



EFFECT OF 2677>T/A NON-SYNONYMOUS SINGLE POLYMORPHISM OF HUMAN MDR1 GENE ON SUBSTRATE BINDING TO PERMEABILITY GLYCOPROTEIN

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Abstract: Background: Non-Synonymous polymorphism in the human MDR1 gene tend to decrease the transporter activity of the P-glycoprotein, a major efflux transporter of endogenous substrates and xenobiotics predominantly cytotoxic agents. Hence this study was determined to assess the effect of 2677 G>T genetic polymorphism of human MDR1 gene on the structure of P-glycoprotein. **Methodology:** Three dimensional structures of MDR variants were ab-initio model using iTASSER server and drug binding residues of transmembrane domain were predicted using Prankweb server. Substrate binding was analyzed by molecular docking using Autodock 4.2.6. Moreover, statistically significant difference was observed between the binding affinities of substrates to wild and mutant variants (One way Analysis of Variance, df=1, 95% CI). **Results & Discussion:** Here in, we have investigated the effect of 2677G>T/A non-synonymous polymorphism on substrate transport via human P-glycoprotein through *in silico* methods. Few molecular modelling studies of human P-gp have stressed the significance of Ser222, Phe303 and Phe343 in substrate conductance. **Conclusion:** The binding energy of P-gp substrates to the mutant (Ala893Ser and Ala893Pro) was found to be less than that of the wild type. Hence genetic testing of *hMDR1* need to be done before initiating therapy with cytotoxic and narrow therapeutic P-gp substrates.

Keywords: MDR1, Pharmacogenomics, P-glycoprotein, Polymorphism

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INTRODUCTION

The multidrug resistance 1 gene MDR1, also known as ABCB1, is localized at chromosome 7q21.1, consisting of 29 exons ranging in size from 49 to 209 bp, encoding an mRNA of 4.5kb(1). P-glycoprotein (Pgp), the product of MDR1 gene is a 170kDa transmembrane protein (Hsia et al., 2002), belongs to ABC super family of transporter proteins which is well recognised for its role in drug transport and chemo resistance(1). It protects the tissues from toxic xenobiotics and other endogenous substances by exporting the substrates from intracellular to extracellular space. The amount of expression, regulation and activity of P-gp influenced by MDR1 gene polymorphisms can directly affect the pharmacokinetics and pharmacodynamics of drugs that are substrates of P-gp(2), leading to inter-individual variation in drug response and toxicity(3,4). The substrate specificity of P-gp is broad including clinically

relevant agents, i.e. anti-neoplastics such as doxorubicin, actinomycin D, paclitaxel, antibiotics such as erythromycin, levofloxacin, sparfloxacin, rifampicin, antihypertensives such as losartan, antivirals such as nelfinavir, indinavir, efavirenz, analgesics such as morphine, antiepileptics such as phenytoin, phenobarbital, antidepressants such as amitriptyline, immunosuppressants such as cyclosporine A, tacrolimus, rapamycin, anti-arrhythmics such as digoxin, verapamil, antilipidemic such as atorvastatin and steroids such as aldosterone, cortisol, dexamethasone(5–7). In addition, the polymorphism exhibited by MDR1 gene is one of the factors responsible for individual susceptibility to various diseases such as breast cancer, colorectal cancer, Parkinson's disease and ulcerative colitis(8–10). Human MDR1 gene is highly polymorphic and over 50 SNPs have been identified(11). Among these, variants 2677G>T/A/C in exon 21, 3435C>T in exon 26 and 1236C>T in exon 12 are the most studied alleles of MDR1 gene(171 (12). MDR1 gene is extensively explored in Indian populations except NEI. In general, the Indians have at least one variant allele of MDR1. A significant inter- and intra-ethnic differences were observed in the allelic and genotype frequencies between North and South Indians(13). On the other hand, the frequency of 1236C>T is available only in North Indian populations (51.9%) and is not available for South Indians. Hence, we designed to study to determine the genotype frequency of MDR1 C1236T genetic polymorphisms in South Indians and thereby predict patient response towards MDR1 substrates.

MATERIALS AND METHODS

Target Modelling:

High resolution crystal structure of human P-gp was queried in RCSB-PDB database and four structures with different resolutions were identified. Identified crystal structures bared the PDB ids 6C0V, 6FN1, 6FN4 and 6QEX with resolutions of 3.40Å, 3.58Å, 4.14Å, and 3.60Å respectively. However, 6C0V, 6FN1 and 6QEX displayed significant number of missing atoms in their structure while 6FN4 had the least resolution. Hence, the human 3D structure of human P-gp was modelled by a threading approach using iTASSER server that predicts protein structure by a hierarchical iterative threading assembly refinement approach (Available at: <https://zhanglab.ccmb.med.umich.edu/I-TASSER/>)(14). The primary sequence of the constructed 3D structure of human P-gp was validated by verifying with the FASTA sequence from UniprotKB (**Identifier:** P08183-1). Three dimensional structures of human P-gp variants with serine and proline at 893 positions were modelled by residue mutation using Swiss PDB viewer. The retrieved and modelled 3D structures were pre-processed further as per the standard methods.

Energy Minimization:

The 3D structures of four target proteins were minimized using the YASARA server that uses YASARA force fields to compute energy functions called knowledge based potentials (Available at: <http://www.yasara.org/minimizationserver.htm>)(15).

Protein Conformation and Stability:

Energy minimized three dimensional structures were subjected to conformational stability analysis. Dihedral angles and atomic contacts were analyzed through Ramachandran Plot using Swiss-PDB viewer(16).

Active Site Prediction:

The drug binding domain of the human P-glycoprotein was determined using Prankweb Server which uses P2 rank, a template-free machine learning method based on local chemical neighbourhood ligandability (Available at: <http://prankweb.cz/>)(17).

Molecular Docking Analysis:

Molecular docking analysis was carried out using Autodock 4.2.6. The drug transporting domain of human P-glycoprotein was pre-defined by a three dimensional grid using Autogrid program(18). Pre-modelled wild and variant proteins were used as targets while 3D structures of ligands were retrieved from ZINC small molecule database. All the targets and ligands in Autodock structure file format (.PDBQT) were employed as inputs while the output

docking log (.dlg) contained data of binding energy, inhibitory constant (KI), bonding interactions and conformational poses of the ligand within the target active site. Genetic algorithm was adopted for conformer search with 25000 evaluations and 27000 generations. Interaction of the docked ligands with the amino acid residues in the active site of the target were visualized using Pymol 2.3 (Schrodinger, LLC). Two-dimensional ligand interactions maps were constructed using LeView software to gain deeper insights on difference in substrate binding to wild type and modelled variants of human P-glycoprotein.

RESULTS& DISCUSSION

The human P-glycoprotein is a transmembrane efflux protein that extrudes toxins and xenobiotics from cells(19). Non-synonymous polymorphisms in the human *MDR1* gene are associated with decreased efflux transport of diverse spectrum of therapeutic agents including cytotoxic agents leading to intracellular drug entrapment and toxicity(20). Here in, we have investigated the effect of 2677G>T/A non-synonymous polymorphism on substrate transport via human P-glycoprotein through *insilico* methods(21).

The Ala893Ser and Ala893Pro mutant variants of human P-glycoprotein were modelled through iTASSER server which employs a mathematical scoring function called C-Score for estimating predicted model quality. C-Score, abbreviated as the confidence score is computed based on the significance of threading template alignments and the convergence parameters of the structure assembly simulations(3). Five models were constructed for each mutant variant and those with highest C-scores were selected for the current study (+0.97 and +1.2 for Ala893Ser and A1a893Pro variants respectively)(22). In order to mimic the biological target protein and validate the stability of the iTASSER predicted models were subjected to energy minimization. The pre- and post-minimization potential energies of the wild type and mutant variants are shown in **Table 1**.

Protein conformation and stability of the energy minimized targets were analysed through dihedral angles and atomic contacts by Ramachandran plot. Ninety eight percent of the residues of were within the allowed region, exception being glycine and proline for wild type, Ala893Ser and Ala893Pro variants suggestive of minimal steric collisions and reliable spatial geometry for the modelled structures. Ramachandran plot of energy minimized structures is shown in **Figure 1**.

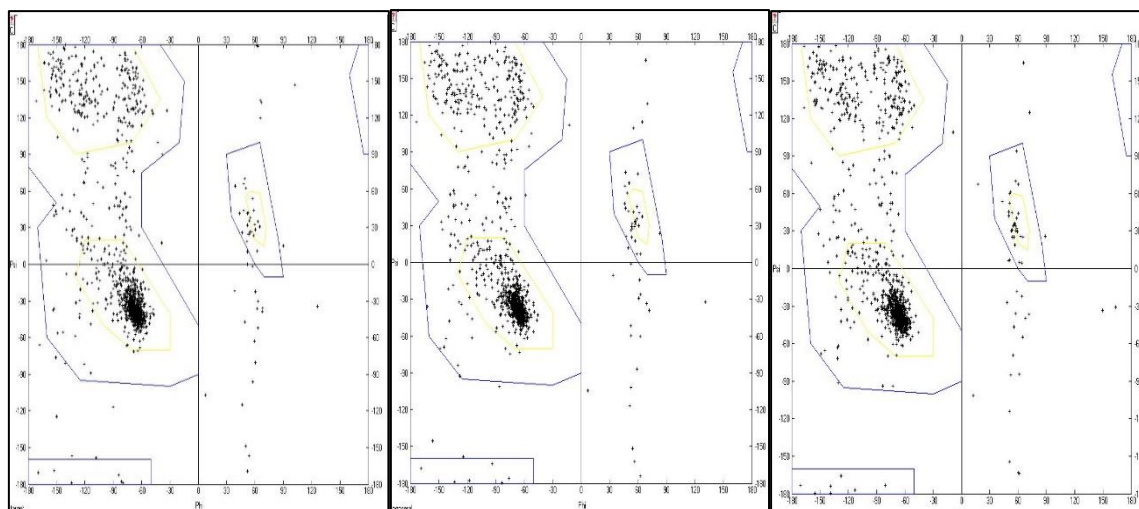


Fig 1: Ramachandran Plot of (a) WILD TYPE (b) Ala893Ser (c) Ala893Pro

Difference in spatial positioning and torsion angles of residues was observed between the wild and mutant variants of human P-gp suggestive of the non-synonymous nature of the 2677G>T/A single nucleotide polymorphism in human MDR1 gene. Moreover, significant differences were

observed between the active site residues of the wild and mutant protein structures, a plausible cause of altered intrinsic activity. The active site residues of the wild type and modelled mutant variants of human P-gp is shown in **Figure 2**.

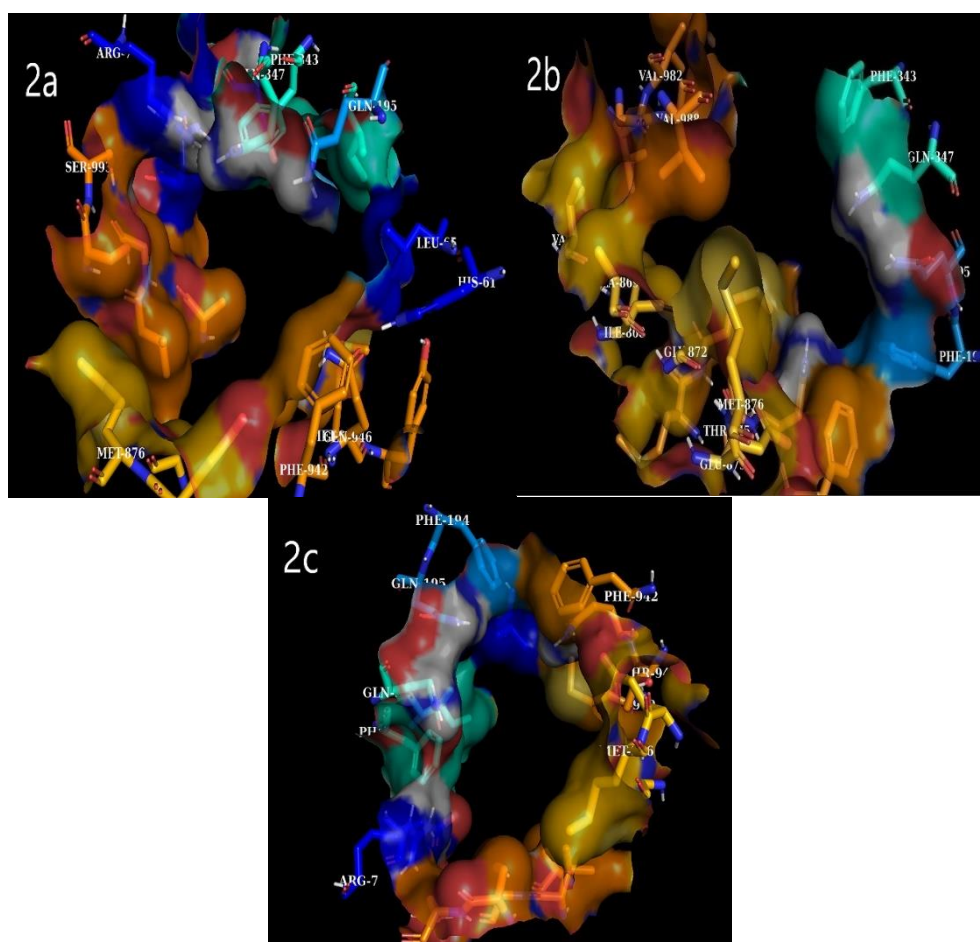


Fig 2: Human PGP Transmembrane Domains involved in Substrate Uptake and Transport

Prankweb predicted active site conformations and amino acid spatial geometries from Ramachandran Plot were analyzed to determine the difference in residues of

transmembrane domain crucial for drug uptake and transport via the human P-gp. Few molecular modelling studies of human P-gp have stressed the significance of

Ser222, Phe303 and Phe343 in substrate conductance. Ser222, Phe303 and Phe343 lined the active site of human P-gp with distances from the centre of active site at 6.8Å, 9.2Å and 7.3Å respectively(23). While Ser222 and Phe343 were present away from the active site of Ala893Ser variant at a distance of 28.3Å and 31.6Å respectively. The amino acid residue Phe303 was observed in the active site at distance of 19.6Å in Ala893Ser variant. Similar differences were observed in residues forming the active site of human P-gp with Ala893Pro variant. The amino acid residue Ser222 was observed to be present at a distance of 14.2Å whereas Phe343 and Phe303 were observed at distances of 31.8Å and 22.9Å from the centre of the active site respectively. From the results obtained, it is clear that the amino acid residue Phe303 in Ala893Ser variant and Ser222

in Ala893Pro variant were confined to the active site of their respective residues after non-synonymous variations. It is clearly evident from the shift in spatial geometry of amino acid residues that the non-synonymous variations Ala893Ser and Ala893Pro induce significant changes in the active site conformation of human P-gp and thereby impair the substrate conductance process.

Effect of non-synonymous polymorphisms on substrate interaction with human P-gp was determined through molecular docking analysis(c). Ligand interaction and binding conformations were analyzed in terms of the following parameters: Binding energy (ΔG Kcal/mol), inhibitory constant (kI), conformational orientation of the ligand in the active site, hydrogen bonding, π - π interactions and root mean square deviation (RMSD) **Table2.**

Table 1: Energy Minimization of the Wild Type, Ala893Ser and Ala893Pro Variants

SNo.	Type of Variant	Pre minimization energy(kcal/mol)	Post minimization energy(kcal/mol)
1	Wild Type	28895.3058	-927.3025
2	Ala893Ser	34411.2056	2127.2448
3	Ala893Pro	28745.2540	2540.2540

Table 2: Molecular Docking Analysis of PGP Substrates with the Wild Type, Ala893Ser and Ala893Pro

Drugs	ΔG (Kcal/mol)			kI(mM)		
	Wild	Ala893Ser	Ala893Pro	Wild	Ala893Ser	Ala893Pro
Cisplatin	-5.62	-2.81	-3.37	52.49	7.24	10.75
Daunorubicin	-4.9	-2.89	-3.29	31.60	7.66	10.16
Daxorubicin	-4.7	-2.87	-3.28	27.45	7.56	10.09
Epirubicin	-4.39	-2.14	-2.86	22.06	4.52	7.50
Geftinib	-3.72	-0.98	-1.37	13.76	1.99	2.63
Imatinib	-2.97	-1.88	-1.03	8.11	3.76	2.07
Paclitaxel	-2.04	-0.05	-1.71	4.21	1.04	3.34
Tamoxifen	-3.85	-2.12	-2.37	15.08	4.45	5.31
Vinblastine	-6.44	-2.62	-0.88	93.54	6.34	1.86
Vincristine	-3.39	1.48	-0.82	10.90	0.35	1.78
P Value [#]	0.000279**			0.00357**		

The mean \pm SD of ΔG (Kcal/mol) of wild type, Ala893Ser and Ala893Pro were -4.2 ± 1.3 , -1.7 ± 1.4 and -2.1 ± 1.1 respectively. The mean \pm SD of kI (mM) of wild type, Ala893Ser and Ala893Pro were 27.9 ± 27.0 , 4.5 ± 2.7 and 5.5 ± 3.7 respectively. [#]represents P-value retrieved through One-way ANOVA ordinary measures, ** represent P-value significant at 95% confidence interval.

Hydrogen bonding interactions with the active site residues of Wild Ala893Ser and Ala893Pro mentioned in the below table

Table 3: Hydrogen Bonding Analysis of PGP Transporter with the Wild Type, Ala893, Ala893Pro

Drugs	Hydrogen bonding		
	Wild	Ala893Ser	Ala893Pro
Cisplatin	GLN152,ASN156	-	GLN152
Daunorubicin	ASN156,GLY477,ASP474	ASN156,ASP174	ASP149
Daxorubicin	HIS48,GLN51,GLN152	HIS48,GLN152,ILE39	ASN156,GLN152
Epirubicin	ILE39, PHE482	-	-
Geftinib	HIS48,GLN152,ASP149	HIS48,GLN152	SER153,ASP149
Imatinib	GLY477,ASN156	ASN156	ASP149
Paclitaxel	PRO481	-	-
Tamoxifen	GLN362	-	-
Vinblastine	ASN156,ASP474,GLY477	ASP149	ASP149
Vincristine	ASN156,ASP357	-	ASN156,ASP474

And the three-dimensional Docked confirmations of P-gp substrates with WILD type, Ala893Ser and Ala893Pro are shown in **Figure 3**

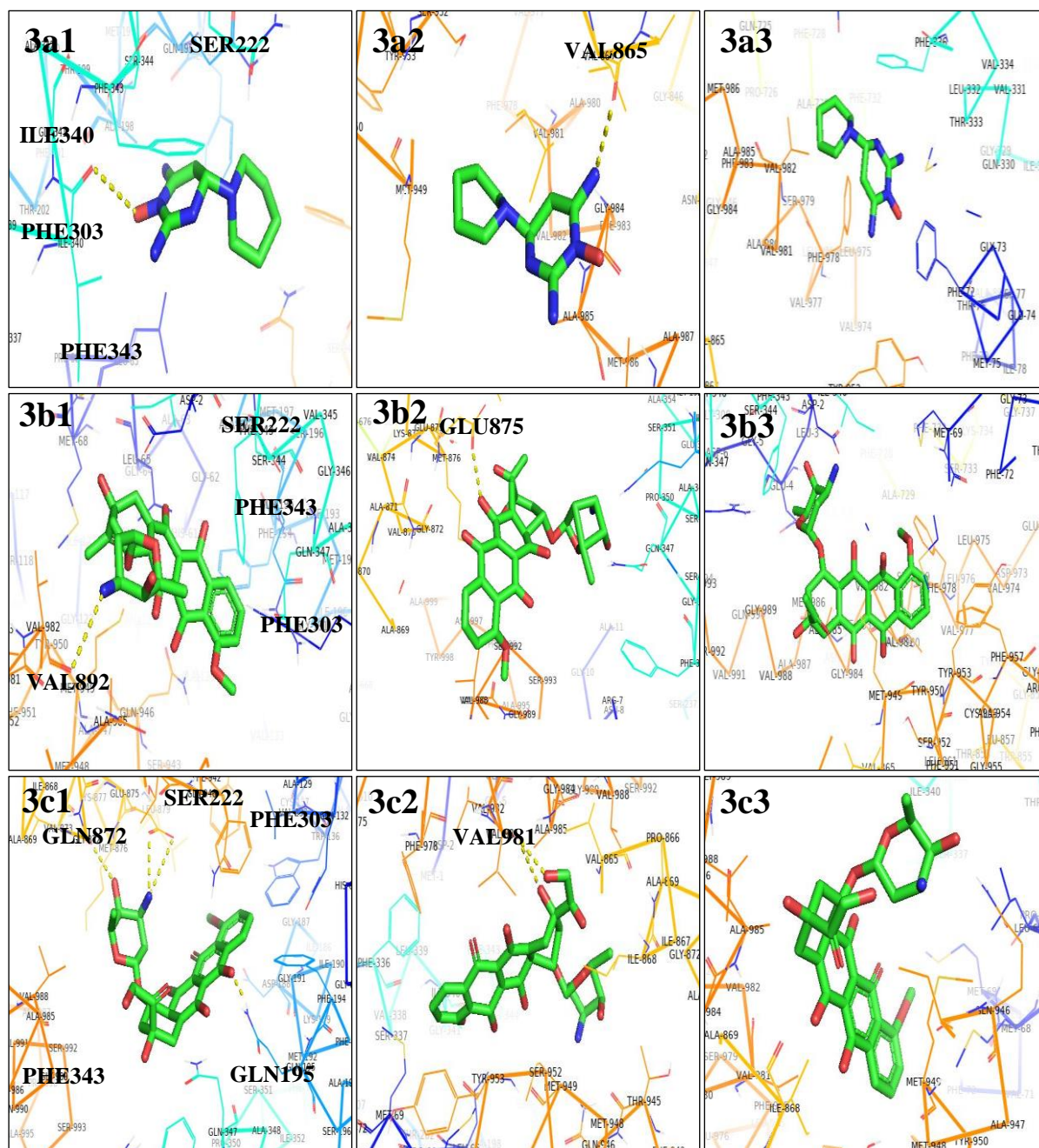


Fig.3: Three dimensional Complexes of Wild and Modeled Mutant PGP structures with docked substrates.

3a1, 3a2, 3a3 represent Cisplatin bound to wild, Ala893Ser, Ala893pro variants respectively
 3b1, 3b2, 3b3 represent Daunorubicin bound to wild, Ala893Ser, Ala893pro variants respectively
 3c1, 3c2, 3c3 represent Doxorubicin bound to wild, Ala893Ser, Ala893pro variants respectively

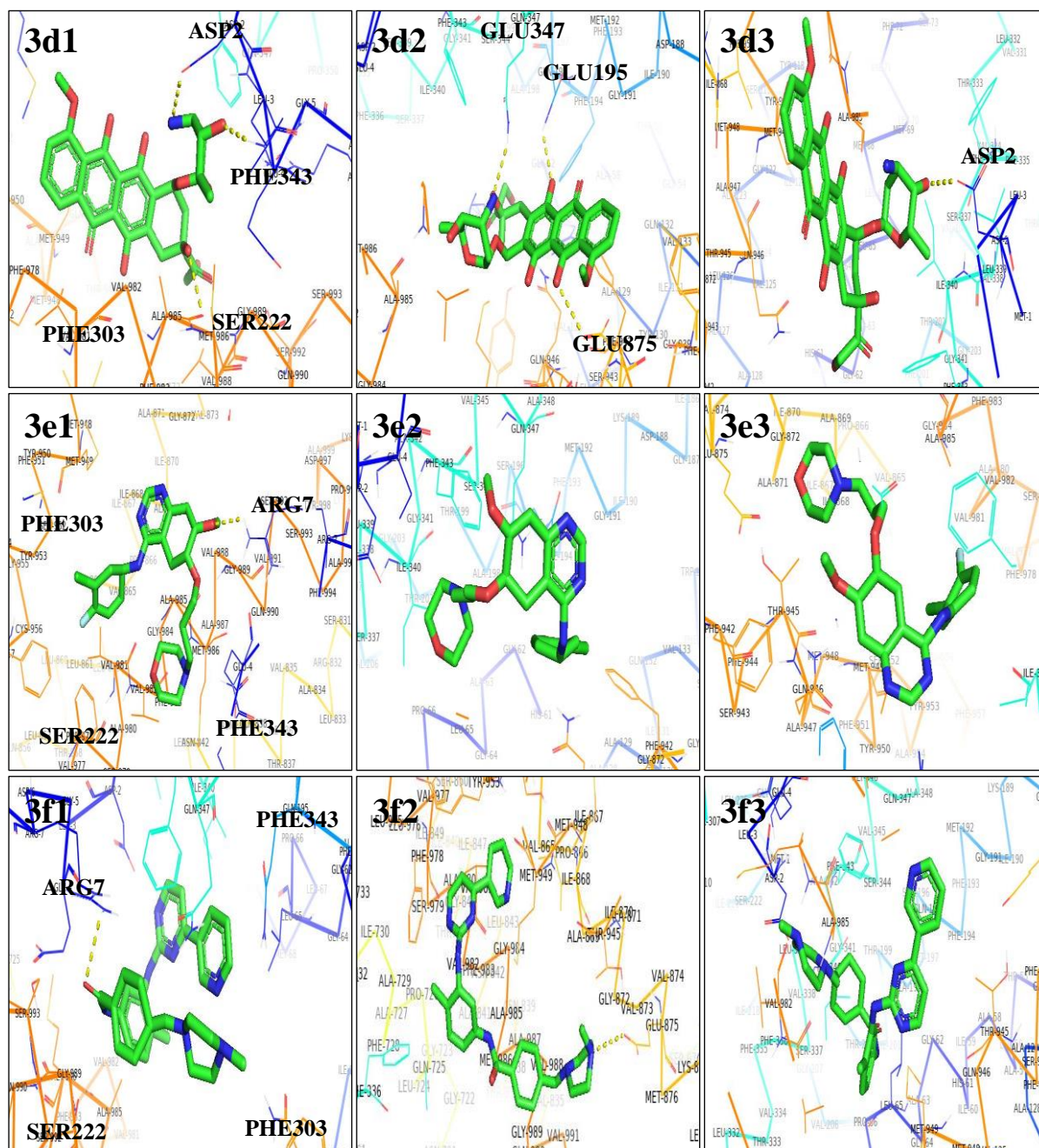


Fig.3: Three dimensional Complexes of Wild and Modeled Mutant PGP structures with docked substrates

3d1, 3d2, 3d3 represent Epirubicin bound to wild, Ala893Ser, Ala893pro variants respectively

3e1, 3e2, 3e3 represent Gefitinib bound to wild, Ala893Ser, Ala893pro variants respectively

3f1, 3f2, 3f3 represent Imatinib bound to wild, Ala893Ser, Ala893pro variants respectively

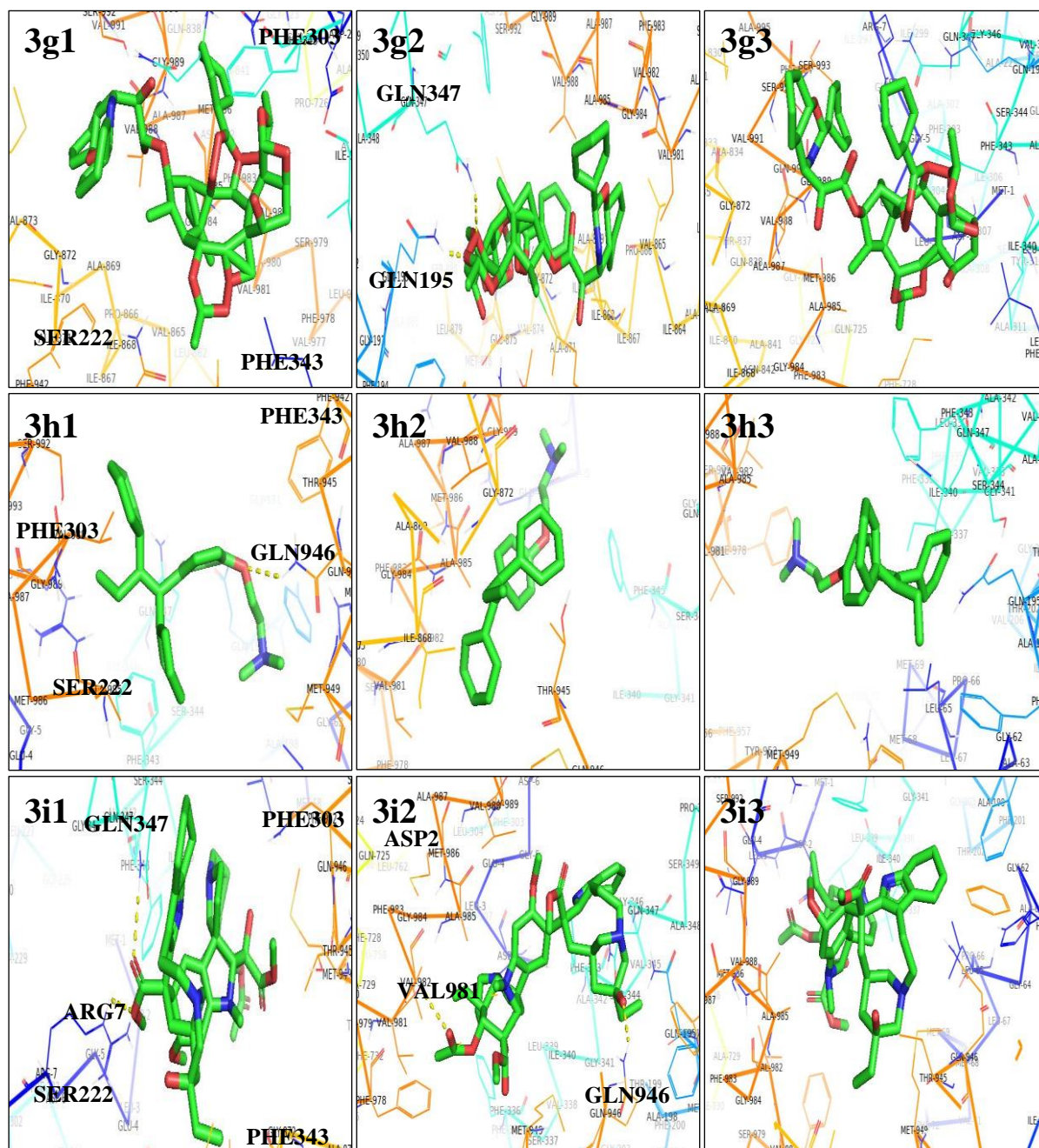


Fig.3: Three dimensional Complexes of Wild and Modeled Mutant PGP structures with docked substrates

3g1, 3g2, 3g3 represent Paclitaxel bound to wild, Ala893Ser, Ala893pro variants respectively
 3h1, 3h2, 3h3 represent Tamoxifen bound to wild, Ala893Ser, Ala893pro variants respectively
 3i1, 3i2, 3i3 represent Vinblastine bound to wild, Ala893Ser, Ala893pro variants respectively

Summary of binding energies of P-gp with the wild type, Ala893Ser and Ala893Pro are shown in **figure 4**

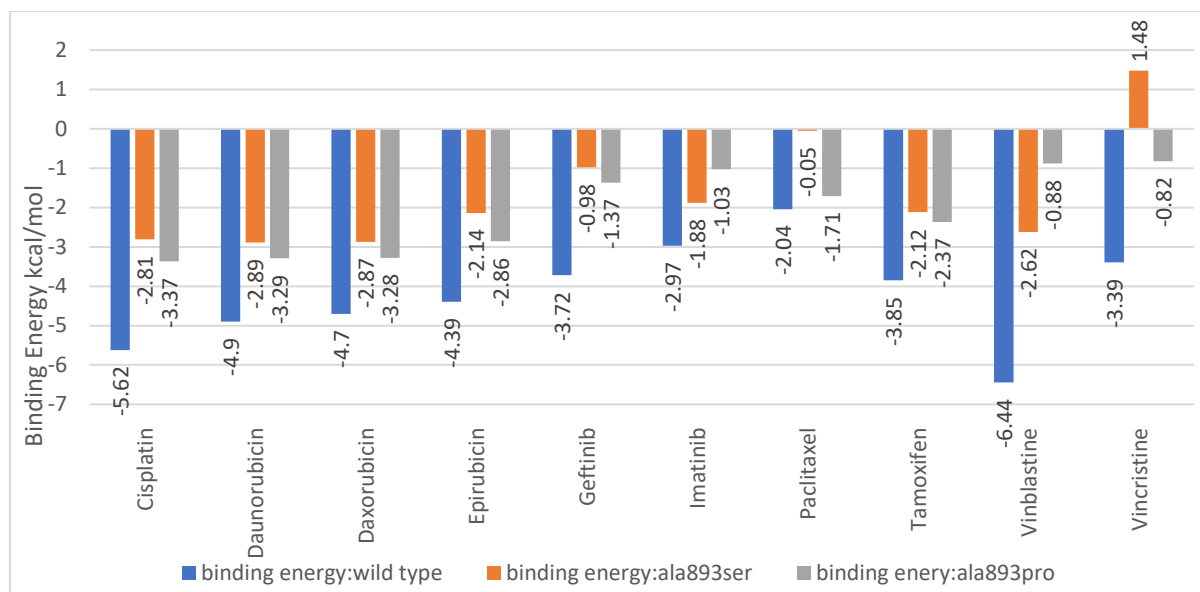


Fig 4: Summary of binding energies of PGP with WILD type, Ala893Ser and Ala893Pro

P value=0.000, 95% CI, P value retrieved through one way Analysis of Variance

CONCLUSION

We observed significant differences in binding energy, inhibitory constant, and hydrogen bonding interactions and binding poses of ligands between the wild type, Ala893Ser and Ala893Pro variants. These differences are highly suggestive of the fact that functional non synonymous polymorphism in the hMDR1, significantly alters the binding of the ligand and impair the substrate conductance process. As human P-gp is the major transporter involved in efflux conductance, loss of P-gp activity may result in intracellular accumulation of endotoxins and xenobiotics causing serious risks such as end organ damage and neuronal toxicity. Hence patients who are carriers of 1236C>T single nucleotide polymorphism would have decreased P-gp function which would result in toxic responses. Hence genetic testing of hMDR1 need to be done before initiating therapy with cytotoxic and narrow therapeutic P-gp substrates.

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Compliance and Ethical standard

I. Ethical approval	-	NA
II. Funding details (In case of Funding)	-	NA
III. Conflict of interest	-	No
IV. Informed Consent	-	NA

Author's contribution

Gautham yerrakula prepared manuscript, docking studies were done by Syed Sadathulla T, Kishor GS and Senthil V, Gramatical corrections were done by Samuel Gideon George

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