Ann Med Res

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In silico characterization of missense mutations in PI3K/AKT/mTOR signaling genes in breast cancer and their role in therapeutic resistance

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MAIN POINTS

- Comprehensive in silico analysis identified recurrent missense mutations in PI3K/AKT/mTOR pathway genes in breast cancer that are predicted to disrupt protein function.
- The PTEN (D92H, D92N, R130G, R130Q, C136R, and C136Y), AKT1 (L52R), and AKT2 (R170W) variants demonstrated strong deleterious effects across multiple computational prediction tools.
- Structural modeling and interaction analysis revealed that these mutations caused significant alterations in protein stability and non-covalent interactions.
- These findings highlight potential biomarkers for therapeutic resistance and provide candidate targets for future breast cancer precision medicine strategies.

Cite this article as: Akcesme B, Lokvančić H, Islam N. In silico characterization of missense mutations in PI3K/AKT/mTOR signaling genes in breast cancer and their role in therapeutic resistance. *Ann Med Res*. 2025;32(12):541--551. doi: 10.5455/annalsmedres.2025.07.185.

ABSTRACT

Aim: This study aimed to investigate the most frequent missense mutations in key genes of the PI3K/AKT/mTOR signaling pathway to evaluate their potential role in the progression of breast cancer and the development of therapy resistance using computational analyses.

Materials and Methods: Nine genes involved in the PI3K/AKT/mTOR pathway were systematically analyzed by screening mutation databases and applying a range of computational prediction tools to assess the possible deleterious effects of missense variants on protein function and pathway regulation.

Results: Several mutations with predicted deleterious effects were identified, including *PTEN* (D92H, D92N, R130G, R130Q, C136R, and C136Y), *AKT1* (L52R), and *AKT2* (R170W). These variants were predicted to contribute to aberrant PI3K/AKT/mTOR pathway activation, enhanced cancer cell survival, and therapeutic resistance in breast cancer.

Conclusion: The findings provided insights into the mutational landscape of breast cancer, proposing potential biomarkers for risk stratification and novel therapeutic targets. Further experimental validation and functional studies are recommended to clarify the clinical significance of these mutations and to guide the development of personalized interventions for breast cancer.

Keywords: Breast cancer, PI3K/AKT/mTOR pathway, Missense mutations, Computational analysis, Therapy resistance

Received: Jul 10, 2025 **Accepted:** Sep 22, 2025 **Available Online:** Dec 25, 2025

INTRODUCTION

Breast cancer (BC) is one of the leading causes of death among females worldwide and although less common, can also affect men. Currently, chemotherapy, endocrine therapy, and targeted therapy have a high success rate in both disease treatment and disease prevention [1]. The PI3K/AKT/mTOR pathway is linked to disease progression and is also responsible for the development of drug resistance [2]. Currently, only a limited number of therapies are available for the treatment of various subtypes of breast cancer. Endocrine therapy (ET) is used to treat HR+ disease, HER2+ disease is treated with HER2-targeted therapy, and chemotherapy and immunotherapy are used to treat patients with BRCA mutations with triple-negative breast cancer (TNBC). Drug resistance eventually leads to tumor relapse in BC, as well as overexpression of the BC resistance protein (BCRP) and a variety of other events [1,3].

The PI3K/AKT/mTOR pathway plays a critical role in various cellular processes, including cell growth and metabolism [4]. Activation of this pathway typically occurs in response to external stimuli, such as growth factors, leading to the activation of phosphoinositide 3-kinase (PI3K). Subsequent PI3K

activation promotes the recruitment of AKT to the plasma membrane, where AKT is then phosphorylated and activated by phosphoinositide-dependent kinase 1 (PDK1) and the mammalian target of rapamycin complex 2 (mTORC2) [5]. Once activated, AKT promotes the activation of the mechanistic target of rapamycin (mTOR), enabling the regulation of downstream signaling events associated with cellular growth and metabolic control [1,3].

PI3KCA leads to PI3K activation and is one of the most frequently mutated genes in BC. The main role of *PTEN* is to dephosphorylate PIP3 molecules, and AKT cannot be phosphorylated by mTORC2 [6]. *AKT1, AKT2*, and *AKT3* are AKT and are major downstream targets of PI3K. IRS1 activates the PI3K/Akt/mTOR pathway, and its variants are the most studied because they are expressed in different tissues [7]. GSK3B, together with *AKT1, AKT2*, and *AKT3*, activates AKT [8]. TSC1 and TSC2 impact the phosphorylation of mTOR and its downstream effector molecules [9].

Dysregulation of the PI3K/AKT/mTOR pathway is seen in various cancer types [10] and this pathway should serve as a target for therapeutic treatment due to its specific role. Studies conducted on several chemotherapeutic drugs, such as doxorubicin, docetaxel, fluorouracil tamoxifen, and paclitaxel, have shown that chemoresistance can develop, and the main cause is mutations in the PI3K/AKT/mTOR pathway [1,11,12].

This study focused on the PI3K/AKT/mTOR pathway and the genes most frequently mutated in patients with BC. The investigation examines the involvement of the PI3K/AKT/mTOR signaling pathway in drug resistance, with particular attention to specific genes within this pathway. In addition, missense variants in these genes were analyzed to better understand their potential contributions to drug resistance processes. By comprehensively examining the PI3K/AKT/mTOR pathway, its associated genes, and the effects of missense variants, the project aims to provide insights into the molecular mechanisms underlying BC treatment resistance.

MATERIALS AND METHODS

This retrospective, in silico study focused on nine key genes of the PI3K/AKT/mTOR pathway (*PIK3CA, PIK3CB, PIK3CD, PIK3R1, AKT1, AKT2, AKT3, PTEN*, and *MTOR*). We collected somatic missense variants reported in breast cancer from mutation databases (cBioPortal). The most frequent variants were prioritized based on recurrence in breast cancer cohorts and functional impact was assessed using multiple computational prediction tools (PredictSNP, SNPs&GO). Variants predicted as deleterious were further mapped to protein domains (DynaMut and DUET) to predict their impact on protein stability, flexibility, and pathway context. The primary endpoint of the study was the identification of the most frequent missense mutations in the nine PI3K/AKT/mTOR pathway genes, while the secondary endpoint was the predicted functional effect of these variants on protein function and pathway regulation using computational tools.

Retrieval of the target genes

Genes for this study were selected based on their well-established involvement in the PI3K/AKT/mTOR signaling pathway, which plays a pivotal role in BC progression and is recognized as a significant contributor to therapeutic resistance. A recent literature review identified nine pertinent genes for analysis: *PIK3CA, PTEN, AKT1, AKT2, AKT3, IRS1, GSK3B, TSC1,* and *TSC2*. By concentrating on these key regulators within the pathway, this study aims to examine their potential contribution to BC treatment resistance.

The cBioPortal for Cancer Genomics (https://www.cbioportal.org/) was used to identify the most frequent genetic alterations in genes involved in the PI3K/AKT/mTOR pathway among patients with BC. This database provides access to large-scale cancer genomics datasets across various cancer types, including tools for visualization, mutation analysis, and data retrieval. For this study, the "Breast Cancer (METABRIC, Nature 2012 & Nat Commun 2016)" dataset, which includes data from 2,509 patients with BC, was selected as one of the largest datasets in the breast cancer portal.

Identification of mutations in selected genes

Mutations in the selected genes associated with the PI3K/AKT/mTOR pathway were analyzed using the cBioPortal database. These genes were chosen based on their recognized roles within this signaling pathway and their documented associations with BC treatment resistance. Only missense mutations were considered for subsequent analysis. Following stratification by mutation type, the most frequently occurring missense mutations within the BC cohort were identified and prioritized for downstream analysis.

Sequence-based methods for predicting gene pathogenicity

The functional impact of the most frequent missense mutations was evaluated using two sequence-based computational tools: PredictSNP (https://loschmidt.chemi.muni.cz/predictsnp/) and SNPs&GO (https://snps-and-go.biocomp.unibo.it/snps-and-go/). To predict whether a given mutation is likely to be disease-causing or benign and to estimate the potential effect of the mutation on protein function, PredictSNP aggregates results from six individual methods: MAPP, PhD-SNP, PolyPhen-1, PolyPhen-2, SIFT, and SNAP. Alongside the classification, PredictSNP provides a prediction score expressed as a percentage, indicating the prediction's expected accuracy. Higher percentages correspond to greater classification reliability; for example, a score of 85% indicates that the prediction is expected to be correct in 85% of cases based on the integrated tools' benchmarking.

https://doi.org/10.5455/annalsmedres.2025.07.185

Variants predicted as deleterious with high percentage scores were prioritized for downstream analyses. SNPs&GO is another predictive platform that classifies single-point protein mutations as either neutral or associated with protein functional changes. For each variant, SNPs&GO provides a probability score and a Reliability Index (RI) value, which reflects the prediction's confidence level. Higher RI values indicate greater reliability of the classification (Usually RI $\geq$ 7 were considered reliable disease-associated predictions).

FASTA sequences for *PTEN* (P60484), *AKT1* (P31749), and *AKT2* (P31751) were retrieved from the UniProt database (https://www.uniprot.org/).

Structure-based methods for protein stability prediction

DynaMut (https://biosig.lab.uq.edu.au/dynamut/), a structure-based computational tool that applies normal mode analysis to predict changes in protein stability resulting from point mutations, was used to evaluate the impact of the selected mutations on protein stability. The predicted $\Delta\Delta G$ values describe the impact of mutations on protein stability: negative values ($\Delta\Delta G < 0$) indicate destabilization, whereas positive values ($\Delta\Delta G > 0$) suggest stabilization of the protein structure. DynaMut provides vibrational entropy ($\Delta\Delta S$) values to estimate the effect of mutations on molecular flexibility. A positive $\Delta\Delta S$ indicates increased flexibility, whereas a negative $\Delta\Delta S$ reflects reduced flexibility and a more rigid structure. Together, these predictions allow the assessment of both the thermodynamic stability and dynamic behavior of the protein upon mutation. The Protein Data Bank in Europe Knowledge Base (PDBe-KB, https://www.ebi.ac.uk/pdbe/pdbe-kb/) was used to obtain accurate protein structures for analysis, prioritizing those with the lowest resolution values and the largest covered sequences. Moreover, PDBe-KB provided information about the ligand binding sites relevant to each structure. The effect of single-point mutations on protein stability was assessed using the DUET web server (https://biosig.lab.uq.edu.au/duet/), an integrated computational approach that combines the results of two independent methods: mCSM and SDM. DUET utilizes a machine-learning framework to improve the accuracy of stability predictions by incorporating both environment-specific substitution matrices and graph-based signatures of protein structure. The output is presented as a change in the Gibbs free energy ($\Delta\Delta G$, kcal/mol), where negative values indicate destabilizing mutations and positive values indicate stabilizing effects on the protein structure. The corresponding protein files were downloaded from the Protein Data Bank in Europe (https://www.ebi.ac.uk/pdbe/) for use in the DynaMut and DUET analyses.

Visualization of interactions in wild type (WT) and mutant structures

BIOVIA Discovery Studio 2021 was employed to facilitate the detailed examination of the selected proteins in both wild-type and mutant configurations. This platform offers robust capabilities for molecular visualization and interaction analysis, enabling comprehensive protein structure and surrounding exploration [13]. The immediate molecular environment of specific residues, both in their native and mutated forms, was visualized using Discovery Studio to investigate potential changes in noncovalent interactions, such as hydrogen bonding, hydrophobic and electrostatic interactions.

RESULTS

According to the literature, *PIK3CA, PTEN, AKT1, AKT2, AKT3, IRS1, GSK3B, TSC1,* and *TSC2* are recognized components of the PI3K/AKT/mTOR signaling pathway. These genes were further investigated to identify relevant mutations using cBioPortal and the "Breast Cancer (METABRIC, Nature 2012 & Nat Commun 2016)" dataset. *PTEN*, *AKT1*,

Table 1. List of the selected genes with the mutation percentages from 2509 BC samples.

No	Gene	Mutation (%)	Domain
1.	*PIK3CA*	40.1%	PI3Ka
2.	*PTEN*	3.9%	DSPc
3.	*AKT1*	4.1%	PH
4.	*AKT2*	0.5%	Pkinase
5.	*AKT3*	/	/
6.	*IRS1*	/	/
7.	*GSK3B*	/	/
8.	*TSC1*	/	/
9.	*TSC2*	/	/

Table 2. List of the selected genes with the corresponding mutations identified in this study.

Gene	Mutation
PIK3CA	H1047R H1047L H1047Y H1047Q E545K E545A E545G E545D E545Q E542K E542A E542V
PTEN	D92A D92E D92G D92H D92N R130Q R130G C136R C136Y
AKT1	E17K L52R Q79K
AKT2	R170W

https://doi.org/10.5455/annalsmedres.2025.07.185

Table 3. Sequence-based prediction results from Predict SNP and consensus tools.

Gene	Mutation	PredictSNP	MAPP	PhD-SNP	PolyPhen-1	PolypPhen-2	SIFT	SNAP
PIK3CA	H1047R	60% (+)	43% (-)	77% (-)	67% (+)	71% (+)	45% (-)	58% (+)
	H1047L	63% (+)	74% (+)	73% (-)	59% (-)	72% (+)	90% (+)	71% (+)
	H1047Y	75% (+)	63% (+)	77% (-)	67% (+)	76% (+)	77% (+)	77% (+)
	H1047Q	74% (+)	73% (+)	51% (+)	67% (+)	71% (+)	46% (-)	58% (+)
	E545K	60% (+)	74% (+)	68% (-)	67% (+)	Unknown	79% (-)	50% (+)
	E545A	63% (+)	71% (+)	58% (+)	67% (+)	54% (-)	79% (-)	50% (+)
	E545G	51% (-)	72% (+)	82% (-)	67% (+)	54% (-)	79% (-)	50% (+)
	E545D	68% (+)	74% (+)	72% (+)	67% (+)	54% (-)	43% (-)	67% (+)
	E545Q	63% (+)	80% (+)	58% (+)	67% (+)	54% (-)	79% (-)	55% (+)
	E542K	65% (+)	76% (+)	51% (+)	67% (+)	43% (-)	46% (-)	58% (+)
	E542A	75% (+)	70% (+)	68% (+)	67% (+)	Unknown	46% (-)	50% (+)
	E542V	61% (-)	72% (+)	68% (-)	67% (+)	68% (-)	79% (-)	56% (-)
PTEN	D92A	76% (-)	78% (-)	88% (-)	67% (+)	68% (-)	79% (-)	85% (-)
	D92E	65% (-)	77% (-)	68% (-)	67% (+)	Unknown	79% (-)	72% (-)
	D92G	76% (-)	76% (-)	88% (-)	67% (+)	68% (-)	79% (-)	81% (-)
	D92H	87% (-)	77% (-)	88% (-)	74% (-)	81% (-)	79% (-)	87% (-)
	D92N	87% (-)	57% (-)	86% (-)	59% (-)	81% (-)	79% (-)	81% (-)
	R130Q	87% (-)	77% (-)	88% (-)	59% (-)	68% (-)	79% (-)	81% (-)
	R130G	87% (-)	84% (-)	86% (-)	74% (-)	43% (-)	79% (-)	85% (-)
	C136R	87% (-)	86% (-)	88% (-)	59% (-)	43% (-)	79% (-)	85% (-)
	C136Y	87% (-)	62% (-)	88% (-)	74% (-)	43% (-)	53% (-)	72% (-)
AKT1	E17K	76% (-)	65% (+)	73% (-)	74% (-)	81% (-)	79% (-)	56% (-)
	L52R	87% (-)	88% (-)	86% (-)	74% (-)	68% (-)	79% (-)	72% (-)
	Q79K	55% (-)	73% (+)	61% (-)	67% (+)	68% (-)	46% (-)	62% (-)
AKT2	R170W	87% (-)	56% (-)	88% (-)	74% (-)	68% (-)	79% (-)	62% (-)

% expected accuracy; (+) neutral; (-) deleterious.

Table 4. Sequence-based prediction results based on the SNP&GO computational tool.

Gene	Mutation	SNPs&GO	
		Effect	RI
PIK3CA	H1047R	Neutral	5
	H1047L	Neutral	6
	H1047Y	Neutral	6
	H1047Q	Neutral	4
	E545K	Neutral	2
	E545A	Neutral	5
	E545G	Disease Related Polymorphism	0
	E545D	Neutral	4
	E545Q	Neutral	5
	E542K	Neutral	3
	E542A	Neutral	5
	E542V	Neutral	3
PTEN	D92A	Disease Related Polymorphism	10
	D92E	Disease Related Polymorphism	10
	D92G	Disease Related Polymorphism	10
	D92H	Disease Related Polymorphism	10
	D92N	Disease Related Polymorphism	10
	R130Q	Disease Related Polymorphism	10
	R130G	Disease Related Polymorphism	10
	C136R	Disease Related Polymorphism	10
	C136Y	Disease Related Polymorphism	10
AKT1	E17K	Disease Related Polymorphism	10
	L52R	Disease Related Polymorphism	10
	Q79K	Disease Related Polymorphism	10
AKT2	R170W	Disease Related Polymorphism	4

RI reliability index.

https://doi.org/10.5455/annalsmedres.2025.07.185

Table 5. Stability results of mutation effects from DynaMut and DUET.

Genes	Substitution	ΔΔG DynaMut (kcal/mol)	ΔΔG ENCoM (kcal/mol)	ΔΔG mCSM (kcal/mol)	ΔΔG SDM (kcal/mol)	ΔΔG DUET (kcal/mol)	ΔΔSVib mCSM (kcal.mol^{-1} K^{-1})
PTEN	D92H	-0.284(-)	-0.119(-)	-0.228(-)	0.740(+)	-0.050(-)	0.149(++)
PTEN	D92N	-0.424(-)	-0.144(-)	-0.351(-)	-0.130(-)	-0.266(-)	0.180(++)
PTEN	R130Q	-0.541(-)	-0.208(-)	-1.302(-)	-1.660(-)	-1.487(-)	0.260(++)
PTEN	R130G	-1.481(-)	-1.090(-)	-1.720(-)	-1.700(-)	-2.008(-)	1.362(++)
PTEN	C136R	-1.146(-)	0.109(-)	-1.378(-)	-1.460(-)	-1.204(-)	-0.137(--)
PTEN	C136Y	-0.754(-)	0.761(+)	-0.989(-)	-1.670(-)	-1.230(-)	-0.952(--)
AKT1	L52R	1.821(+)	2.189(+)	-1.236(-)	-1.860(-)	-1.211(-)	-2.736(--)
AKT2	R170W	0.051(+)	0.073(-)	-0.505(-)	0.320(+)	-0.438(-)	-0.091(--)

ΔΔG change in the Gibbs free energy; ΔΔSVib change in vibrational entropy energy between wild-type and mutant; (+) stabilizing; (-) destabilizing; (++) increased molecule flexibility; (–) decreased molecule flexibility.

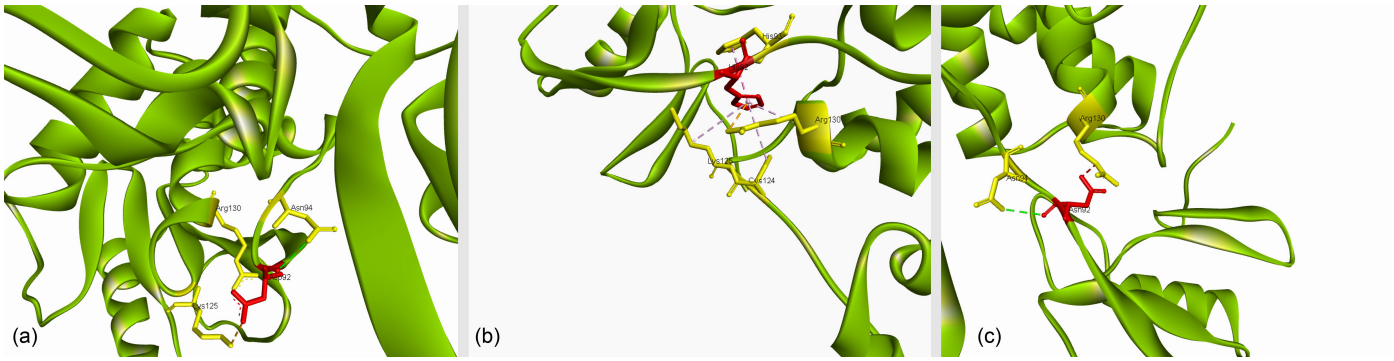

Figure 1. Structural visualization of PTEN at residue 92. (a) Wild-type Asp92 and its interactions. (b) D92H and (c) D92N mutants, illustrating the differences in local residue interactions resulting from these point mutations. The target amino acids are highlighted in red, and the interacting residues are shown in yellow.

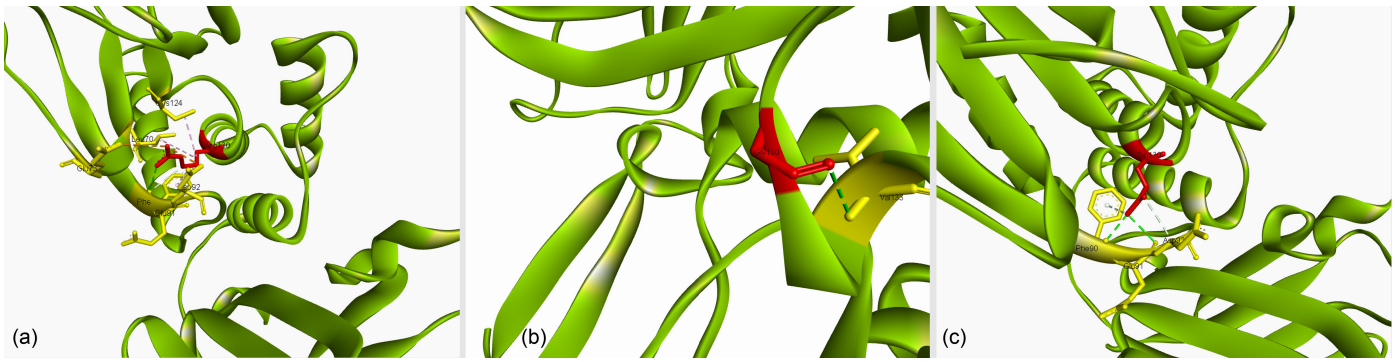

Figure 2. Structural visualization of PTEN at residue 130. (a) Interaction network of wild-type Arg130. (b) and (c) R130Q and R130G mutants, respectively, demonstrating the alteration of molecular interactions following mutation. The target amino acids are highlighted in red, and the interacting residues are shown in yellow.

and *AKT2* were selected as the primary target genes for detailed analysis. The selected target genes are presented in Table 1.

The subsequent step involved identifying the most frequent missense mutations in the four selected genes (*PIK3CA, PTEN, AKT1*, and *AKT2*) by analyzing the cBioPortal database. For *PIK3CA*, H1047R/L/Y/Q mutations were observed in 471 patients, E545K/A/G/D/Q in 191 patients, and E542K/A/V in 101 patients. In *PTEN*, D92A/E/G/H/N mutations were present in 5 patients, R130Q/G in 3 patients, and C136R/Y in 2 patients. For *AKT1*, the E17K, L52R, and Q79K mutations were identified in 79, 5, and 2 patients, respectively, whereas the *AKT2* R170W mutation was detected in 1 patient. Table 2 lists the selected genes with the corresponding mutations identified in this study.

Sequence-based prediction using the PredictSNP computational tool, in conjunction with consensus-based methods, indicated that only *PTEN* (D92H, D92N, R130Q, R130G, C136R, and C136Y), *AKT1* (L52R), and *AKT2* (R170W) mutations are likely to be deleterious (Table 3). The SNPs&GO computational method classified 14 mutations as disease-related polymorphisms (Table 4).

Based on comprehensive sequence-based predictions (Tables

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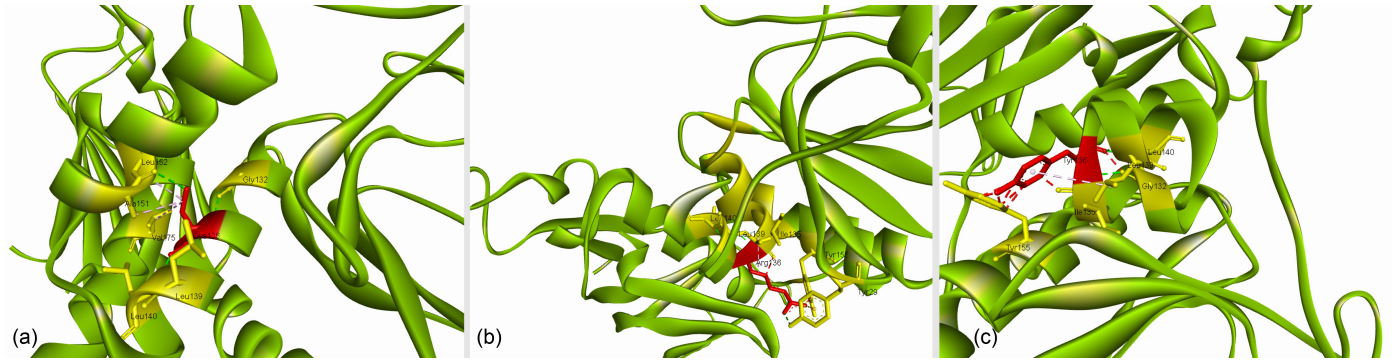

Figure 3. Structural visualization of PTEN at residue 136. (a) Wild-type Cys136 and its associated interactions. (b) and (c) Effects of C136R and C136Y mutations on the interaction profile of this site. The target amino acids are highlighted in red, and the interacting residues are shown in yellow.

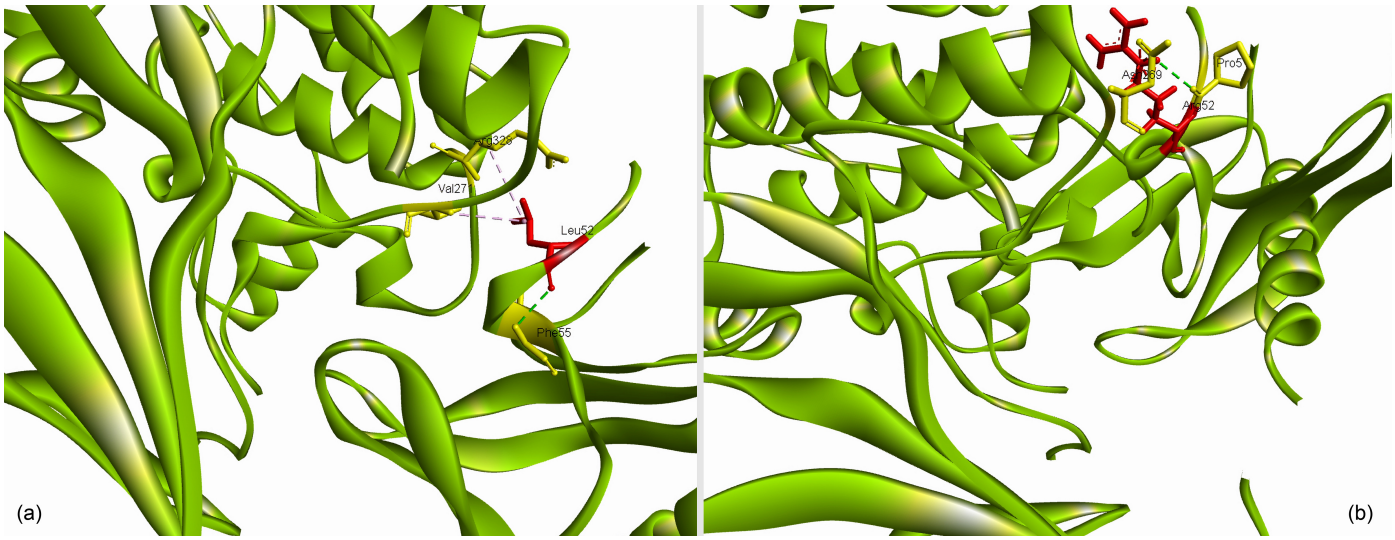

Figure 4. Structural visualization of AKT1 at residue 52. (a) Wild-type Leu52 interactions. (b) L52R mutant and resultant changes in local residue interactions. The target amino acids are highlighted in red, and the interacting residues are shown in yellow.

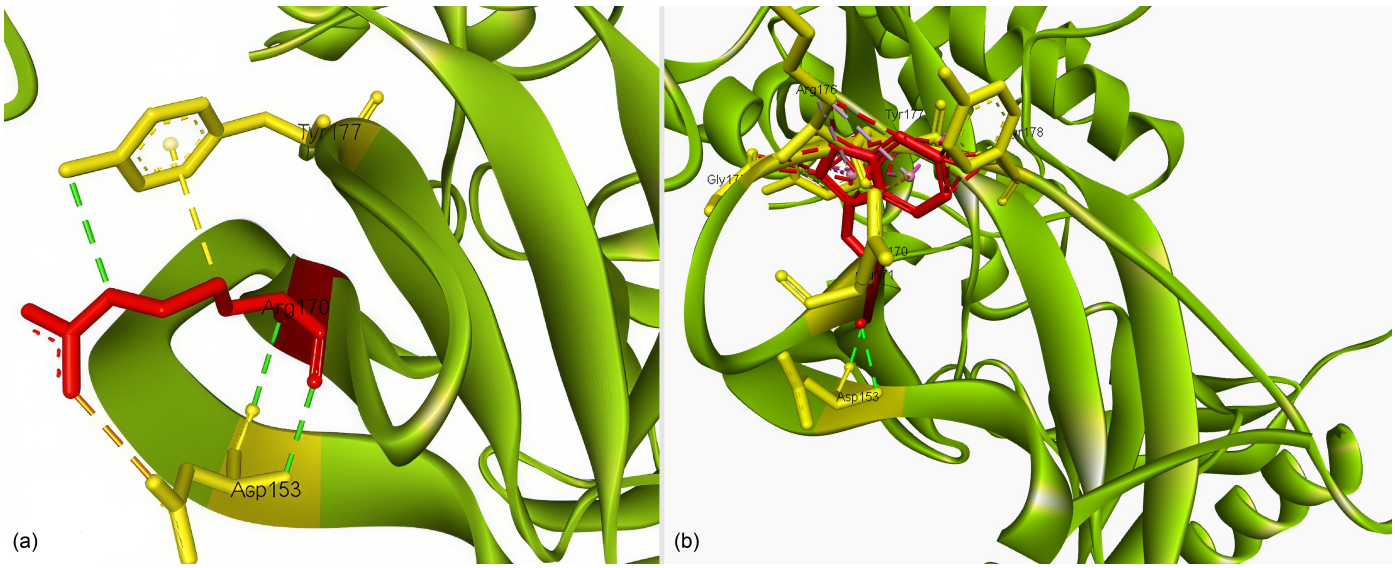

Figure 5. Structural visualization of AKT2 at residue 170. (a) Interactions of wild-type Arg170. (b) R170W mutant, highlighting differences in the interaction landscape. The target amino acids are highlighted in red, and the interacting residues are shown in yellow.

3 and 4), only the *PTEN* mutations D92H, D92N, R130Q, R130G, C136R, and C136Y; the *AKT1* mutation L52R; and the *AKT2* mutation R170W were consistently classified as deleterious by all computational tools employed (PredictSNP,

https://doi.org/10.5455/annalsmedres.2025.07.185

MAPP, PhD-SNP, PolyPhen-1, PolyPhen-2, SIFT, SNAP, and SNP&GO). The unanimous "deleterious" and "disease-related polymorphism" scores across these algorithms indicate that these variants are highly likely to impact protein function, thereby warranting their selection for further structural analysis. Table 5 summarizes the deleterious missense variants prioritized for structural analysis, along with consensus prediction, stability outputs, and representative interaction changes.

Representative high-resolution protein structures were obtained from the PDBe-KB and PDBe databases, with *PTEN, AKT1*, and *AKT2* modeled using 1D5R (chain A), 7NH5 (chain A), and 8Q61 (chain A), respectively. Structural impact assessments (Table 5) revealed that most of the evaluated mutations destabilized the protein structure or significantly altered molecular flexibility.

Differences in the interactions between wild-type and mutant structures were identified for *PTEN*, *AKT1*, and *AKT2* (Figures 1–5). The target amino acids are depicted in red and interacting residues are highlighted in yellow in each case.

Asp92 forms three key interactions in the *PTEN* wild-type structure: a conventional hydrogen bond with Asn94, a salt bridge with Lys125, and an attractive charge interaction with Arg130 (Figure 1a). The D92H mutation results in a pi–pi stacked interaction with His93, accompanied by three pi–alkyl interactions with Lys125, Cys124, and Arg130 (Figure 1b). The D92N mutant retained a conventional hydrogen bond with Asn94 and gained an unfavorable bump interaction with Arg130 (Figure 1c).

Wild-type Arg130 in *PTEN* participates in two attractive charge interactions with Glu73 and Asp92, two alkyl interactions with Cys124 and Leu70, a pi–alkyl interaction with Phe90, and a carbon hydrogen bond with Glu91 (Figure 2a). The R130Q mutant exhibited a pi donor hydrogen bond with Phe90, a carbon hydrogen bond with Asp92, and a conventional hydrogen bond with Glu91 (Figure 2b). The R130G mutation results in a single conventional hydrogen bond interaction with Val133 (Figure 2c).

Wild-type Cys136 in *PTEN* forms four conventional hydrogen bond interactions with Ala151, Gly132, Leu139, and Leu140 and two alkyl interactions with Val175 and Leu152 (Figure 3a). The C136R mutation results in conventional hydrogen bonds with Tyr29 and Leu140, two unfavorable bump interactions with Tyr155 and Ile135, and an alkyl interaction with Leu139 (Figure 3b). The C136Y mutant forms a pi–alkyl interaction with Leu139, two unfavorable bump interactions with Tyr155 and Ile135, and two conventional hydrogen bonds with Gly132 and Leu140 (Figure 3c).

In *AKT1*, wild-type Leu52 establishes two alkyl interactions with Arg328 and Val271 and a conventional hydrogen bond with Phe55 (Figure 4a). The L52R mutant forms a conventional hydrogen bond with Pro51 and an unfavorable bump interaction with Asn269 (Figure 4b).

For *AKT2*, wild-type Arg170 displays a salt bridge and two conventional hydrogen bond interactions with Asp153, an additional conventional hydrogen bond with itself, and both a conventional hydrogen bond and pi–alkyl interaction with Tyr177 (Figure 5a). The R170W mutation introduces a conventional hydrogen bond with Asp153, an unfavorable bump interaction with Gly175, a pi–alkyl interaction with Arg176, two amide–pi stacked interactions with Tyr177 and Tyr178, and a pi–anion interaction with Glu171 (Figure 5b).

DISCUSSION

The activation of the PI3K/AKT/mTOR signaling pathway has been widely linked to BC cell growth, survival, and drug resistance, making it a critical focus for cancer research. Dysregulation of this pathway is frequently observed in multiple forms of BC, contributing to tumor development, disease progression, metastasis and resistance to various therapies [14]. The PI3K/AKT/mTOR pathway interacts with numerous other signaling cascades, adding to the BC's molecular complexity and heterogeneity. Targeting specific components (nodes) of this pathway holds promise for the advancement of more personalized and effective treatments for patients with BC [1,3]. Many genes, including but not limited to *PIK3CA, PTEN, AKT1, AKT2, AKT3, IRS1, GSK3B, TSC1*, and *TSC2*, are involved in the PI3K/AKT/mTOR pathway [1,3]. These genes play important regulatory roles at different points within the pathway. *PTEN* functions as a tumor suppressor by dephosphorylating phosphatidylinositol (3,4,5)-trisphosphate (PIP3) in the cell membrane [15], which inhibits AKT activation and downstream signaling. *PTEN* contains domains such as the phosphatase domain that are critical for its tumor-suppressive activity [16]. Loss of *PTEN* function, often due to missense mutations, leads to uncontrolled activation of the PI3K/AKT/mTOR pathway, promoting cell growth and survival and increasing cancer risk, including in BC [3]. *AKT1* and *AKT2* are serine/threonine kinases and major downstream effectors of PI3K signaling [17]. *AKT1* mainly regulates cell proliferation and survival, while *AKT2* plays an essential role in cell metabolism and migration. Both proteins contain a PH domain, a kinase domain, and a regulatory domain, all of which contribute to their activities and regulation [18]. *AKT1* or *AKT2* mutations can disrupt normal signaling, contributing to tumor growth and therapeutic resistance in BC. *PTEN, AKT1*, and *AKT2* were selected for analysis, and their most frequent missense mutations were examined to better understand their potential contributions to BC progression and drug resistance.

In this study, we identified the *PTEN* variants D92H, D92N, R130G, R130Q, C136R, and C136Y as deleterious. Several of these mutations are located within regions critical for protein-ligand interactions, affecting both PIP2 binding and inhibition of downstream pathways. Karn et al., performed molecular docking analyses and found that D92H and R130Q mutations disrupt PTEN's ability to bind PIP2.

https://doi.org/10.5455/annalsmedres.2025.07.185

The loss of PIP2 interaction primarily impairs the dephosphorylation process, directly affecting the phosphatase function of *PTEN* [19]. Matreyek et al., reported that the D92H variant represents a highly abundant yet phosphatase-inactive form of *PTEN*. This mutation can dysregulate AKT signaling even in the presence of wild-type *PTEN*, indicating that D92H can act in a dominant-negative manner. Importantly, these dominant-negative variants were observed in BC according to cancer genomics datasets, highlighting their potential relevance in the pathogenesis of BC [20]. Researchers investigated the clinical overlap between *PTEN* deficiency and activated PI3K delta syndrome 1 (APDS1). Their analysis included a patient with a pathogenic *PTEN* mutation (C136R), one of the deleterious variants identified in this study. The patient exhibited immune dysregulation phenotypes similar to APDS1, including hyper IgM, low IgG, increased transitional B cells, and lymphoproliferation. Functional assays confirmed dysregulation of the PI3K/AKT/mTOR pathway. Importantly, treatment with sirolimus, an mTOR inhibitor, effectively resolved lymphoproliferation and splenic abnormalities. These findings highlight that *PTEN* loss-of-function mutations can phenocopy PI3K pathway activation and indicate that targeted therapies may benefit patients with BC harboring similar *PTEN* mutations [21]. The *PTEN* C136R mutation compromises phosphatase activity, contributing to increased proteasome activity and protein instability [22]. Several studies have classified C136R as a pathogenic alteration, including in patients with BC from diverse populations [23,24]. Gervas et al., specifically identified this mutation in a patient with hereditary BC of mongoloid origin, emphasizing the relevance of *PTEN* analysis in addition to *BRCA1* and *BRCA2* screening. While *BRCA1/2* mutations are well-characterized in hereditary BC, the importance of investigating alternative pathways that contribute to cancer development is becoming increasingly recognized. The PI3K/AKT/mTOR signaling cascade is central to key cellular processes such as growth, survival, and metabolism, and disruptions in this pathway are frequently linked to tumorigenesis and BC progression [1,3,6,7]. Cetintas et al., conducted detailed computational and experimental analyses of several *PTEN* missense mutations. According to multiple computational tools, their investigation demonstrated that certain mutations, including C136Y, displayed strong deleterious effects. Molecular dynamics simulations indicated that the C136Y mutation resulted in significant residual fluctuations in the protein structure and was associated with the least compact conformation among the variants analyzed. These structural alterations were correlated with decreased protein stability and were predicted to impair *PTEN* function. Given the central role of *PTEN* in regulating cell proliferation and survival, such mutations are likely to contribute to protein dysfunction and promote tumorigenic processes. The findings support the disease relevance of C136Y and other *PTEN* alterations in cancer development and provide further evidence for their potential pathogenic impact in BC when present [25].

Beyond regulating cell growth and survival, the PI3K/AKT/mTOR pathway is implicated in the development of chemoresistance, presenting substantial challenges for the treatment of BC. All genes evaluated in this study are connected to the emergence of therapy resistance in different ways. Huang et al., (2021) reported C136Y in patients with BC, often alongside other *PTEN* mutations such as D92G, D92V, K125T, G127V, and G129E, emphasizing the diversity of variants contributing to disease complexity [26]. Additionally, researchers described C136Y among mutations found in patients with BC with disease progression, together with variants such as G127E, R130Q, G36E, and Q97* [24]. These findings support the clinical significance of C136Y and related *PTEN* mutations in BC, particularly in the context of aggressive disease and therapeutic resistance.

Sharma et al., identified several harmful *PTEN* nsSNPs, including R130G and R130Q, which overlapped with mutations classified as deleterious in this study. Their computational analysis showed that these variants disrupt the structural stability of *PTEN*, compromise catalytic sites, and impair the PI3K/AKT pathway regulation. These results further validate the pathogenic potential of R130G and R130Q in BC and underscore the broader impact of *PTEN* missense mutations on tumor suppressor function [27]. The *PTEN* R130Q mutation has also been closely associated with the BC phenotype, and several research groups are investigating PTEN-targeted strategies for treating BC [28]. In their study, Naidu and Suneetha characterized R130Q as a deleterious variant that reduces protein stability. As noted by Kim et al. [29], R130Q, along with R130G, represents one of the most common oncogenic mutations in *PTEN*. Functional studies further indicate that arginine at position 130 is essential for the catalytic activity and overall protein function of *PTEN*, highlighting the pathogenic significance of alterations at this site [30].

The other two proteins in our study: *AKT1* and *AKT2* belong to the AKT family of serine threonine protein kinases, which is known as protein kinase B (PKB). They regulate a variety of biological activities, including cell survival, proliferation, growth, metabolism, and angiogenesis. *AKT1* is involved in cell survival pathways, promoting cell survival, and regulating protein synthesis [31]. Furthermore, *AKT2* shares almost all functional similarities with *AKT2* but plays some distinct roles in specific cellular processes, such as glucose metabolism regulation and insulin signaling. *AKT1* and *AKT2* are involved in tumor initiation, metastasis, and therapeutic resistance in cancer [32].

Several *AKT1* mutations, including D32Y, K39N, P42T, L52R, C77F, and Q79K, have been reported in recent BC studies [33,34]. Among these, the L52R variant is a recurrent, activating mutation that enhances Akt phosphorylation and is classified as oncogenic [34]. This specific mutation

https://doi.org/10.5455/annalsmedres.2025.07.185

has been identified in patients with BC, further implicating *AKT1* in disease progression [35]. Structural and biochemical analyses indicate that such activating mutations can lead to constitutive kinase activity, promoting downstream signaling even in the absence of upstream stimuli. This dysregulation contributes to uncontrolled proliferation and survival, which are central to malignant transformation and therapeutic resistance. Recent functional and clinical investigations have further defined the significance of the *AKT1* L52R mutation. Shrestha Bhattarai et al., demonstrated that L52R is among the activating, non-E17K *AKT1* mutations capable of driving growth factor-independent AKT activation. Their study revealed that such mutations can sensitize tumor cells to ATP-competitive AKT inhibitors, such as capivasertib. In a phase II clinical trial that included patients with various solid tumors harboring AKT1-3 alterations, an individual with an *AKT1* L52R-mutant cancer exhibited a durable partial response to capivasertib, highlighting the therapeutic potential of directly targeting these activating mutations. These findings indicate that allele-specific activation profiles influence pharmacological sensitivities and suggest that tailored AKT inhibitor therapy may benefit BC cases with *AKT1* L52R or similar mutations [36]. Recent in vitro research has demonstrated that *AKT1* L52R mutation contributes to paclitaxel resistance in BC cells. The study found that the L52R variant, along with other PH domain mutations, was associated with increased resistance to paclitaxel in MDA-MB-231 cells compared to wild-type *AKT1*. Although *AKT1* L52R protein levels were lower than those of the wild-type, this mutation still resulted in enhanced AKT pathway activation and cell survival. These findings underscore the functional impact of the L52R variant in chemoresistance promotion and highlight its potential as a biomarker for treatment response in BC [37].

Although the *AKT1* E17K mutation is more frequently reported and widely studied in BC, emerging evidence highlights the clinical relevance of other activating mutations, such as L52R. Both E17K and L52R have been associated with increased resistance to multiple chemotherapeutics, not only in BC but also in other types of cancer, such as colorectal cancer [38]. These mutations often co-occur with additional driver alterations but may also act independently to promote oncogenic signaling and contribute to therapy resistance [39]. Moreover, the feasibility of liquid biopsy for monitoring disease and guiding targeted therapeutic strategies in advanced-stage BC is demonstrated by the blood-based detection of *AKT1* mutations [39]. Taken together, these findings support the use of *AKT1* L52R, alongside E17K, as a potential predictive biomarker for drug resistance and treatment planning in BC, reinforcing the importance of precision medicine approaches for patients with *AKT1* pathway alterations [38].

AKT2, although highly homologous to *AKT1*, is distinguished by its essential role in glucose metabolism and insulin signaling, processes that are often altered during tumorigenesis. Although rare in human cancers, the R170W mutation in *AKT2* affects regulatory mechanisms by potentially disrupting kinase activity and substrate specificity [40]. Notably, our study did not find any existing research directly connecting the *AKT2* R170W variant to BC in the experimental or clinical settings. This highlights a significant gap in the literature and underscores the need for targeted in vivo studies to clarify the functional and clinical relevance of this particular mutation. The *PTEN* and *AKT1* mutations identified in our analysis are well supported by in vivo and clinical evidence, reinforcing their established pathogenic and therapeutic roles in BC. Therefore, our findings provide a rationale for future translational investigations, particularly regarding the potential impact of *AKT2* R170W in BC biology and treatment resistance.

Although the present study focused on BC, the PI3K/AKT/mTOR axis is relevant in other malignancies. This pathway has been identified as a therapeutic target in chordoma [9] and dysregulation has been reported across diverse cancers [10]. Pathogenic *PTEN* missense variants have been functionally characterized in glioblastoma [25], reinforcing the broader importance of PTEN-AKT signaling in oncogenic processes. These pathway-wide observations support the wider interest of the present mutation-focused findings for additional tumor types.

Conclusion

Identifying deleterious genetic mutations in BC using computational methods represents a significant step in understanding the molecular basis of this disease. Researchers have identified specific variants with potential functional impact using computational tools. These findings offer key insights into the genetic landscape of BC, supporting risk stratification and development of targeted therapeutics. The PI3K/AKT/mTOR pathway requires further investigation to clarify its role in the pathogenesis and progression of BC. Progress in this area could improve the clinical outcomes of patients with BC. This study focuses on *PTEN, AKT1*, and *AKT2* and assesses their most frequent mutations and pathogenic effects. Further research is necessary to achieve more clinically relevant results, and integrating bioinformatics and genomics remains vital for uncovering BC genetics and advancing personalized medicine.

Ethics Committee Approval: This study did not involve human participants, animal experiments, or the use of personal identifiable data. Therefore, approval from the ethics committee was not required.

Informed Consent: Not required for this study.

Peer-review: Externally peer-reviewed.

Conflict of Interest: The authors declare that there are no existing or potential conflicts of interest, including financial, consultancy, institutional, or other relationships, that could be

https://doi.org/10.5455/annalsmedres.2025.07.185

perceived as leading to potential bias or a conflict of interest regarding the submitted manuscript.

Author Contributions: Conceptualization: B.A.; Investigation: B.A., H.L., N.I.; Data curation: H.L.; Formal analysis: H.L.; Methodology: B.A., H.L. Software: H. L ; Visualization: H. L and N. I. ; Writing–original draft: B.A., H.L., N.I. ; Writing, review, and editing: B.A., H.L., N.I. . All authors have approved the final version of the manuscript.

Financial Disclosure: The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

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