity (Shaw and Ras, 2006; Engelman *et al.*, 2006). Besides its involvement in signaling regulation, PTEN contributes to genomic stability and DNA repair by facilitating RAD51 recruitment following DNA damage (Ma *et al.*, 2019). Additionally, hereditary cancer syndromes like Cowden syndrome, which increases the risk of breast and other cancers, are associated with germline PTEN mutations (Song *et al.*, 2012).

Despite PTEN's known involvement in the disease,

there is still little information on exon-specific mutations, especially exon 5, in Pakistani breast cancer patients. Thus, the goal of this study is to look into exon 5 mutations of the *PTEN* gene in women with breast cancer from Pakistan. To support future diagnostic and treatment approaches, this work aims to improve understanding of PTEN's role in breast cancer development and progression and to provide baseline molecular data for the local population.

MATERIALS AND METHODS

Study design and place of work

This study was carried out using both field and lab work. Samples of breast cancer tissue were obtained from patients who were admitted to different hospitals in Lahore, Pakistan. All molecular analyses were conducted at the Molecular Biology Laboratory, Department of Zoology, University of Education (DSNT), Lahore.

Sample collection and storage

Fifteen tissue samples of breast cancer were obtained intraoperatively during surgical procedures. Fresh tumor tissues were placed in sterile biopsy jars without formalin added to prevent DNA cross-linking and degradation. Samples were brought to the lab in an ice box as soon as they were collected and stored at -20 °C in a varioline freezer until further processing. Every sample handling procedure was carried out in an aseptic environment. A unique identification code was given to every tissue sample. The hospital source and patient age were recorded for reference (Supplementary Table I).

Genomic DNA extraction

Genomic DNA was isolated from breast cancer tissue samples by two methods: The organic (phenol-chloroform) method and a commercial genomic DNA extraction kit (GeneJET™ Genomic DNA Purification Kit).

Organic DNA extraction method

Frozen tissue samples were thawed at room temperature and lysed using a solution containing 375 µL of 0.2 M sodium acetate, 50 µL of 10% SDS, and 10 µL of proteinase K (10 mg/mL). The samples were vortexed briefly and incubated at 37 °C for 24 h. Following incubation, 120 µL of phenol: chloroform: isoamyl alcohol (PCI=25:24:1) was added, mixed gently, and centrifuged at 13,500 rpm for 20 to 30 min. The aqueous phase was carefully transferred to a fresh tube, and 95% chilled ethanol was used to precipitate the DNA. After 10 min of incubation at -20°C, the samples were centrifuged once more. The DNA pellet was washed with 70% ethanol, air-dried, and resuspended in 100 µL of TE buffer. Extracted

DNA was stored at -20 °C.

Kit-based DNA extraction

A small section (<1 cm) of thawed tissue was excised, ensuring removal of fatty material, and mechanically crushed. The tissue was digested in 180 µL digestion buffer with 20 µL proteinase K and incubated at 56 °C until complete lysis. RNase treatment was performed, followed by addition of lysis buffer and ethanol. The lysate was moved to a DNA purification column and treated in accordance with the manufacturer's guidelines. DNA was kept at -20°C after being eluted in 200 µL of elution buffer.

DNA quantification and quality assessment

Extracted DNA was assessed qualitatively and quantitatively by agarose gel electrophoresis using a 2% agarose gel prepared in TAE buffer containing ethidium bromide. Samples of DNA were placed next to a molecular weight ladder and electrophoresed at 120 V and 500 mA for 35 min. DNA bands were visualized under UV illumination using a gel documentation system, where band intensity reflected DNA concentration.

Primer design and optimization

The genomic sequence of the *PTEN* gene was retrieved from Ensembl (Ensembl ID: ENST00000371953.8; Ref. Seq: NM_000314.8). Forward and reverse primers targeting exon 5 were designed using Primer3 software and validated through in silico BLAST analysis (Table I).

Primers were synthesized commercially and diluted to working concentrations. Gradient PCR was performed to optimize annealing temperature, with the optimal temperature determined to be 59 °C.

Table I. Primer sequences used for PTEN exon 5 amplification.

Length (bp)	Tm (°C)	GC (%)	Sequence (5'-3')
20	58.6	55.0	F= TGACCTATGCTACCAGTCCG
23	57.6	43.5	R= AAATTCTCAGATCCAGGAAGAGG

PCR amplification

PCR amplification was performed using a Simple Amplify PCR system. Each 20 µL reaction contained 10 µL of 2× PCR master mix, 1 µL of forward primer, 1 µL of reverse primer, 2 µL of genomic DNA, and 6 µL of nuclease-free water. There were 35 cycles of amplification, each including denaturation, annealing, and extension. To verify successful amplification, PCR products were examined on a 2% agarose gel.

DNA sequencing and sequence analysis

Forward primers and PCR products were sent to a commercial sequencing facility (Lab Genetics, Lahore) for Sanger sequencing. Sequencing results were provided as chromatograms.

Chromas software was used to analyze sequencing chromatograms. To find mutations in exon 5, the obtained sequences were compared with the *PTEN* reference sequence using NCBI nucleotide BLAST.

RESULTS

Figure 1 illustrates the PCR amplification and sequencing analysis of *PTEN* exon 5 in samples UEBCT36, UEBCT37, and UEBCT38. As shown in Figure 1, PCR amplification of exon 5 produced clear and distinct bands of the expected size on a 2% agarose gel, confirming the specificity and efficiency of the amplification process. These results verified the integrity of the extracted genomic DNA and confirmed its suitability for sequencing analysis. The absence of non-specific bands or smearing further indicates optimal PCR conditions. Figure 1c-h present the Sanger sequencing chromatograms and corresponding BLAST alignments for samples UEBCT36, UEBCT37, and UEBCT38. The chromatograms exhibited well-defined, sharp nucleotide peaks without background noise, indicating high-quality sequencing data. BLAST analysis of the query sequences against the reference exon 5 sequence of the *PTEN* gene (Transcript ID: ENST00000371953.8) revealed a 100% similarity index for all three samples. No nucleotide substitutions, insertions, or deletions were observed, demonstrating the absence of mutations in exon 5 of the *PTEN* gene in these patients.

Supplementary Figure 1 shows the chromatogram and BLAST alignment analyses of samples UEBCT39, UEBCT40, UEBCT41, and UEBCT42, while those of UEBCT43, 44 and 52 are shown in Supplementary Figure 2 and these of UEBCT53, 54, 55 and 56 are depicted in Supplementary Figure 3. The sequencing chromatograms in all these samples displayed clear, uninterrupted peak patterns, indicating accurate base calling throughout the amplified exon 5 region. These results confirm the reliability of the sequencing procedure and exclude the presence of technical artifacts. The corresponding BLAST alignments in all these samples demonstrated complete alignment of each query sequence with the reference exon 5 sequence of the *PTEN* gene. All four samples showed a 100% sequence identity, confirming that exon 5 remained genetically conserved in these patients. This finding was consistent across patients with varying tumor grades, lymphovascular invasion status, and treatment histories, suggesting that

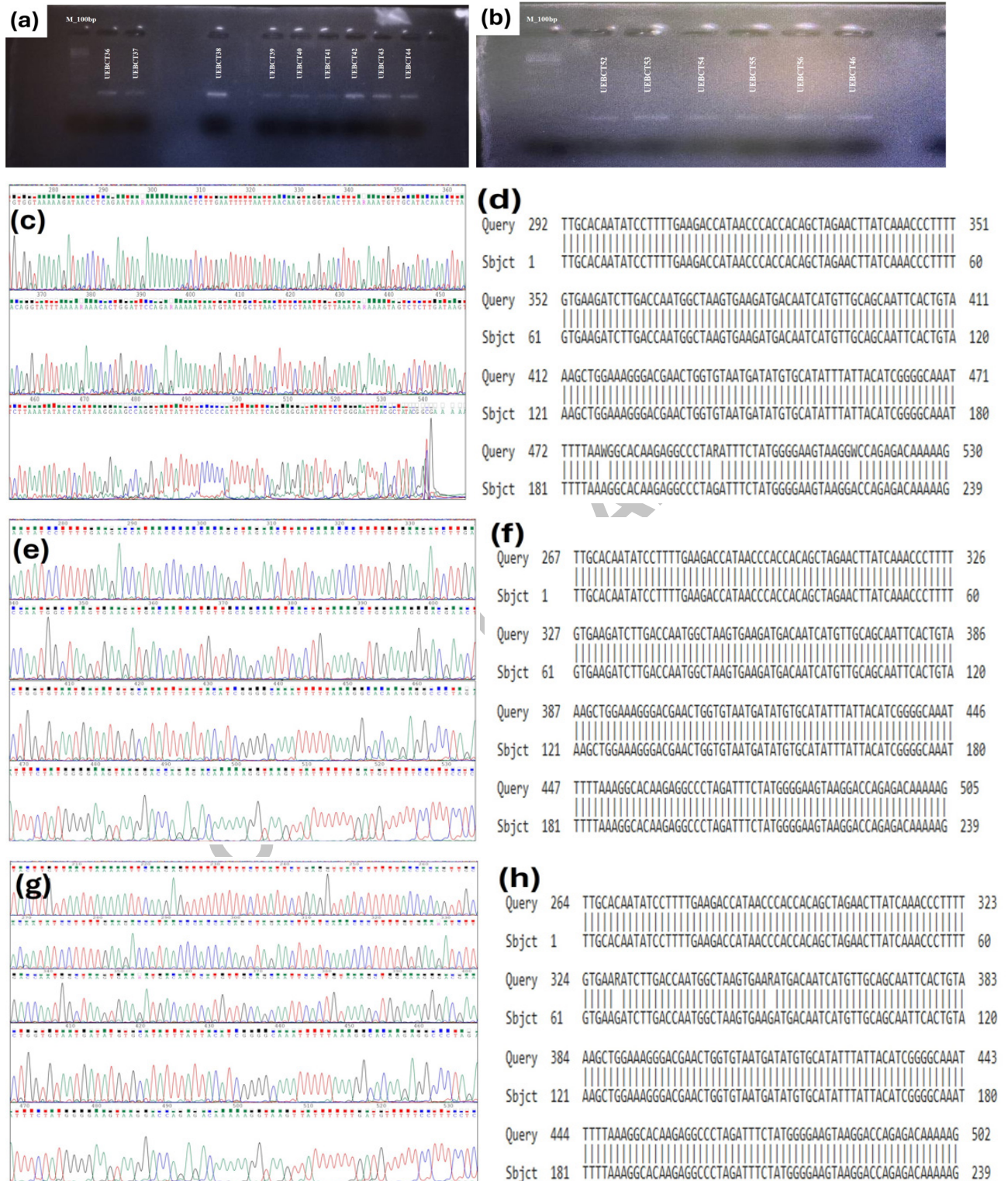


Fig. 1. (a, b) Amplified PCR Product Bands were fractioned on 2% Agarose Gel, (c, d) Chromatogram and BLAST alignment of query sequence of sample UEBCT36, (e, f) Chromatogram and BLAST alignment of query sequence of sample UEBCT37, and (g, h) Chromatogram and BLAST alignment of query sequence of sample UEBCT38 with reference sequence of Exon 5 of PTEN gene.

exon 5 mutations were not present despite clinical heterogeneity.

These findings further support the absence of exon 5 mutations across different histological subtypes, including invasive ductal and invasive lobular carcinoma.

DISCUSSION

Breast cancer represents the most frequently diagnosed malignancy among women all over the world, and its ratio is high in lower- and middle-income countries, including Pakistan. Pakistan has the highest frequency of breast cancer in Asia, according to GLOBOCAN 2022, thus it is important to investigate its molecular and genetic aspects. Breast cancer is caused by multiple factors which include environmental, genetic, and epigenetic (Xiong *et al.*, 2025). Among the several genes that cause breast cancer, *PTEN* gene is essential as a tumor suppressor. *PTEN* gene is linked to breast cancer. It has a pivotal role in controlling cell survival and multiplication via PI3K/AKT signaling pathway. This pathway becomes hyperactive when *PTEN* function is lost and it supports oncogenesis. Inactivation of *PTEN* can result from mutations, deletions, epigenetic silencing, or post-translational modifications (Wander *et al.*, 2020).

Importantly, exon 5 is regarded as a mutational hotspot among its nine exons due to the fact that it produces the phosphatase catalytic domain, which is necessary for its tumor-suppressive activity. Mutations in this exon can affect *PTEN*'s function. Due to the functional importance of exon 5, it has been the subject of many studies (Baig *et al.*, 2011). 19 distinct *PTEN* gene mutations, including three missense mutations (319G>A (Asp106Asn), 389G>A (Arg129Gln), and 482G>A (Arg160Lys), were found in a study that analyzed DNA from 350 breast cancer patients and 400 healthy controls. The 389G>A mutation is correlated with R130Q in exon 5. The results indicate that a variety of germline *PTEN* mutations, including those in exon 5, may have role in occurrence of breast cancer in the Pakistani population (Baig *et al.*, 2011).

Recent research has identified missense mutations in exon 5 of the *PTEN* gene, namely R130G and R130Q, both at codon 130, a crucial residue in the *PTEN* protein's phosphatase domain. The tumor suppressor activity of *PTEN* is affected by these mutations. To determine the functional importance of these mutations next generation sequencing (NGS) was performed by researchers along with in-silico structural analysis. The results emphasize the possible adverse effects of exon 5 mutations in breast cancer progression within the Pakistani community (Iqbal *et al.*, 2024). The biological significance of exon 5 led us to select it. This exon encodes a portion of the phosphate

domain that is crucial for tumor suppressor activity and has been identified as a hotspot for mutations in a variety of cancer types. In the present study, we analyzed the exon 5 of the *PTEN* gene in 15 breast cancer samples collected from Pakistani patients.

Interestingly, none of the samples showed any mutation by Sanger sequencing. Although this negative result was originally unexpected, it coincides with several indicators that demonstrate its impact and significance. It is important to recognize the scientific importance of these findings and place them in the broader framework of breast cancer genetics. The results in this study correspond with a number of other studies that have documented missing mutation frequencies in *PTEN* exon 5 in breast cancer. A study conducted in Sweden revealed that *PTEN* protein loss was prevalent in breast cancer, but gene alterations were rare. They conclude that loss of *PTEN* function is more often caused by deletions than by point mutations and consequently discovered no mutation in exon 5 (Saal *et al.*, 2007).

In breast cancer, LOH at the *PTEN* loci frequently results in decreased gene function. Research indicates that LOH at this location is linked to increased tumor size, grade, ER expression loss and metastasis to lymph nodes. This means that even in the absence of direct mutations, chromosomal alterations may affect *PTEN* function (Zhang *et al.*, 2013). Expression of *PTEN* may be downregulated by microRNA disruption, post-translational modifications, and promoter hyper methylation, this may lead to decreased protein levels without changing the DNA sequence. Studies have shown that a considerable percentage of TNBC have *PTEN* protein loss even in the absence of mutations (Chen *et al.*, 2023). TP53, PIK3CA and *PTEN* gene mutations were examined in a study of breast cancer patients in Khyber Pakhtunkhwa (KPK, Pakistan). By using whole exome sequencing (WES) and other bioinformatics methods, the study sought to confirm the mutational spectrum of here important genes and find potential biomarkers. Instead of finding any mutations in exon 5, they found mutations in other exons (Ahmad *et al.*, 2023).

Several factors can help explain our negative findings. First of all, *PTEN* inactivation in breast cancer is not necessarily caused by mutations in exon 5. Different exons, regulatory regions, or even whole different pathways may result in loss of function. One well-known mechanism that results in decreased gene expression is *PTEN* promoter hyper methylation. Similarly, *PTEN* function can be suppressed without changing its sequence by post-translational modifications or interactions with microRNA. Secondly, most of our patients were possibly sporadic cases, and the published literature suggests

that PTEN mutations are more often found in hereditary or familial breast cancers, particularly those associated with PTEN hamartoma tumour syndrome (PHTS) or Cowden syndrome. Sporadic cases may exhibit distinct genetic profiles. Thirdly, the study included 15 breast cancer samples from the Pakistani population. Although this sample size provided preliminary insight into PTEN exon 5 mutations, it is relatively small and may not fully represent the genetic diversity of the entire population. As our sample size (n=15) was limited, which may have limited our ability to detect rare mutations. Unfortunately, we were unable to collect additional samples due to illiteracy and limited knowledge. The patients were unwilling to provide their samples and did not cooperate with us properly. Since PTEN mutations are generally not common in breast cancer, a larger cohort may be required to record these occurrences. Additionally, sample collection was not subtype dependent, as PTEN mutations are more common in patients with TNBC.

Sanger sequencing is widely accepted and reliable for detecting point mutations and small sequence variations in targeted regions of DNA. It has limitations, especially when it comes to detect large deletions or low-level alterations. For comprehensive mutation profiling, techniques such as multiplex ligation-dependent probe amplification (MLPA), quantitative PCR, or NGS might be more suitable. Despite the lack of mutations, our research remains highly valuable. It adds to the expanding database of research challenging the uniqueness of mutation-based PTEN inactivation and offers initial understanding into the genetic background of breast cancer in a Pakistani cohort. It also emphasizes the need for more thorough approaches to studying tumor suppressor genes, such as analysis of protein levels, methylation and gene expression.

CONCLUSION

In conclusion, although our study samples did not contain any mutations in exon 5 of the *PTEN* gene, this result is consistent with several studies and highlights the complex nature of cancer genetics. According to our findings, non-mutational occurrences in our population might be more frequently responsible for PTEN dysfunction in breast cancer. This points out that in order to fully understand PTEN's role in Pakistani breast cancer patients, extensive research using whole-gene sequencing, larger sample sizes, epigenetic tests and molecular subtype analysis is required. Even though mutations in PTEN gene account for 5-10% of sporadic breast tumors globally, the probability of discovering a mutation in such a small sample size is already low. There is evidence that the frequencies and patterns of mutations can vary

greatly across ethnic and geographic groups. The rate of PTEN mutations in breast cancer has been found to be significantly greater in Western populations compared to South Asian communities.

By combining all these techniques, a more in-depth evaluation of PTEN's role in the Pakistani population and its contribution to the development of breast cancer will be possible.

DECLARATIONS

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IRB approval

This study was approved by the Chairman Ethical Committee/EIRB of the University vide UE/S&T/2023/105 dated 30th August, 2023.

Ethical approval and patient enrollment

The Ethical Review Board of the University of Education, Lahore gave approval for this study. Subsequently, permission for sample collection was granted by the ethical committees of the respective hospitals. Patients with breast cancer were found once the diagnosis was confirmed by routine clinical and pathological examinations. Prior to sample collection, each subject gave written informed consent.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Supplementary material

There is supplementary material associated with this article. Access the material online at: <https://dx.doi.org/10.17582/journal.pjz/20260416110135>

Statement of conflict of interest

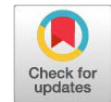
The authors have declared no conflict of interest.

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Supplementary Material

Investigating the Exon 5 *PTEN* Gene Mutations in Human Breast Cancer: Hospital-Based Study from Lahore, Pakistan

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Supplementary Table I. Assigned sample IDs and patient information.

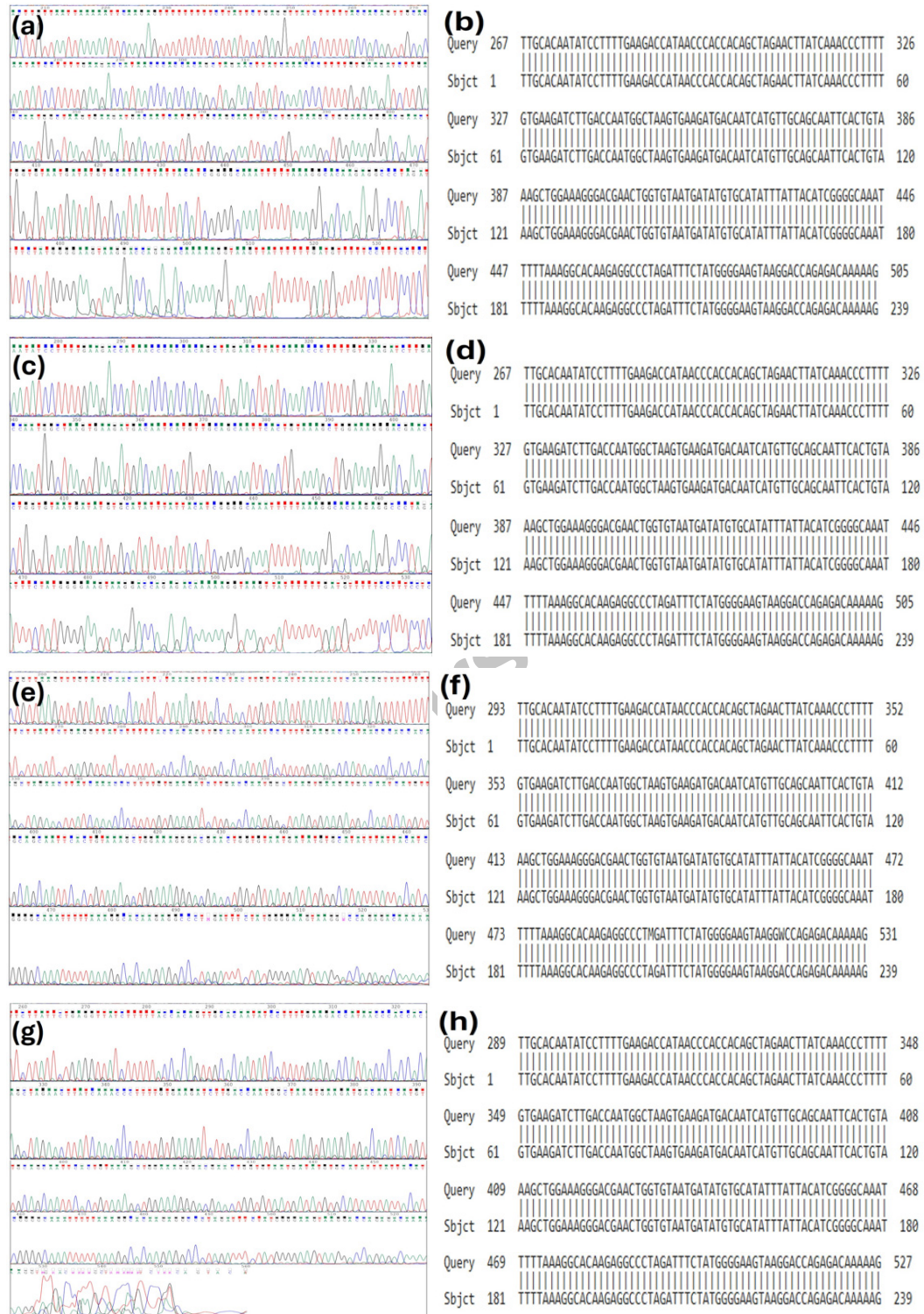
Sample ID	Hospital Name	Age
UEBCT36	Jinnah Hospital, Lahore	51
UEBCT37	Sir Ganga Ram Hospital, Lahore	45
UEBCT38	Sir Ganga Ram Hospital, Lahore	40
UEBCT39	Sir Ganga Ram Hospital, Lahore	45
UEBCT40	Sir Ganga Ram Hospital, Lahore	62
UEBCT41	Sir Ganga Ram Hospital, Lahore	42
UEBCT42	Sir Ganga Ram Hospital, Lahore	46
UEBCT43	Sir Ganga Ram Hospital, Lahore	43
UEBCT44	Sir Ganga Ram Hospital, Lahore	60
UEBCT46	Sir Ganga Ram Hospital, Lahore	35
UEBCT52	Jinnah Hospital, Lahore	54
UEBCT53	Jinnah Hospital, Lahore	50
UEBCT54	Gulab Devi Chest Hospital, Lahore	71
UEBCT55	Jinnah Hospital, Lahore	63
UEBCT56	Gulab Devi Chest Hospital, Lahore	62

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0030-9923/2026/0001-0001 \$ 9.00/0

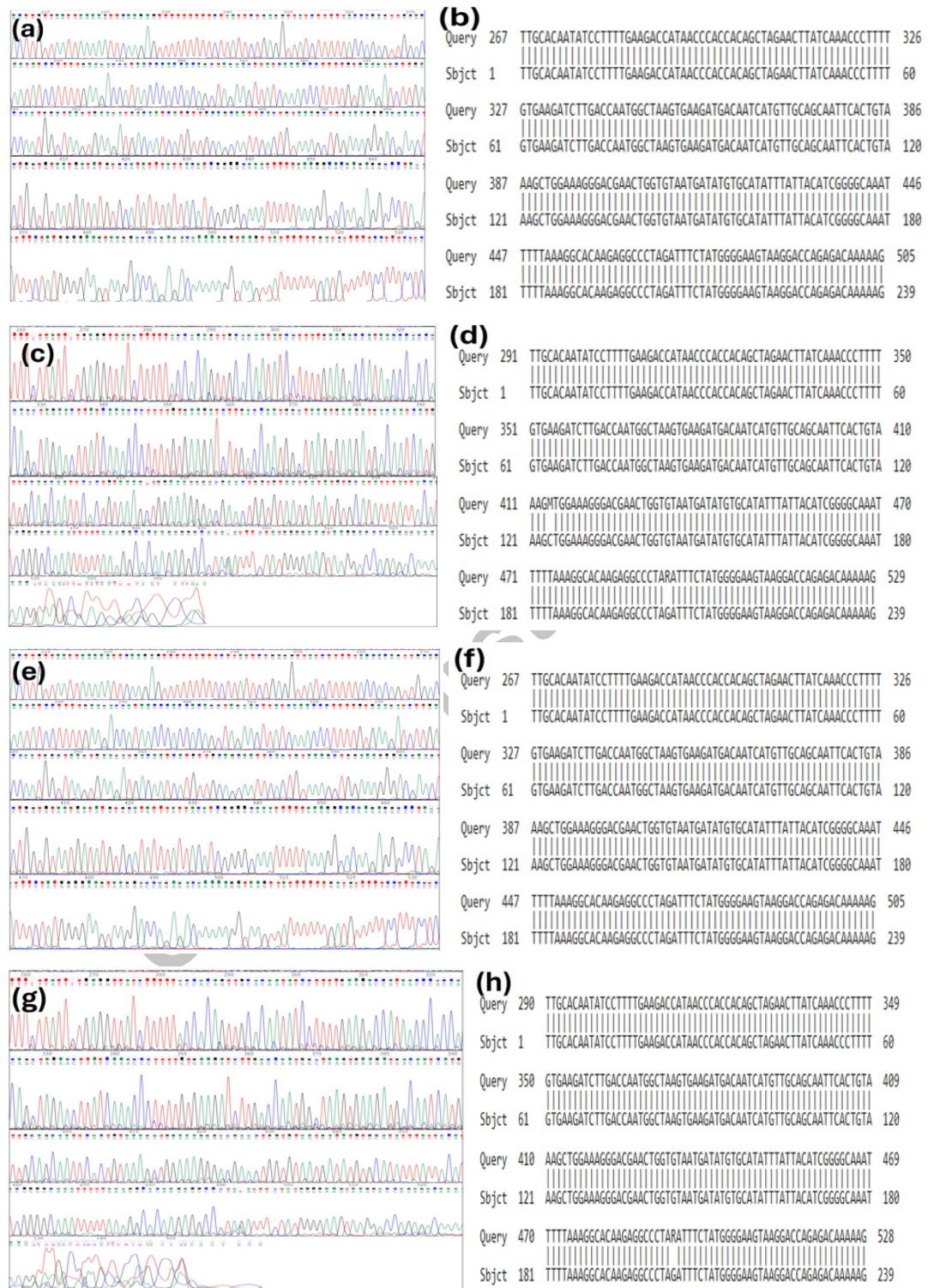


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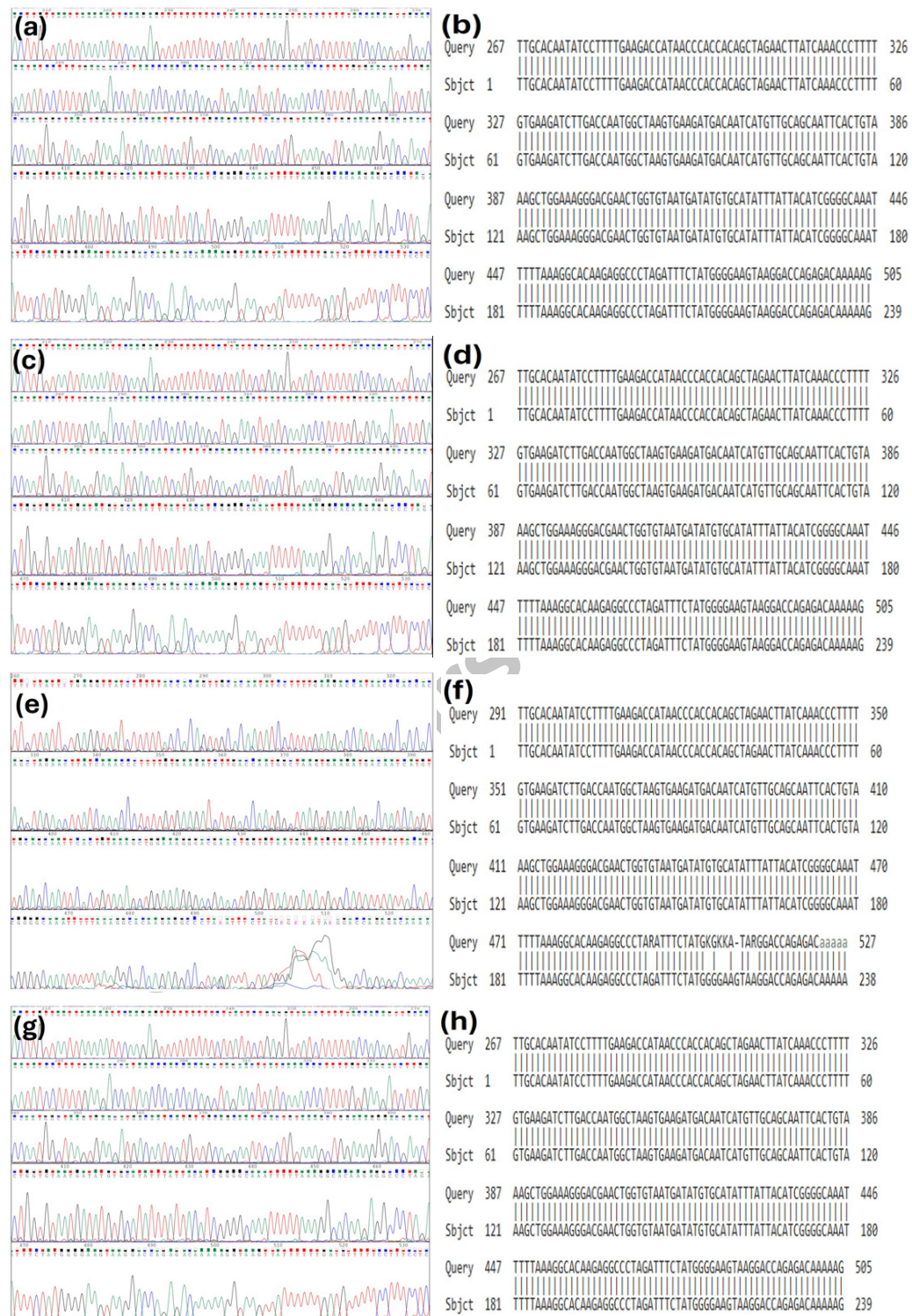
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Supplementary Fig. 1. (a, b) Chromatogram and BLAST alignment of query sequence of sample UEBCT39, (c, d) Chromatogram and BLAST alignment of query sequence of sample UEBCT40, (e, f) Chromatogram and BLAST alignment of query sequence of sample UEBCT41, and (g, h) Chromatogram and BLAST alignment of query sequence of sample UEBCT42 with reference sequence of Exon 5 of *PTEN* gene.



Supplementary Fig. 2. (a, b) Chromatogram and BLAST alignment of query sequence of sample UEBCT43, (c, d) Chromatogram and BLAST alignment of query sequence of sample UEBCT44, (e, f) Chromatogram and BLAST alignment of query sequence of sample UEBCT46, and (g, h) Chromatogram and BLAST alignment of query sequence of sample UEBCT52 with reference sequence of Exon 5 of *PTEN* gene.



Supplementary Fig. 3. (a, b) Chromatogram and BLAST alignment of query sequence of sample UEBCT53, (c, d) Chromatogram and BLAST alignment of query sequence of sample UEBCT54, (e, f) Chromatogram and BLAST alignment of query sequence of sample UEBCT55, and (g, h) Chromatogram and BLAST alignment of query sequence of sample UEBCT56 with reference sequence of Exon 5 of *PTEN* gene.