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IN SILICO ANALYSIS, HOMOLOGY MODELLING AND MOLECULAR DOCKING OF SINGLE NUCLEOTIDE POLYMORPHISMS IN HUMAN HER2/ERBB2 GENE

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ABSTRACT
HER2/ErbB2 Gene is known to be a key player in the pathogenesis of several human cancers. The *HER2* receptors were amplified and resulted in overexpression of *HER2* protein, which has been associated with tumor cell proliferation and cancer progression. Our study's main objectives are to evaluate and find the various tolerated, deleterious, damaging *nsSNPs*, their disease-causing ability, and the effect of those mutations on the stability of protein structure to find the molecular phenotypic effects of these nsSNPs and perform homology modeling and docking of *ErbB2* gene. In our study, different variants of the *Erbb2* gene associated with the other diseases were obtained using dbSNP and Disgenet. Variants associated with the disease were processed through SIFT analysis, and the variants with the lowest score (0.00-0.05) were selected. They were further analyzed through SNP & GO, and the mutation with the highest score (> 0.5) was found to be a disease-associated mutation. I-Tasser platform was used to perform homology modeling and was further validated by the Ramachandran plot. Molecular Docking of Model (associated disease) against drugs was performed by Py-Rx. The above analyses found that the mutation with the lowest score (SIFT) and highest Score (SNP and Go) were associated with Breast Cancer and Glioma. Drugs that showed the highest binding affinity with the model protein provide a new insight for *ErbB2* as a drug target. Thus, it is concluded that several damaging, deleterious nsSNPS are highly disease-causing, changing the stability of protein structure, and different synthetic and natural drugs showed a high binding affinity with the protein model. So these drugs can be used in determining an effective treatment in the future.

KEYWORDS: Erbb2 Gene; Variants; Mutation; Disease; Cancer.

INTRODUCTION
In a genome, a single nucleotide polymorphism (SNP) is a source of variation. Single nucleotide polymorphisms are crucial in deciphering the causes of a variety of complex human diseases. Single nucleotide polymorphisms (SNPs) are modifications of the single nucleotide in the genomic sequence of individuals. Several public databases for SNPs are open, including dbSNP, GWAS Central SwissVar, and ClinVar. Only missense variants and nonsynonymous SNPs (nsSNPs) are significant because they display changes in the translated amino acid residue sequence. nsSNPs have been related to various diseases and play an essential role in the functional diversity of coded proteins in human populations. nsSNPs can affect protein function by lowering protein solubility or destabilizing protein structure and gene regulation by altering transcription and translation.[1-4] Nearly half of all disease-related genetic variations are caused by nonsynonymous SNPs.[5] Many types of cancer and normal tissues express the human epidermal growth factor receptors 1,2,3,4.[6] *HER2* is a 185kd transmembrane glycoprotein expressed in many tissues and plays a significant role in excessive, uncontrolled cell growth and tumorigenesis.[7-9] Amplification, overexpression, mutation, and polymorphism of *ErbB2* can cause various cancer.

HER2 has no direct activating ligand of its own and becomes active because of heterodimerization with other ligand-bound family members such as *HER1* and *HER3*. *ErbB2* polymorphism is associated with various cancer like ovarian cancer, colorectal cancer, lung carcinoma, urothelial bladder carcinoma, glioma, and the most important, breast cancer. Receptor homo and heterodimerization lead to downstream signaling pathways that promote cell growth, proliferation, and survival.[10] *HER2/ERBB2* activates some of the signaling pathways such as MAPk, P13/Akt, among which activation of MAPK leads to breast cancer.[11] In breast cancer, *HER2/ERBB2* is the dominant tyrosine kinase receptor. Taking all of these factors into account, as well as *ErbB2's* central function in many forms of cancer, this study aimed to determine the impact of various polymorphisms in ErbB2 on its protein structure,

which may play a significant role in disease susceptibility, as well as the ligand's binding affinity to the protein.

MATERIALS AND METHODS

The information on the human ErbB2 Gene was gathered from the National Center for Biotechnology Information (NCBI) website's Entrez gene. The workflow of the study is shown in figure 1.

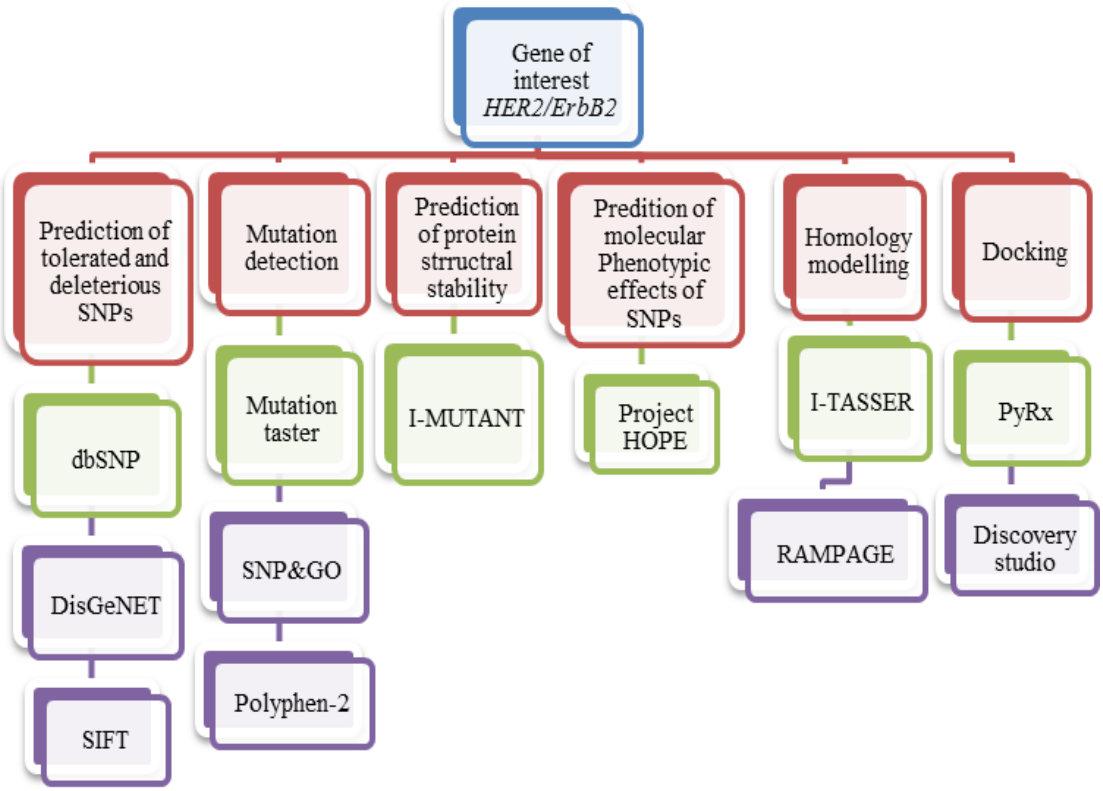


Figure 1: Workflow of study.

A. Mining the database for SNPs

The data on the human *ErbB2* Gene were gathered from Entrez Gene on the National Centre for Biological Information (NCBI) site. The SNP data (protein accession no and SNP ID) of the gene was retrieved from dbSNP (http://www.ncbi.nlm.nih.gov/projects/snp/) and Swissprot databases (http://expasy.org/). We searched for ErbB2 and divided the results into 3' UTR SNPs, 5' UTR SNPs, Missense SNPs, and intronic SNPs. It should be noted that dbSNP has a high rate of false positives, but this was partially mitigated by using several other SNP impact prediction methods, which are mentioned below. In this study, the freely available online tool DisGeNET was also used to determine the disease associated with *ErbB2*, their score, and SNPs responsible for each of them.

B. Mutation detection

Mutations such as deletions, insertions, substitutions, and others are retrieved from submitted literature and sequences submitted in online databases like NCBI (dbSNP, ClinVar, PMC), Uniprot, DisGeNET.

PolyPhen-2 stands for Polymorphism Phenotyping v2. It's a modern version of the previous PolyPhen tool that uses a few physical and comparative factors to predict the effects of amino acid substitutions in coding nonsynonymous SNPs (nsSNPs) on the structure and function of human proteins.[12] The tool calculates the score difference between variants by estimating the PSIC score for each variant.

SNPs &GO is used to predict the involvement of point mutations in proteins with functional annotations in causing disease in humans.

PROVEAN stands for Protein Variation Effect Analyzer, and it is used to predict whether a mutation has any impact on the functioning of a protein.[13] The individual results of all mutations are displayed in tabular form within a few seconds after the submission.

C. Prediction of protein structural stability

I-Mutant (version2.0) (http://folding.uib.es/i-mutant/i-mutant2.0.html) is a neural network-based tool that

analyses protein stability and alterations by taking the single-site mutations into account. To predict the mutational effect on protein stability, the fasta sequence of a protein retrieved from Uniprot is used as an input. The result is displayed in the form of scores.

D. Predicting the molecular phenotypic effects of deleterious SNPs

Project HOPE (http://www.cmbi.ru.nl/hope/) is an online web-server that collects structural information from various sources to search protein 3D structures. We entered the four SNPs (rs121913471, rs121913468, rs121913470, rs28933368, and rs121909107) that we obtained from the last step together with the primary structure of the *ErbB2* protein (obtained from the Protein Data Bank (http://www.rcsb.org/pdb/explore/explore.do?structureId =1D5V) into HOPE20.

HOPE allows you to choose the amino acid that was affected by the mutation, alter the specific amino acid, and then observe the resulting change in the 3D (tertiary/quaternary) structure, producing the expected change as well as an explanation for it (in both structure and function of the protein).

E. Homology modeling

Homology modeling refers to constructing the 3D structure of a novel or mutated protein based on the known structure of a protein resembling it (template protein). I-TASSER software was used for performing homology modeling. It's a various leveled protein structure demonstrating technique dependent on optional construction upgraded Profile-Profile stringing Alignment (PPA) and iterative Threading gathering refinement (TASSER) programming usage.[14]

The fasta sequence retrieved from the Uniprot or NCBI (dbSNP) is submitted. After filling in the remaining details, the process is terminated at Run I-tasser, and results are sent to the user-submitted institute Email id. The result briefs the predicted protein secondary structure models, information about the secondary structure and ligand binding sites of protein, and ligands that bind to it. Based on the c-score between 5 to 2, the homolog model having the highest score is selected for further study.

The Ramachandran plot is a method of noticing vigorously allowed regions in protein structure for backbone dihedral angles psi averse to phi of amino acid residues. It says about predicted structure with intrigue amino acids in a mirror plot of -180 degree to +180-degree rotation of psi and phi between torsion angles [15]. *(http://mordred.bioc.cam.ac.uk/~rapper/rampage.php).*
The Ramachandran plot can be viewed by both rampage and discovery studio.

F. Docking

PyRx0.8 (http://pyrx.sourceforge.net/downloads) is a virtual screening tool for computational drug discovery that can be used to screen library compounds against potential drug targets.[16] Molecular Docking of *ErbB2* and its mutated isoforms was done by Auto Dock vina in PyRx. For docking, drug structures are required in the PDB format. This was done by downloading drugs from the chEMBL or Pub Chem database. These files were then changed over to PDB format. Subsequently, ligands were obtained in PDB format.

The ligands in PDB format were docked with macromolecule (*ErbB2*) degrees, and the score represented by binding affinity was noted. The interactions were visualized using Discovery Studio and Pymol software. The results were saved in png (Portable Network Graphics) format.

RESULTS

The dbSNP comprises both validated and non-validated polymorphisms. There was a total of 150 nonsynonymous missense SNPs were determined using dbSNP. Missense nsSNPs, 3'UTR SNPs, and intronic SNPs were selected for an examination. These 150 SNPs were sorted out into 37 SNPs based on their scores. The variants' data were compared with ClinVar and DisGeNET data, and out of this 37 nsSNPs, only 5 SNPs were sorted out for further analysis.

A. Deleterious nsSNPS by SIFT

Out of 150 SNPs, some were deleterious, and some were tolerated based on C-score (ranges from 0 to 1). SNPs with a <0.05 score is considered deleterious. Whereas the score with >0.05 is deemed to be tolerated.[17] From these 150 SNPs, only 5 SNPs were chosen based on their scores.
According to the classification proposed by Ng and Henikoff 2003 lower, the tolerance index greater will be the functional impact.[18]

Among the five nsSNPs, 4nSNPs were perceived to be profoundly deleterious with a tolerance index score of 0.00. One nsSNPs was found to be tolerated but was identified to be highly disease-causing by other further analysis.

B. Evaluation of damaging effects of nsSNPS related to mutation by SNPs & GO, PolyPhenv2, PROVEAN

All the 5 SNPs submitted to the SIFT server were also submitted to the PolyPhen server. For PolyPhen-2, scores range from 0-1. Higher the score more is the disease-causing ability of mutation. Four nsSNPs were found highly damaging, and rs121913471 was identified as benign. These damaging SNPs are said to affect the structure and stability of the *ErbB2* gene.

SNP & GO is a prediction tool to find which particular mutation among the submitted mutations is responsible for causing disease. Output in SNPs & GO consists of many terms like effect, Reliability index (RI), Biological process (BP), Molecular function (MF), Cellular

components (CC). The effect column signifies the ultimate effect due to mutation. It can either be neutral or disease-causing polymorphism. Furthermore, the reliability index is displayed in the range of 0-10, where 0 stands for unreliable and ten stands for reliable prediction of effect. Scores above 0.5 are predicted to be disease-causing polymorphism, and scores below 0.5 are predicted to be neutral, or they can not cause any disease.

Provean results are obtained in the form of a table where all the listed query mutations, their PROVEAN score, and prediction. The prediction cut-off in the case of this tool is -2.5, i.e., when prediction scores are less than 2.5, the mutations are deleterious, and if greater than -2.5, it is neutral. According to the obtained result, four mutations are deleterious, and the other one mutation is neutral. The results of SIFT, PolyPhen, SNPs & GO and PROVEAN are given in table1.

Table 1: List of nssnps analysed by sift, Polyphen, Snps and Go and Provean.

SNP	*Amino acid change*	*Protein ID*	*Amino acid*	*Effect (SIFT)*	*SIFT Score*	*Polyphen Score*	*Effect (Polyphen)*	*SNPs and GO (score)*	*Effect (SNPs and GO)*	*Provean Score*	*Effect (Provean*
rs121913470	L755S	ENSP00000463714	L S	Deleterious	0	1	Damaging	0.947	Disease	-5.027	Deleterious
rs121913471	V777L	ENSP00000463714	V L	Tolerated	0.309	0.013	Benign	0.842	Disease	-0.973	Neutral
rs28933368	E914K	ENSP00000269571	E K	Deleterious	0	1	Damaging	0.944	Disease	-3.554	Deleterious
rs121913468	D769H	ENSP00000269571	D H	Deleterious	0	1	Damaging	0.802	Disease	-6.130	Deleterious
rs61737968	E79A	ENSP00000269571	E A	Deleterious	0	1	Damaging	0.87	Disease	-5.171	Deleterious

C. Effect of mutation on the stability of protein structure

I-Mutant 2.0 is a neural network-based tool for analyzing protein stability and alterations by taking single point mutations into account. I-Mutant 2.0 permits the choice of the prognostication of protein stability changes at different levels of temperature and Ph [19]. The DDG score (<0) determines a decrease in stability and the other way round. However, the RI (Reliability Index) range is 0-9. The fasta sequence of the protein obtained from UniProt is used as an input to predict the mutational effect on protein stability. The results are shown in Table 2.

Table 2: Outpt for I-Mutant.

rs _id	*Mutation*	*Wild Type*	*New*	*Stability*	*DDG*	*Temperature*	*pH*
rs121913470	L755S	L	S	Decrease	-2.87	25	7
rS121913471	V777L	V	L	Increase	0.76	25	7
rs121913468	D769H	D	H	Decrease	-2.74	25	7
rs28933368	E914K	E	K	Decrease	-3.32	25	7
rs61737968	E79A	E	A	Increase	0.44	25	7

From this above analysis, we have found that four nonsynonymous missense mutations L755S, D769H, V777L, and E914K, are found out to be highly disease-causing mutations. L755S, V777L, D769H mutations are responsible for causing breast cancer, and E914K mutation is responsible for causing glioma.

D. Molecular phenotypic effects of SNPs

Project HOPE displays a 3D model of rs121913470, rs12913471, rs121913468, rs28933368. The spatial effects of each of the individual domains they are a part of and their results on closest domains. The 3D representation of the nsSNPs and their effects on *ErbB2* is shown in Figures 2,3,4, and 5.

Fig. 2: 3D representation of rs121913470 and their effect on ErbB2. The first overview shows the protein in, grey and amino acid in magenta, the closeup shows the side chain of both the wild type (Leucine) and the mutant residue (Serine) at position 755 and green and red respectively.

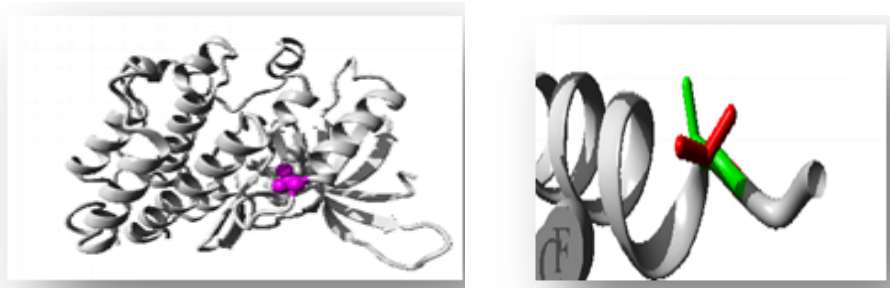


Fig. 3: 3D representation of rs121913471 and their effect on ErbB2. The first overview shows the protein in, grey and amino acid in magenta, the closeup shows the side chain of both the wild type (valine) and the mutant residue (Leucine) at position 777 and green and red respectively.

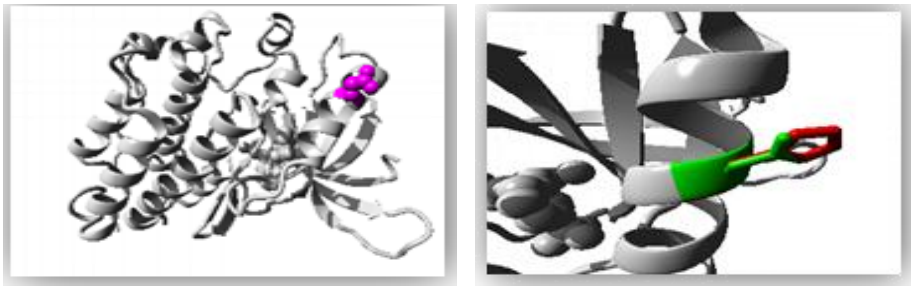


Fig. 4: 3D representation ofrs121913468 and their effect on ErbB2. The first overview shows the protein in, grey and amino acid in magenta, the closeup shows the side chain of both the wild type {Aspartic acid) and the mutant residue (Histidine) at position 769 and green and red respectively.

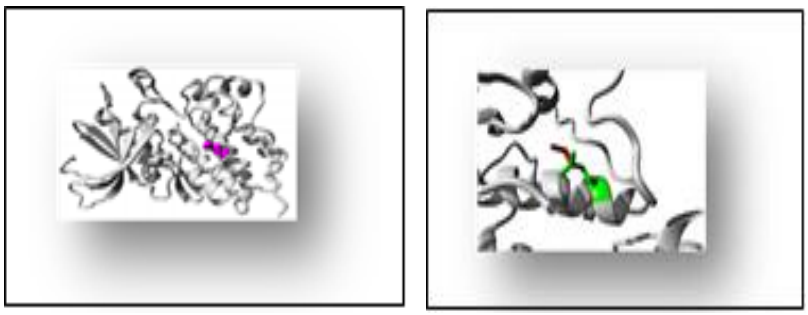


Fig. 5: 3D representation of rs 28933368 and their effect on ErbB2. The first overview shows the protein in, grey and amino acid in magenta, the closeup shows the side chain of both the wild type (Glutamic acid) and the mutant residue (Lysine) at position 914 and green and red respectively.

E. Homology modeling

Preparation of target protein structure: After selecting the desired protein, the protein's FASTA format was used to determine its structural prediction. The sequence is submitted to the database of Zhanglab (I-TASSER).

Structure prediction models by I-TASSER (For normal protein sequence and mutated protein sequence): The homolog of an ErbB2 was built by using the I-TASSER online server. The protein sequence of the ErbB2 Gene (both normal and mutated) was submitted to I-TASSER to visualize the protein structure of the gene and obtain

the 5-model system. The homology model of normal and mutated ErbB2 protein sequence is shown in figure 6.

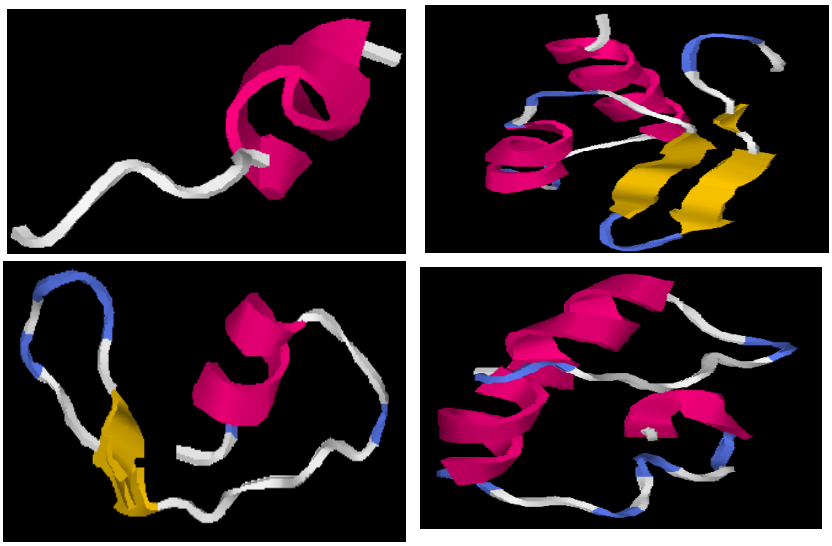


Fig. 6.: (a) Homolog model of ErbB2 obtained from I-TASSER with a c-score -1.29 (rs121913470), (b) Homolog model of ErbB2 obtained from I-TASSER with a c-score -4.00 (rs121913471), (c) Homolog model of ErbB2 obtained from I-TASSER with a c-score -1.53 (rs121913468), (d) Homolog model of ErbB2 obtained from I-TASSER with a c-score 0.47 (rs28933368).

F. Rampage and Discovery Studio

The Ramachandran plot for the ErbB2 protein structure causing breast cancer and glioma is shown in Figure 7. The residues in the allowed, favored and outliers' regions for both the protein structure are given in Table.

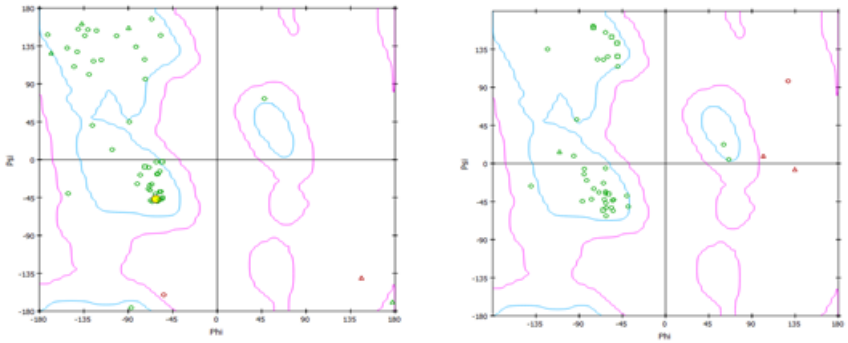


Fig. 7:(a) Ramachandran plot for ErbB2 protein structure causing breast cancer (b) Ramachandran plot of ErbB2 protein structure causing glioma.

Table 3: The table shows the no of favored regions, allowed regions and outlier region of the submitted protein structure (breast cancer) and protein structure (glioma).

Breast cancer			**Glioma**		
Number of residues in allowed region	2.0% expected	9.4%	Number of residues in allowed regions	2.0% expected	7.0%
Number of residues in outlier region	—	1.9%	Number of residues in outlier regions		2.3%
Number of residues in favoured region	98% expected	88.7%	Number of residues in favoured regions	98.0% expected	90.7

G. Molecular docking

The protein-ligand binding interactions were analyzed using PyRx software. The main aim of docking is in structure-based virtual screening for discovering new active drugs towards a particular target protein [20]. We use different synthetic drugs, and we compared their binding affinity with natural compounds such as spinasterol, turmerones, furanodiene, cyclocurcumin. The list of drugs with their structure and binding affinity

is shown in table 4. The pictorial representation of the docked structure is shown in figure 8.

Table 4: Lists of drugs along with its Structure and Binding affinity used for docking.

PubChem ID	*Name of compound*	*Binding affinity (Kcal/mol)*	*RMSD UB*	*RMSD LB*
51039094	Irbinitinib	-9.6 and -8.9	0.0	0.0
11620908	TAK-285	-8.4 and -8.0	0.0	0.0
5281331	Spinasterol	-7.7 and -7.9	0.0	0.0
69879809	Cyclocurcumin	-8.0 and -8.5	0.0	0.0

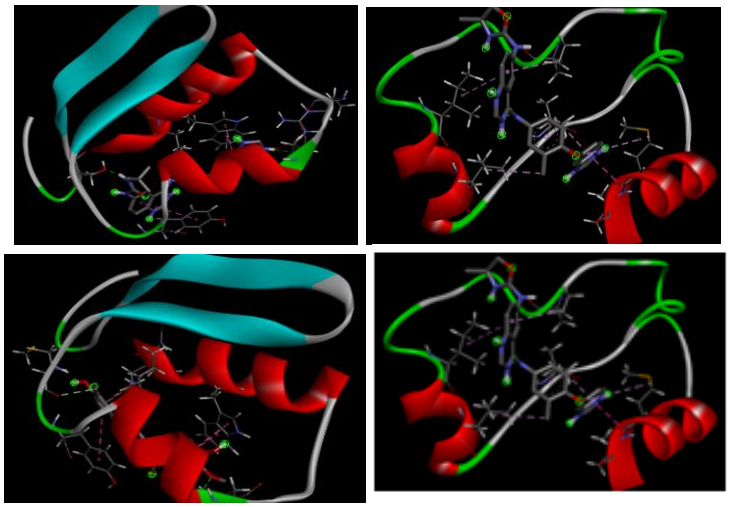
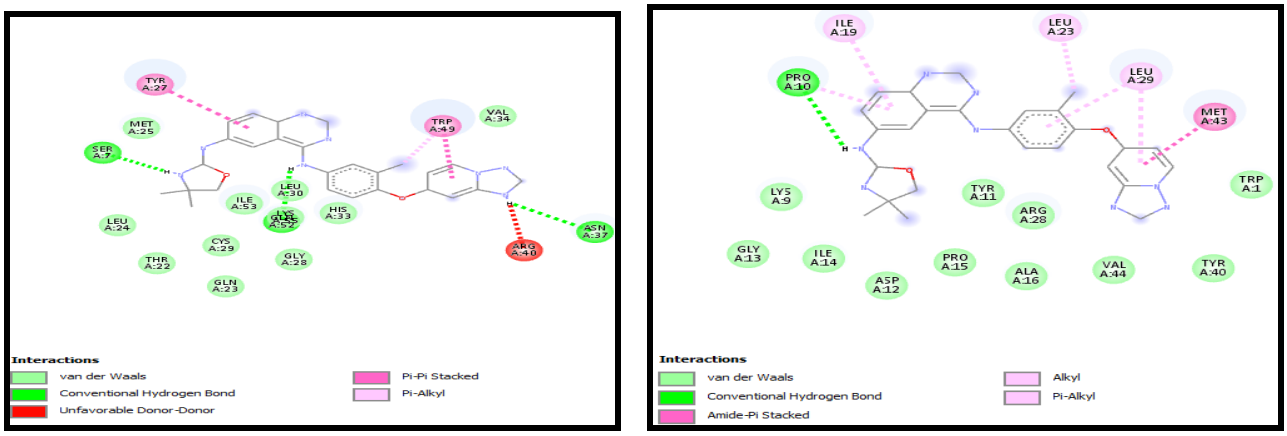


Figure 8: (a)ErbB2 protein model (breast cancer) docked with irbinitinib, (b) ErbB2 protein model (glioma) docked with irbinitinib, (C) ErbB2 protein model (breast cancer) docked with cyclocurcumin (D) ErbB2 protein model (glioma) docked with cyclocurcumin.

The 2D diagram represents the binding and interactions of amino acids with the ligand. The dark green color represents the hydrogen bond interaction. The light green color interaction represents the van der Waals interaction; the pink color represents the Pi- Alkyl interaction. The orange color indicates the pi-cation interaction. The red color indicates the unfavorable donor- donor interaction. The 2D interaction of ErbB2 variants is shown in figure 9.



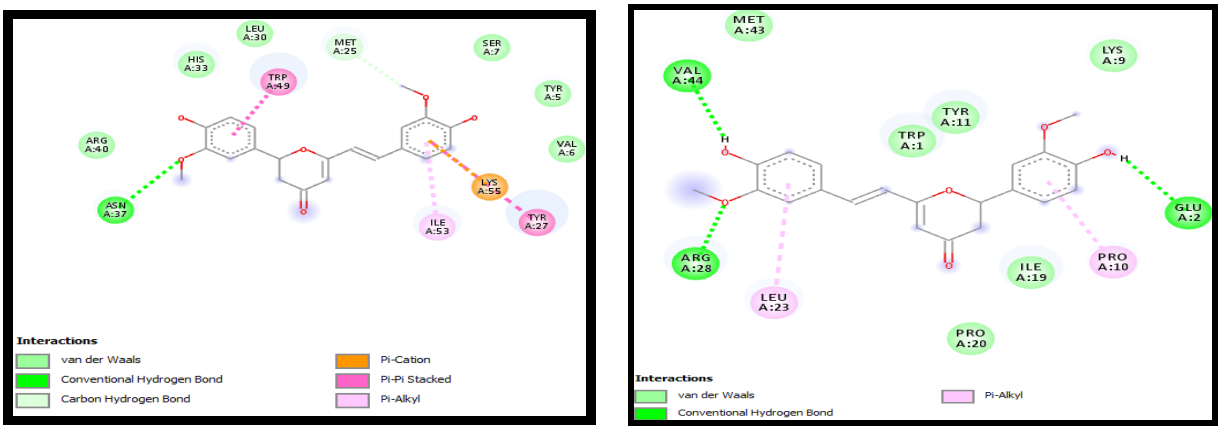


Fig. 9: (a) 2-dimensional interaction of ErbB2 variants responsible for causing breast cancer docked with synthetic ligand Irbinitinib, (b) 2-dimensional interaction of ErbB2 variants responsible for causing glioma docked with synthetic ligand irbinitinib, (c) 2-dimensional interaction of ErbB2 variants responsible for causing breast cancer docked with natural ligand cyclocurcumin, (d) -dimensional interaction of ErbB2 variants responsible for causing glioma docked with natural ligand cyclocurcumin.

H. Protein-Protein interaction

The proteins rarely act alone and usually function better in groups as one protein's function tends to regulate the other proteins' expression or suppression. That is why the molecular processes within a cell are carried out by molecular machines built from a vast number of proteins. Here, *ErbB2* protein interacts directly with other proteins such as EGF, GRB2, GB7, BTC, SHC1, PTPN11, and other proteins, as shown in figure 10.

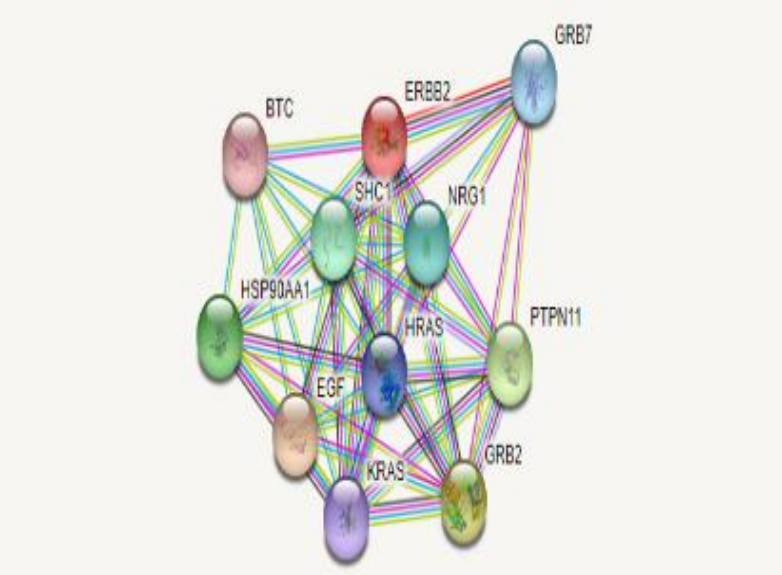


Fig. 10: Protein-Protein interaction network of ErbB2 protein.

DISCUSSION

To put it in a nutshell, the targeting *ErbB2* protein may also affect other proteins that are interacting with it. Hence, the target drug designing may find this valuable information for modeling and designing the drug appropriately. The *ErbB2* gene is a transmembrane glycoprotein that plays a vital role in many cancers like gastric cancer, ovarian cancer, colorectal cancer, and the most important, breast cancer. The most common form of adult brain tumor is glioma, and ErbB2 plays a crucial role in tumor progression. [21]. Many human SNPs are now recognized (above 4-million unique SNPs) (http://www.ncbi.nlm. nih.gov/SNP/index.html), alongside the genome sequence and other proteome details, they leave a much more comprehensive understanding of the genotype-phenotype relationship. Therefore, an effort was made to identify SNPs that can modify the structure, function, and expression of the ErbB2 gene. There were 37 nsSNPs submitted to the SIFT, and from that, 37 nsSNPs, 9 SNPs were introduced to the PolyPhen server; out of these 4 SNPs were found to be highly deleterious by SIFT and highly damaging by PolyPhen. The 4 SNPs were highly disease-causing and associated with breast cancer (rs121913470, rs121913468, rs121913471) and glioma (rs28933368). No study was reported, which shows the association

between damaging nsSNP (rs61737968) and any disease. Therefore, the validation of those nsSNPs in any disease is required to enrich this finding. The study of human genetic variations in the ErbB2 Gene, combined with a computational method to predict their potential functional influence, will make the ErbB2 gene variant and its effects on protein functional characteristics easier to understand. Structural validations tools like I-TASSER RAMPAGE and Discovery studio were used for protein structure predictions and helped find the association of protein in various disease development and the development in potent drug discovery. I-TASSER built the protein model. The built protein model is docked. It showed that synthetic drug irbinitinib and natural drug cyclocurcumin interacted more effectively with mutated ErbB2 protein**,** and these drugs can be used to treat breast cancer and glioma.

CONCLUSION

HER2/ErbB2 gene is associated with various cancer but mainly causes breast cancer. However, the present study aims to find out the association of the *ErbB2* Gene with breast cancer and various other cancers and evaluate the role of Single-nucleotide Polymorphisms (SNPs) in cancer.

In this study, different types of cancer associated with ErbB2 mutations were obtained, most of which were breast cancer. Glioma was also found. Involvement of the ErbB2 gene in breast cancer and glioma was selected for further analysis. SNPs are usually uniformly present, abundant, and widely utilized genomic markers used for whole-genome population structure analysis. These SNPs result from imprecision during DNA replication at a specific position, causing substitution carried by future generations. Moreover, HER2/ErbB2 gene is a 185kd transmembrane glycoprotein, which contained many nonsynonymous missense SNPs, out of which 30 were deleterious (SIFT), and we selected five high -risk disease-causing damaging SNPs which is responsible for causing breast cancer and glioma. The evaluation of the damaging effect was carried out using PolyPhen2, which showed that a high percentage of SNPs were observed to be damaging. Furthermore, I-Mutant was used to check changes in protein stability caused by point mutations, and it showed that most SNPs were responsible for decreasing structure stability. Structural validations tools like I-TASSER RAMPAGE and Discovery studio were used for protein structure predictions. It helps find the association of protein in various disease development and the development in potent drug discovery. PyRx is a Computational Drug Discovery (CDD) virtual screening program used to screen libraries of compounds against potential drug targets. PyRx was used in building the mutated structure of *the ErbB2* gene. The built model showed that the synthetic drug irbinitinib and natural drug cyclocurcumin interacted more effectively with *ErbB2* protein. Based on the binding affinity, these drugs can be used to treat breast cancer and glioma. However, the assurance for the treatment of glioma is still under investigation. This study helps in approaching the future prospects of computational drug discovery and in determining effective therapies in the future by using in-silico research methodology. This whole-genome research is done by using various databases, software, and bioinformatic tools.

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Declarations

1. Funding

Not applicable

2. Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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