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Original Research Article

IN-SILICO CHARACTERIZATION OF HUMAN HER2 GENE TO PREDICT THE BREAST CANCER ASSOCIATED BIOMARKERS

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Abstract: *HER2 gene hyperactivation is responsible for the incidence of invasive breast cancer (BC). It leads to over expression of HER2 receptors in breast cells resulting in abnormal receptor dimerization and signal transduction cascade initiation. It ultimately causes uncontrolled proliferation of breast cells. Therefore, this is the most susceptible target gene for BC treatment. HER2 gene transcript with accession ID ENST00000269571.10 was selected for association analysis in present study. A total of ten single nucleotide polymorphisms (SNPs) of gene were analyzed using in-silico tools like CELLO, PROTPARAM, SOPMA, and SWISSMODEL. These tools helped in determining the effect of variants on sub-cellular localization, physiochemical properties, and secondary (2D) and tertiary (3D) structures of mutated proteins. Variants rs1249755832, rs769703068, rs1567913219, and rs1213602748 altered the localization of mutated proteins. SNPs rs1213602748, rs769703068, and rs1249755832 considerably changed the isoelectric point (pI), extinction coefficient, instability index, aliphatic index, and GRAVY. Variant rs769703068 caused a change in all four properties of the 2D structure, i.e., alpha helix, extended strand, beta-turn, and random coil. Polymorphisms that caused a significant deviation in 3D configuration were rs769703068, rs144434331, and rs146177313. These variants can be recommended as biomarkers for HER2$^+$ BC cell diagnosis.*

Keywords: Breast Cancer, Biomarkers, Single Nucleotide Variations, HER2, Configuration

Introduction

Breast cancer (BC) is characterized by the uncontrolled proliferation of the endothelial lining of lobules, milk ducts, and other supporting tissues. Its symptoms include breast swelling, swollen lymph nodes, bloody or clear nipple discharge, retracted and scaly nipples, and discomfort in breast tissue (Irvin Jr et al., 2011; Luo et al., 2023). Possible causes of BC might be genetics, family history, hormonal abnormalities, and environmental factors (Roheel et al., 2023). BC is the major cause of female cancer-associated mortality (de la Peña et al., 2023). In the United States, new cases and deaths of BC have been estimated to be 297,790 and 43,170, respectively (Siegel et al., 2023). There are three types of invasive BC, i.e. ER or estrogen receptors, HER2 or human epidermal growth factor 2, and PR or progesterone receptors (Roheel et al., 2023).

Human epidermal growth factor receptor tyrosine kinase 2 (HER2) gene is also symbolized as C-ERB-2, CD340, MLN-19, NEU, NGL, P185, and ERBB2. It is located on the chromosome 17q21 and encodes for HER2/neu protein. These are the receptor proteins localized on the breast cells. In healthy females, these proteins are associated with the average growth of breast cells (Aertgeerts et al., 2011). HER2 receptor protein comprises of three domains, i.e., tyrosine kinase (intracellular domain), trans-membrane domain, and ligand binding (extracellular domain). Binding of ligands with the extracellular domain causes conformational change and dimerization of receptors. This results in the activation of a signal transduction cascade, which plays a role in the growth of cells and apoptosis inhibition (Bai et al., 2023).

BC cells exhibiting the overexpression of HER2 gene are termed HER2-positive breast cancer (HER2$^+$ BC) cells. These cells divide at a comparatively higher rate and are more malignant than the HER2-negative breast cancer (HER2$^-$ BC) cells (Ivanova et al., 2023; Wu et al., 2023). Among BC patients, the incidence of HER2$^+$ BC is 10 to 20% (Zakaria et al., 2023). In HER2$^+$ BC cells, HER2 undergoes heterodimerization, which may be ligand-dependent or independent. This generates abnormal signals, causing tumorigenesis in breast cells (Jost et al., 2013; Swain et al., 2023).

Literature reports various single nucleotide polymorphisms (SNPs) in the HER2 gene closely associated with BC incidence and progression in human. These SNPs include Ala117Pro and Ile655Val, (Cresti et al., 2016; Furrer et al., 2016; Kallel et al., 2010), P1170A (Han et al., 2005), rs2910164 (Meshkat et al., 2016), rs1058808 and rs2517956 (Su et al., 2015) and S310F, S310Y, R678Q, D769H, I767M, L755S, D769Y, V842I and K753I (Gaibar et al., 2020).

Considering this close association between HER2 gene mutations and BC occurrence and the importance of HER2 as the most sensitive target for BC treatment, the present study was initiated. In this study, HER2 gene was characterized to get an insight into BC-associated biomarkers. SNPs were analyzed using multiple

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bioinformatics tools, and the effect of mutations on localization, physiochemical attributes, and configuration of mutated proteins was recorded.

Methodology

Retrieving the CDS and SNPs from ENSEMBL database

ENSEMBL database (https://www.enxembl.org/index.html, accessed in August 2023) was used to retrieve the coding sequence (CDS) and SNPs of HER2 transcript ERBB2-201, Accession # ENST00000269571.10. Total ten SNPs were retrieved. Out of which six were missense, two were stop gained and two were frame shift mutations (Table 1).

Table 1: SNPs of HER2 gene retrieved from the ENSEMBL database for documentation in present study, along with the consequence type, codon position, nucleotides change, and deleterious status

Case #	SNP ID	Consequence type	Codon position	Change of nt.	Change of amino acid	SIFT	Poly-Phen
1	rs1284110310	missense	28	GGC>GAC	G > D	0	0.998
2	rs1253654469	missense	112	TAT>TGT	Y > C	0	0.993
3	rs1249755832	Stop gained	143	CGA >TGA	R > *		
4	rs769703068	Frame shift	334	TGC > TC	C > X		
5	rs144434331	missense	776	GGT > GTT	G > V	0	0.916
6	rs1567913219	Stop gained	825	TGG > TGA	W > *		
7	rs747974836	missense	880	GAT>TAT	D > Y	0	0.988
8	rs1213602748	Frame shift	905	GTG>GT	V > X		
9	rs146177313	missense	1141	AAC>AAA	N > K	0	1
10	rs568793816	missense	1221	TAT>TGT	Y > C	0	0.915

Case Designing

To create the mutated cases, SNPs were incorporated at the corresponding codon positions in the CDS of nucleotide sequences. Normal as well as mutated CDS comprising of nucleotide sequences were translated into amino acids sequences using the EXPASY translate tool (https://web.expasy.org/translate/, accessed in August 2023). CDS nucleotide and amino acid sequences are shown in Supplementary Data Figure S1 and Supplementary Data Figure S2.

CELLO Tool

To assess the effect of SNPs on the sub-cellular localization of mutated proteins, Subcellular Localization Predictive System, the CELLO tool (cello.life.nctu.edu.tw, accessed in August 2023), was consulted. The tool predicted localization scores in the extracellular environment, nucleus, cytoplasm, plasma membrane, lysosome, mitochondria, endoplasmic reticulum, golgi complex, and cytoskeleton. The scores were compared between normal and mutated proteins.

PROTPARAM Tool

To predict the effect of mutations documented in the present study on the physicochemical properties of the protein, the EXPASY PROTPARAM tool (https://web.expasy.org/protparam/, accessed in August 2023) was used. This tool calculated normal and mutated proteins' molecular weight, isoelectric point (I), extinction coefficient, half-life, instability index, aliphatic index, and grand average of hydropathicity (GRAVY).

SOPMA Tool

Secondary structure prediction method SOPMA tool (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=NPSA/npsa_sopma.html, accessed in August 2023) was consulted to predict the change in the secondary (2D) structure of HER2 encoded protein that might be induced by SNPs documented in the present study.

SWISSMODEL

SWISSMODEL, an automated 3D protein structure homology modeling server (https://swissmodel.expasy.org, accessed in August 2023), was consulted to determine the effect of SNPs on the 3D configuration of protein. The configurations of normal and mutated proteins were compared.

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Table 2: Assessment of the effect of SNPs in the HER2 gene on sub-cellular localization of mutated proteins

Case #	SNPs ID	EX	NU	CY	PM	LY	MT	ER	GL	CT
Normal		1.884	1.080	0.752	0.636	0.296	0.123	0.049	0.041	0.029
1	rs1284110310	1.904	1.087	0.751	0.629	0.282	0.121	0.049	0.042	0.029
2	rs1253654469	1.935	1.063	0.738	0.631	0.287	0.121	0.048	0.041	0.029
3	rs1249755832	3.291	0.292	0.362	0.213	0.181	0.416	0.052	0.024	0.010
4	rs769703068	4.802	0.027	0.013	0.018	0.083	0.010	0.011	0.003	0.001
5	rs144434331	1.883	1.073	0.764	0.643	0.288	0.120	0.050	0.042	0.029
6	rs1567913219	3.040	0.392	0.346	0.645	0.343	0.048	0.014	0.018	0.016
7	rs747974836	1.883	1.077	0.737	0.643	0.308	0.123	0.049	0.040	0.029
8	rs1213602748	2.674	0.767	0.526	0.434	0.303	0.100	0.020	0.022	0.016
9	rs146177313	1.851	1.107	0.774	0.619	0.291	0.129	0.050	0.041	0.029
10	rs568793816	1.952	1.062	0.733	0.633	0.277	0.120	0.047	0.041	0.029

EX = extracellular, NU = nuclear, CY = cytoplasm, PM = plasma membrane, LY = lysosomal, MT = mitochondria, ER = endoplasmic reticulum, GL = golgi complex, CT = cytoskeleta

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Results

Prediction of SNPs effect on sub-cellular localization

CELLO tool revealed the effect of SNPs on mutated proteins localization (Table 2). Normal protein was abundantly localized in the extracellular environment (score = 1.884), followed by the nucleus (score = 1.080). Localization of mutated proteins in cases 1, 2, 5, 7, 9, and 10 was consistent with the standard protein. However, the results were contrary to the normal protein in cases 3, 4, 6, and 8. Highest score was observed in extracellular environment, i.e... 3.921, 4.802, 3.040, and 2.674, respectively. Hence, four SNPs, rs1249755832, rs769703068, rs1567913219, and rs1213602748, changed the localization of mutated proteins from extracellular and nuclear to extracellular only

Prediction of SNPs' effect on physicochemical properties

To assess the mutations effect on physicochemical attributes of proteins, PROTPARAM tool was employed. Highest values of pI were observed in cases of SNPs rs1567913219 and rs1213602748, i. e. 6.36 and 6.51, respectively. The extinction coefficient values in cases 3 (11585), 4 (42535), 6 (84040), and 8 (98145) exhibited significant deviation from the normal value of 138275. Highest and lowest values were found to be induced by SNPs rs747974836 (case 7) and rs769703068 (case 4), i.e.. 139765 and 42535, respectively. No change in half-life was observed in any of the cases documented in present study. Significant variation in the instability index from the normal value (56.13) was observed in cases 3 (41.00), 6 (49.74), and 8 (50.17). The only mutation that caused a significant change in the aliphatic index of mutated proteins was rs1249755832 reported in case 3. The value was 110.56 versus the normal value of 83.35. SNPs documented in case 3 caused a significant change in GRAVY, i.e. 0.020 from the normal value of -0.247. Mutations reported in cases 4, 6, and 8 also caused some deviation, with values recorded as -0.166, -0.120, and -0.120, respectively (Table 3).

Table 3: Prediction of physicochemical properties of normal and mutated proteins using the PROTPARAM tool

Cases	Mol. wt.	pI	Ext. coefficient ($M^{-1}cm^{-1}$)	Half-life (hr)	Instability index	Aliphatic index	GRAVY
Normal	137910.50	5.58	138275	30	56.13	83.35	-0.247
1	137968.53	5.55	138275	30	56.15	82.35	-0.250
2	137850.46	5.58	136910	30	55.92	82.35	-0.244
3	15560.93	5.48	11585	30	41.00	110.56	0.020
4	38537.02	5.87	42535	30	51.71	81.48	-0.166
5	137952.58	5.58	138275	30	56.13	82.58	-0.244
6	90590.24	6.36	84040	30	49.74	88.62	-0.120
7	137958.58	5.62	139765	30	56.13	82.35	-0.246
8	100334.60	6.51	98145	30	50.17	89.87	-0.120
9	137924.57	5.62	138275	30	56.36	82.35	-0.248
10	137850.46	5.58	136910	30	56.04	82.35	-0.244

Table 4: Assessment of SNPs effect on 2D configuration of mutated proteins using SOPMA tool

Case #	SNPs ID	Alpha helix (%)	Extended strand (%)	Beta turn (%)	Random coil (%)
Normal	rs1284110310	24.54	15.62	4.86	54.98
1	rs1253654469	25.02	15.22	5.02	54.74
2	rs1249755832	24.62	15.46	5.10	54.82
3	rs769703068	30.99	23.94	7.75	37.32
4	rs144434331	21.59	19.60	5.68	53.12
5	rs1567913219	24.86	15.38	5.02	54.74
6	rs747974836	25.00	18.45	6.19	50.36
7	rs1213602748	24.78	15.46	4.78	54.98
8	rs146177313	27.72	17.93	5.83	48.51
9	rs568793816	24.46	15.86	5.10	54.58
10	rs1284110310	24.62	15.78	5.10	54.50

Prediction of SNP effect on 2D structure

The SOPMA tool was accessed to document the change in the 2D configuration of mutated proteins. Three cases, i. e., 3, 4, and 8, documenting SNPs rs769703068, rs144434331, and rs146177313, were found to induce variation in alpha helix as compared to the regular protein. SNPs documeneted in cases 3, 4, 6, and 8 caused variation in extended strand values, i.e.. 23.94, 19.60, 18.45, and 17.93%, respectively,

as compared to the normal protein (15.62%). SNPs reported in cases 3 (rs769703068) and 6 (rs747974836) altered the value of random coil (Table 4).

Prediction of SNPs effect on 3D structure

Comparison of the 3D configuration of normal and mutated proteins using SWISSMODEL revealed significant deviation induced by SNPs reported in cases 3 (rs769703068), 4 (rs144434331), and 8 (rs146177313). SNPs rs1567913219 and rs747974836 reported in cases 5 and 6 caused changes in 3D structure differently than the variants of cases 1, 2, 7, 9, and 10. SNPs of the cases mentioned later altered the structure from the normal one. However, the resulting structures were closely similar (Figure 1)

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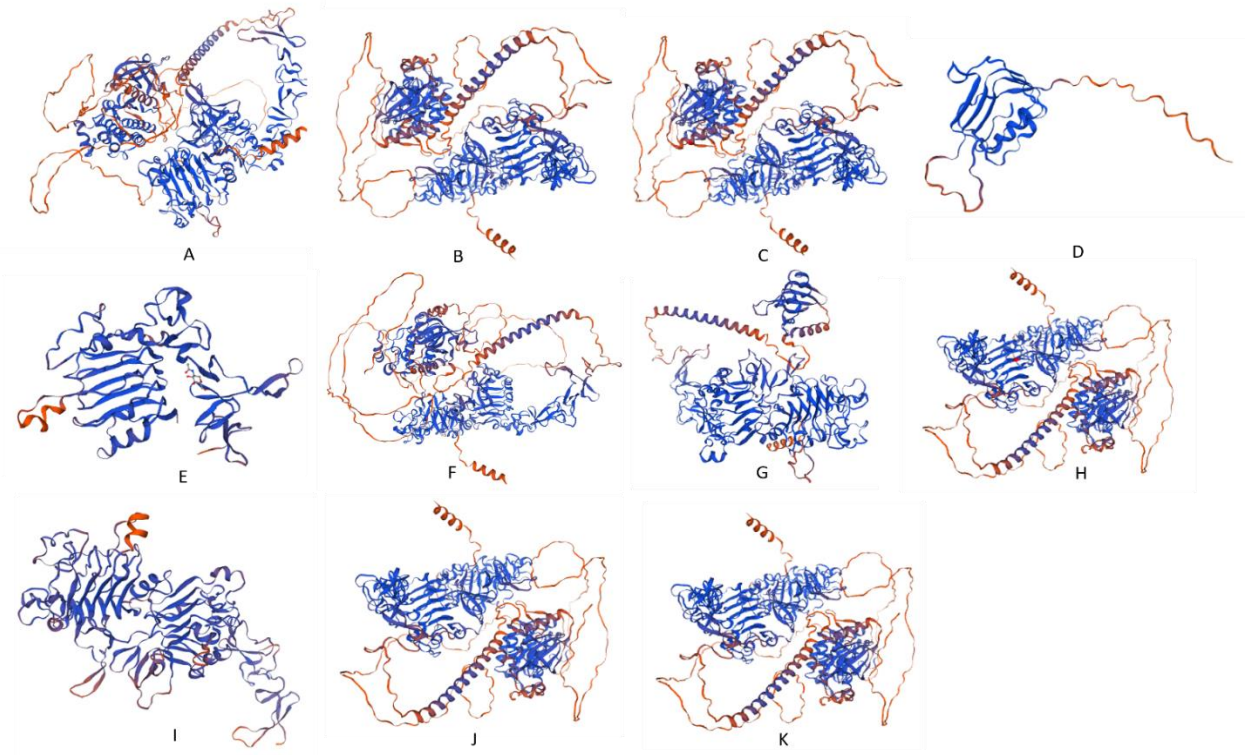
Figure 1: Assessment of the effect of SNPs on 3D configuration of mutated proteins using SWISS-MODEL



A: normal protein, B: case 1, C: case 2, D: case 3, E: case 4, F: case 5, G: case 6, H: case 7, I: case 8, J: case 9, K: case 10

Discussion

Present study has evaluated the importance of HER2-associated SNPs as diagnostic and prognostic BC biomarkers. Ten SNPs addressed were rs1284110310, rs1253654469, rs1249755832, rs769703068, rs144434331, rs1567913219, rs747974836, rs1213602748, rs146177313 and rs568793816. Mutations were analyzed for their impact on localization, physical and chemical properties, and configuration (2D and 3D) of protein.

Localization of HER2 protein was predicted to be extracellular in all the cases, which is consistent with the previously reported literature (Jeong et al., 2017; Wakefield et al., 2023).

In all the cases except 6 and 8, the mutated proteins were slightly acidic, just like normal proteins, with pI ranging between 5.48 and 5.87. However, in cases 6 and 8, proteins were comparatively alkaline pI closer to 7 (Righetti, 2004). In all cases, mutated and the normal proteins showed high aliphatic index values corresponding to their good thermostability (Pack and Yoo, 2004). However, SNP documented in case 3 considerably enhanced thermal stability of encoded mutated protein with the value of 110 versus the average value of 83.35 for normal. Normal and the mutated proteins exhibited in-vitro instability with instability index values above 40 (Gamage et al., 2019). All the mutated proteins were found non-polar, just like normal one with negative GRAVY values, while the SNP documented in case 3 rendered the mutated protein polar with positive GRAVY value (Babnigg and Joachimiak, 2010). This is the first study to report the physicochemical properties of HER2 protein receptors.

Seven SNPs rs1249755832, rs769703068, rs1567913219, rs1213602748, rs1213602748, rs144434331, and rs146177313 of HER2 gene documented in the present study were found to alter the characteristics of mutated proteins as compared to normal one. No study in the literature has documented these SNPs as biomarkers for BC. However, multiple studies have documented several variants in the HER2 gene as biomarkers. Like a study targeted 361 BC patients and analyzed the association of the Ala1170Pro variant with HER2 hyperactivation (Cresti et al., 2016). Similarly, another study analyzed the HER2 gene in seventy-three females infected with HER2+ BC and

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found the prognostic value of Ala1170Pro (Furrer et al., 2016).

Conclusion

Present study findings document rs1249755832, rs769703068, rs1567913219, rs1213602748, rs1213602748, rs144434331, and rs146177313 SNPs of HER2 gene as the possible predictors of BC because of the alteration in HER2 receptor protein caused by them. These variants can be analyzed in BC patients by sequencing the HER2 gene and compared with the normal individuals' genes. This will help in further confirming the biomarker potential of these variants.

Declarations

Data Availability statement
All data generated or analyzed during the study are included in the manuscript.
Ethics approval and consent to participate
Approved by the department Concerned.
Consent for publication
Approved
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Conflict of interest

The authors declared absence of conflict of interest.

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