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## Predicting drug-target interaction in cancers using homology modeled structures of MTHFR gene

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### Abstract

Pharmacogenomics uses bioinformatics tools to study how an individual's genetic makeup affects the body's response to drugs. Our aim was towards the understanding of the possible role of structural variations in breast cancer drug metabolising gene such as MTHFR that closely interact with the neoplastic drugs viz. Cyclophosphamide, 5-Fluorouracil, Methotrexate and others. We investigated the polymorphism in the gene that might affect the drug binding capacity of these molecules. We used VEGA genome browser for obtaining information about the gene-structure and dbSNPs of NCBI for SNPs. The Protein sequence was retrieved from NCBI database and using Swiss Homology method the protein structure was constructed. Linux based software TRITON was used to study and determine the mutations in the structure. For predicting the variations of drug binding capacities of the chemotherapeutic agents, we used Molegro Virtual Docker. This study revealed that the binding energy for mutated MTHFR structures of the proteins was greater than that of the wild type proteins. This indicates that mutations causing structural modifications modulated the drug binding energies with various ligands (drugs). It therefore shows that the variations in the structure of the proteins influences the drug-binding capacity and also influences drug toxicity related to drug-gene interactions. This is the first computational report for these drug-gene interactions. This study determined the pharmaco-genomic interactions in chemotherapeutic drugs commonly used for breast cancer. This could be a model study for drug designing or selecting a drug suitable to the individual patient's genomic response.

**Keywords:** 5-Fluorouracil; cyclophosphamide; methotrexate; MTHFR; SNPs; drug binding; homology modeling; breast cancer; chemotherapy.

### Introduction

Cancer is a complex disease that occurs due to the accumulation of errors in the genes of some individuals. Some of these genetic errors are inherited while others are spontaneously acquired as a result of certain environmental exposures or individual behaviors, usually coupled with inherited susceptibility. Chemotherapy involving the use of cytotoxic antineoplastic agents remains an important strategy in the overall management of patients with malignant tumors. Drug-metabolizing enzymes and drug transporters play key roles in determining the pharmacokinetics and overall disposition of antineoplastic agents in the body. The structural information from the theoretically modeled complex can help us to understand the catalytic mechanism of enzymes. Amino acid mutations/substitutions may have diverse effects on protein structure and function. Hence, reliable

information about the protein sequence variations is essential to gain insights into disease genotype-phenotype correlations. With the recent availability of the complete genome sequence and the accumulation of variation data, determining the effects of amino acid substitution will be the next challenge in mutation research (Chen and Shen, 2009). Although single nucleotide polymorphisms (SNPs) may be potentially important pharmacogenetic determinants of cancer therapy, functional evidence regarding their relevance is currently lacking. Polymorphisms in drug metabolizing genes may modulate cytotoxic effect of commonly used chemotherapy drugs. Molecular Docking allows the scientist to virtually screen a database of compounds and predict the strongest binders based on various scoring functions. It explores ways in which two molecules, such as drugs and

an enzyme/or estrogen receptor fit together and dock into each other, like pieces of a three-dimensional jigsaw puzzle.

MTHFR gene is located on chromosome 1p36.3. MTHFR C677T polymorphism, results in decreased MTHFR activity and increased thermolability. This leads to lower levels of 5-methylTHF and an accumulation of 5,10-methyleneTHF (Kumar and Jamil, 2006; Reddy and Jamil, 2006; Khan and Jamil, 2008). 5-Fluorouracil (5FU) exerts a cytotoxic effect by mediating the formation of an inhibitory ternary complex involving its metabolite 5-fluoro-2'-deoxyuridine-5'-monophosphate (5FdUMP), thymidylate synthase, and 5, 10-methyleneTHF, thereby inhibiting thymidylate synthase activity with consequent depletion of intracellular thymidylate and ultimately resulting in the suppression of DNA synthesis. Increased intracellular concentrations of 5, 10-methyleneTHF enhances the formation and stability of this inhibitory ternary complex, thereby augmenting the cytotoxic effect of 5-FU (Sohn *et al.*, 2004; Kumar *et al.*, 2010; Suman and Jamil, 2006; Jamil *et al.*, 2009).

DNA has been a major target for anticancer drugs. The effects of nucleic acid binding drugs are known for various diseases such as cancer. The different modes of drug binding to DNA include intercalation between adjacent base pairs, intrusion into the minor groove and into the major groove. In silico molecular docking is one of the most powerful techniques of structure-based drug design (Kotra *et al.*, 2008; Brooijmans and Kuntz, 2003).

To understand the possible role of structural variation (non-synonymous substitution) of MTHFR we carried out the homology modelling of the proteins so that its interaction with the drug molecules like Cyclophosphamide, Doxorubicin, and 5-Fluorouracil could be evaluated, since it is hypothesised that the polymorphism in MTHFR gene might affect the drug efficacy, using molecular docking methods.

## Materials and Methods

### (i) Retrieval of Exons and Introns of MTHFR from Vega Genome Browser

The information regarding the exons and introns was retrieved from The Vertebrate Genome Annotation (VEGA) (Wilming *et al.*, 2008) database which is a central repository for high

quality, frequently updated, manual annotation of vertebrate finished genome sequence. Details of the projects for each species are available through the homepages for human, mouse, zebra fish, pig and dog. The website is built upon code from the Ensemble project.

### (ii) Protein Sequence Retrieval from NCBI

The amino acid sequence of the proteins was retrieved from the National Center for Biotechnology Information (NCBI) GenBank database (Benson *et al.*, 2011).

### (iii) Retrieval of SNP from dbSNP

The information regarding the SNPs of MTHFR was retrieved from The Single Nucleotide Polymorphism database (dbSNP) (Sherry *et al.*, 2001) which is a public-domain archive for a broad collection of simple genetic polymorphisms. This collection of polymorphisms includes single-base nucleotide substitutions (also known as single nucleotide polymorphisms or SNPs), small-scale multi-base deletions or insertions (also called deletion insertion polymorphisms or DIPs), and retroposable element insertions and microsatellite repeat variations (also called short tandem repeats or STRs). In this we can substitute any class of variation for the term SNP. Each dbSNP entry includes the sequence context of the polymorphism (i.e., the surrounding sequence), the occurrence frequency of the polymorphism (by population or individual), and the experimental method(s), protocols, and conditions used to assay the variation. This database as described above has been most useful for designing our study.

### (iv) Protein Homologous Structure Searching by BLASTP against PDB Database

The homologous protein structures to the MTHFR protein were retrieved using BLASTP (Altschul *et al.*, 1990) against the PDB database. The Standard protein-protein BLAST (blastp) was used for both identifying a query amino acid sequence and for finding similar sequences in protein databases. Like other BLAST programs, blastp is designed to find local regions of similarity. When sequence similarity spans the whole sequence, blastp will also report a global alignment, which is the preferred result for protein identification purposes.

### (v) Protein Homology Modeling by SPDBV

Actual modeling of the MTHFR protein to the template structures was done using SPDBV

(Guex and Peitsch, 1997) viewer and the SWISS MODEL. Deep View - Swiss-PdbViewer is an application that provides a user friendly interface allowing analyzing several proteins at the same time. The proteins can be superimposed in order to deduce structural alignments and compare their active sites or any other relevant parts. Amino acid mutations, H-bonds, angles and distances between atoms are easy to obtain due to the intuitive graphic and menu interface. DeepView - Swiss-PdbViewer, developed by Nicolas Guex, is tightly linked to SWISS-MODEL, an automated homology modeling server developed within the Swiss Institute of Bioinformatics (SIB) at the Structural Bioinformatics Group at the Biozentrum in Basel. Working with these two programs greatly reduces the amount of work necessary to generate models, as it is possible to thread a protein primary sequence onto a 3D template and get an immediate feedback of how well the threaded protein will be accepted by the reference structure before submitting a request to build missing loops and refine side chain packing. Swiss-PdbViewer can also read electron density maps, and provides various tools to build into the density. In addition, various modeling tools are integrated and command files for popular energy minimization packages can be generated.

#### (vi) Mutagenesis by TRITON

The mutant MTHFR structure was generated based on the wild type using TRITON (Prokop *et al.*, 2008). The program TRITON is a graphical tool for computational aided protein engineering. It implements methodology of computational site-directed mutagenesis to design new protein mutants with required properties. It uses the external program MODELLER (Eswar *et al.*, 2007) to model structures of new protein mutants based on the wild-type structure by homology modeling method. Subsequently, properties of these protein mutants are modeled. For enzymes, chemical reactions are modeled using semi-empirical quantum mechanics program MOPAC. Qualitative prediction of mutant activities can be achieved by evaluating

the changes in energies of the system and partial atomic charges of the active site residues during the reaction. Ligand-protein binding properties can be studied by docking methodology using the external program AutoDock. Ligand binding modes and affinities of individual protein mutants are obtained as a result. Binding properties can be also analyzed by visualization of affinity maps or by calculation of electrostatic potential interactions between ligand and individual residues of binding site. The program TRITON offers graphical tools for preparation of the input files and for visualization of output data. The program uses hierarchical projects to organize computational data. The program TRITON is an excellent system which has been used in this study.

#### (vii) Protein Molecular Binding Studies by Molegro Virtual Docker

Molegro Virtual Docker (Thomsen and Christensen, 2006) handles all aspects of the docking process from preparation of the molecules to determination of the potential binding sites of the target protein, and prediction of the binding modes of the ligands. Molegro Virtual Docker offers high-quality docking based on a novel optimization technique combined with a user interface experience focusing on usability and productivity. The Molegro Virtual Docker (MVD) has been shown to yield higher docking accuracy than other state-of-the-art docking products (MVD: 87%, Glide: 82%, Surflex: 75%, FlexX: 58%).

## Results

Computational analysis of the results obtained are presented in Figures (1-6) and Tables (1-3) as mentioned below for the known chemotherapeutic drugs such as, Cyclophosphamide, 5-fluorouracil and Methotrexate, interacting with their target MTHFR.

#### (a) Construction of homology models of MTHFR

The protein sequence of MTHFR, obtained from NCBI, is presented below:

```
>gi|87240000|ref|NP_005948.3| 5, 10-methylenetetrahydrofolate reductase (NADPH) [Homo sapiens]
MVNEARGNSSLNPCLEGSASSGSESSKDSRRCSTPGLDPERHERLREKMRRRLES GDKWFSLEFFPPRTA
EGAVNLISRFDRMAAGGPLYIDVTWHPAGDPGSDKETSSMMIASTAVNYCGLETILHMTCCRQRLEEITG
HLHKAKQLGLKNIMALRGDPIGDQWEEEGGFNYAVDLVKHIRSEFGDYFDICVAGYPKGHPAEGSFEAD
LKHLEKVSAGADFIITQLFFEADTFRFVKACTDMGITCPIVPGIFPIQGYHSLRQLVKLSKLEVPQEI
KDVIEPIKDNDAAIRNYGIELAVSLCQELLASGLVPGLHFYTLNREMATTEVLKRLGMWTEDPRRPLPWA
LSAHPKRREEDVRPIFWASRPKSYIYRTQEWDFPNRGRWGNSSSPAFGELKDYLYFLYLSKSPKEELLKM
```

WGEELTSEESVFEVFLVLYLSGEPNRRNGHKVTCLPWNDEPLAAETSLKELLRVNRQGITINSQPNING  
 KPSSDPIVWGPGSGGYVFQKAYLEFFTSRETAEALLQVLKKYELRVNYHLVNVKGENITNAPELQPNVAVT  
 WGIFPGREIIQPTVVDPVSEMFVKDEAFALWIERWGWKLYEEESPSRTIIQYIHDNYFLVNLVDNDFPLDN  
 CLWQVVEDTLELLNRPTQNARETEAP

*(b) Protein 3D Homology Modeling: Homologous Structures of MTHFR*

MTHFR protein databases are more specialized. They contain information derived from the primary sequence databases, protein translations of the nucleic acid sequences and sets of patterns and motifs derived from sequence homologs. The three-dimensional structure of MTHFR was generated by homology modeling based on the MTHFR structure as a

unique template with such a homology (Fig. 1-A & 1-B) the resulting model produced by SPDBV according to the Modeler, objective function can be considered a high-quality model as assessed by the structure-quality checking programs used. (Fig. 2-A & 2-B). C677T mutation (NCBI SNP ID: 1801133) in exon 4 (Ala 222 Val). Mutagenesis was performed by TRITON software. Energy after Minimization was - 7440.173 (Fig. 3-A & 3-B).

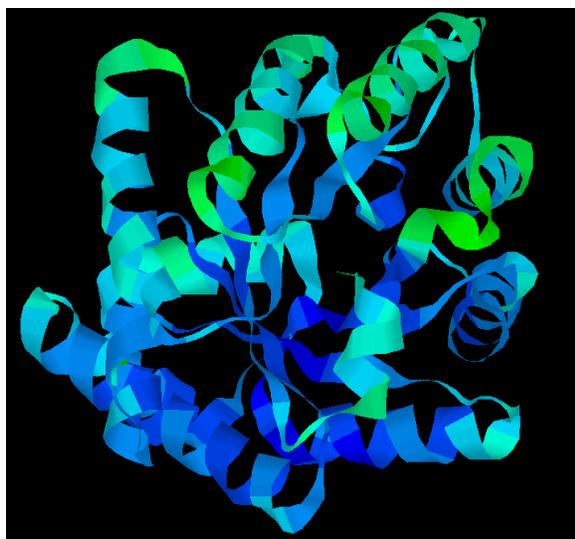


Figure 1-A: First Template, 1V93 A chain.

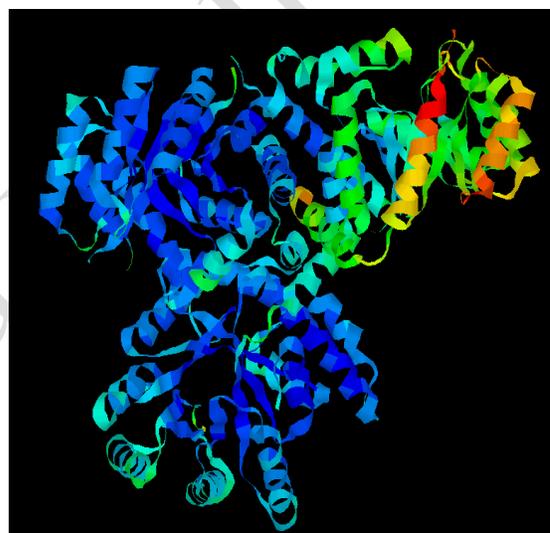


Figure 1-B: Second Template, 1ZP3 A chain

**Homology Modeling of MTHFR performed using Accelrys Ds ViewPro View**

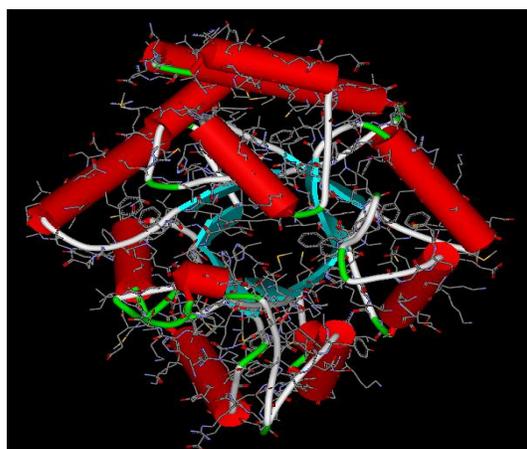
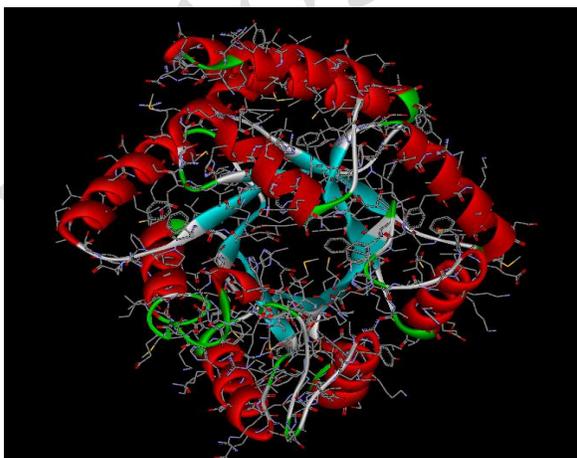


Figure 2-A & 2-B: Mutating the wild type structure of MTHFR with the given polymorphism.

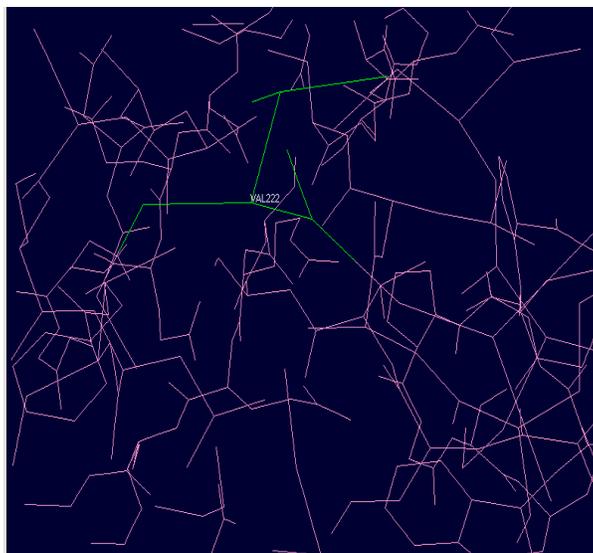


Figure 3-A: Mutated structure of MTHFR (Ala222Val) Display in SPDBV.

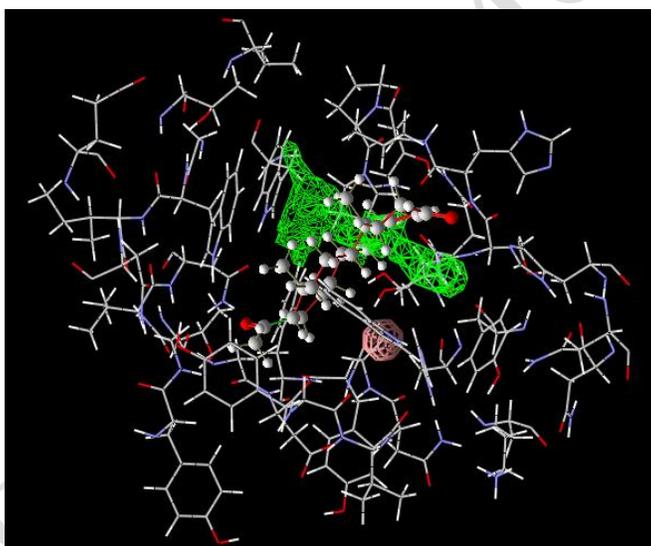


Figure 3-B: Drug binding on the active site of MTHFR shown in green.

### (c) Binding Studies of MTHFR

#### Analysis of Drug Binding Energies of Chemo Drugs and Protein

The chemotherapeutic drugs which we have studied for the present investigation were Cyclophosphamide, 5-Fluorouracil (5-FU) and Methotrexate (MTX) that are linked to the enzyme Methylenetetrahydrofolate Reductase (MTHFR) and also the diseases. For predicting the variations of drug binding capacities, Molecular binding was done by Molegro Virtual

Docker. Drug binding on the Active Site of MTHFR is shown in green in the figure. This study showed that average energy required for binding for the mutated MTHFR structure was greater than that for the wild type structure as shown below:

- (i) The binding energy for Cyclophosphamide to the wild type structure of MTHFR was -67.1793 and for mutated structure (with A222V polymorphism) it was -62.2292 (Figure 4-A & 4-B, Table 1).

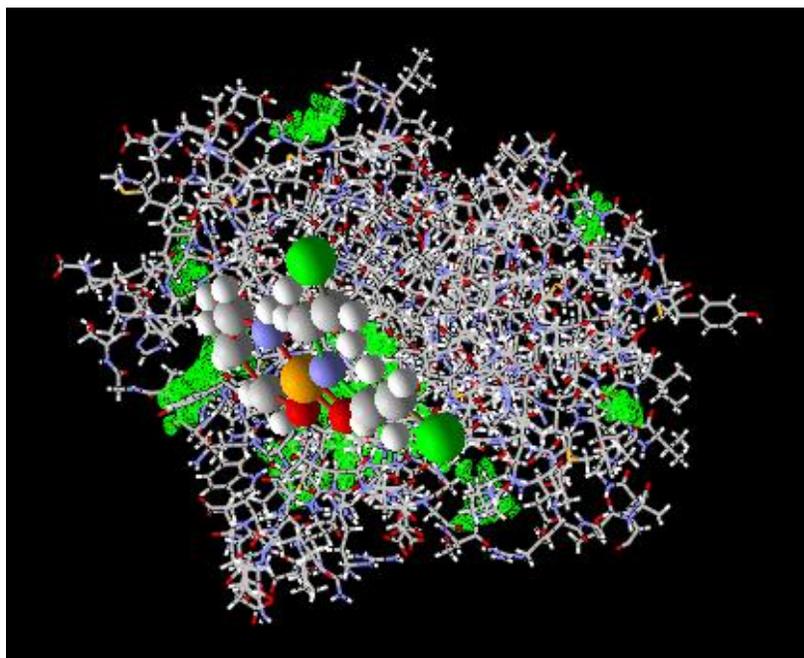


Figure 4-A: Cyclophosphamide binding on the active site (green colour indicates active sites) of wild type MTHFR.

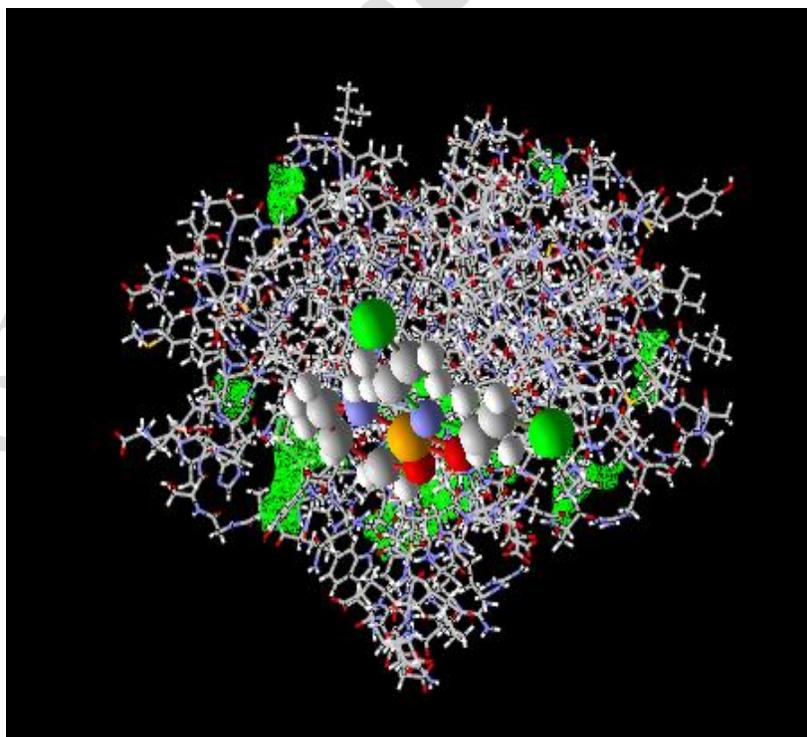


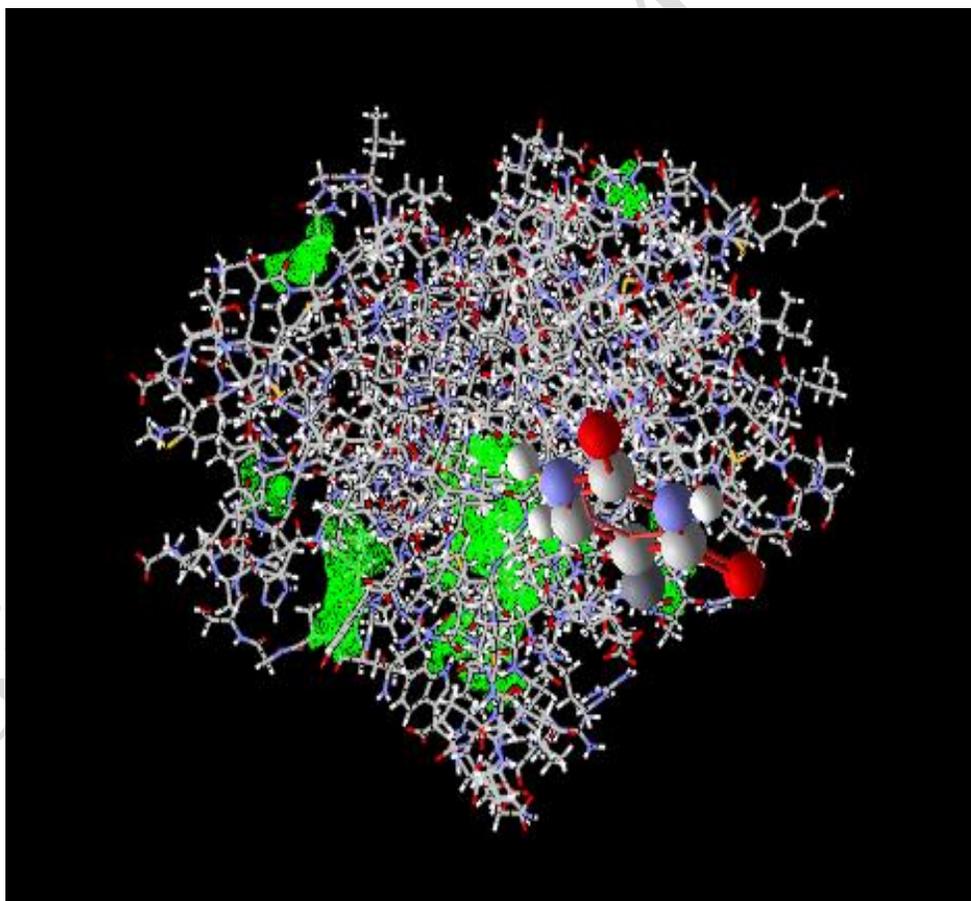
Figure 4-B: Cyclophosphamide binding on the active site (green color indicates active sites) of mutated structure of MTHFR.

### I. Cyclophosphamide Drug Binding with Wild type MTHFR

**Table 1: Showing the binding energies with which cyclophosphamide binds to the MTHFR protein.**

| Wild type                 | Wild type    | Mutant type               | Mutant type  |
|---------------------------|--------------|---------------------------|--------------|
| Drug Name                 | Energy Value | Drug Name                 | Energy Value |
| Cyclophosphamide Analog-1 | -68.0028     | Cyclophosphamide Analog-1 | -68.0747     |
| Cyclophosphamide Analog-2 | -66.3558     | Cyclophosphamide Analog-2 | -66.0382     |
| Cyclophosphamide Analog-3 | -57.9299     | Cyclophosphamide Analog-3 | -56.8783     |
| <b>Average Energy</b>     | -67.1793     | <b>Average Energy</b>     | -62.2291     |

(ii) Whereas for 5-Fluorouracil, the binding energy was -16.1972 (for wild type structure) and for the mutated type (with A222V polymorphism) it was -15.0211 (Figure 5-A & 5-B; Table 2).



**Figure 5-A: 5-Fluorouracil binding on the active site (green colour indicates active sites) of wild type MTHFR.**

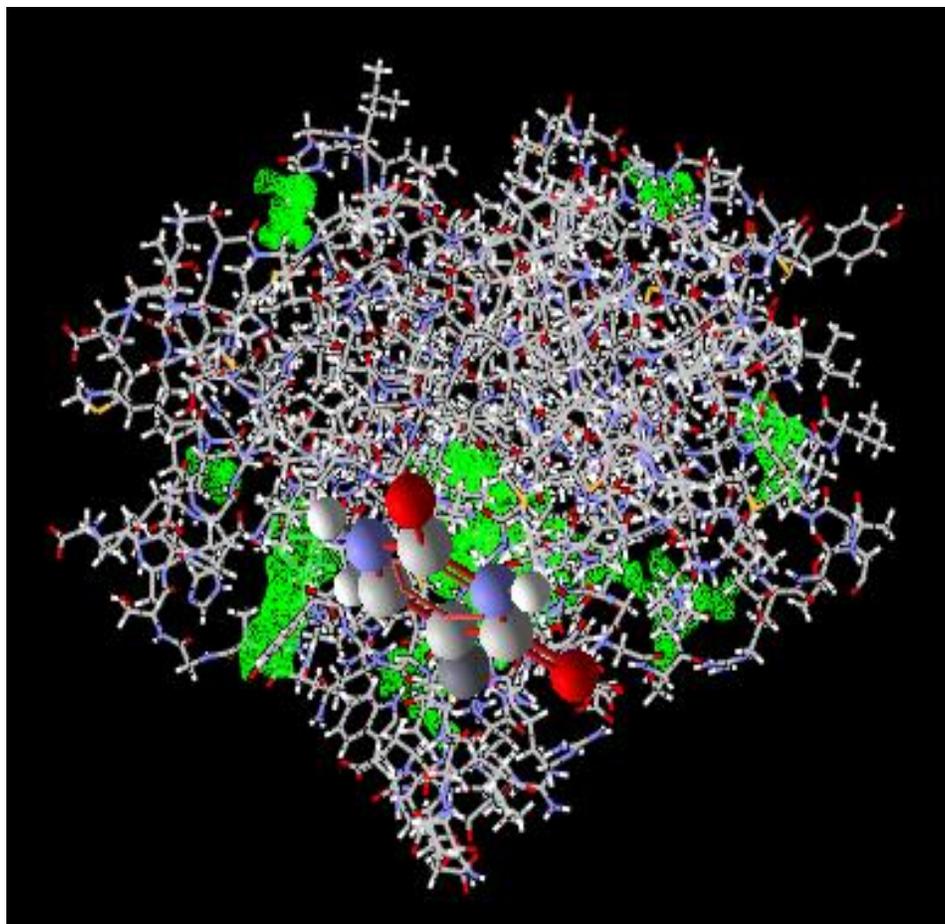


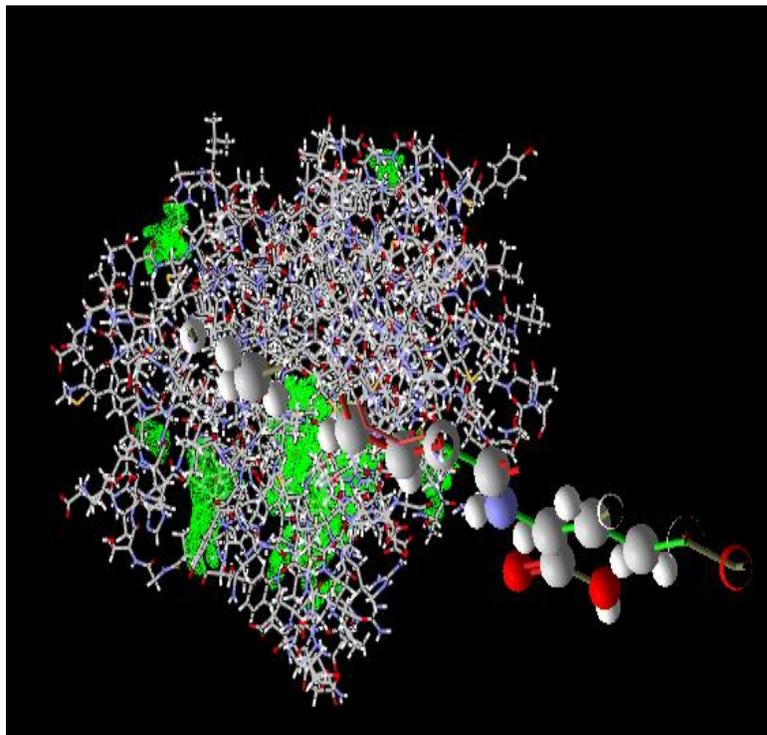
Figure 5-B: 5-Fluorouracil binding on the active site of mutated structure of MTHFR (green color indicates active sites).

## II. 5-Fluorouracil Binding with Wild type MTHFR

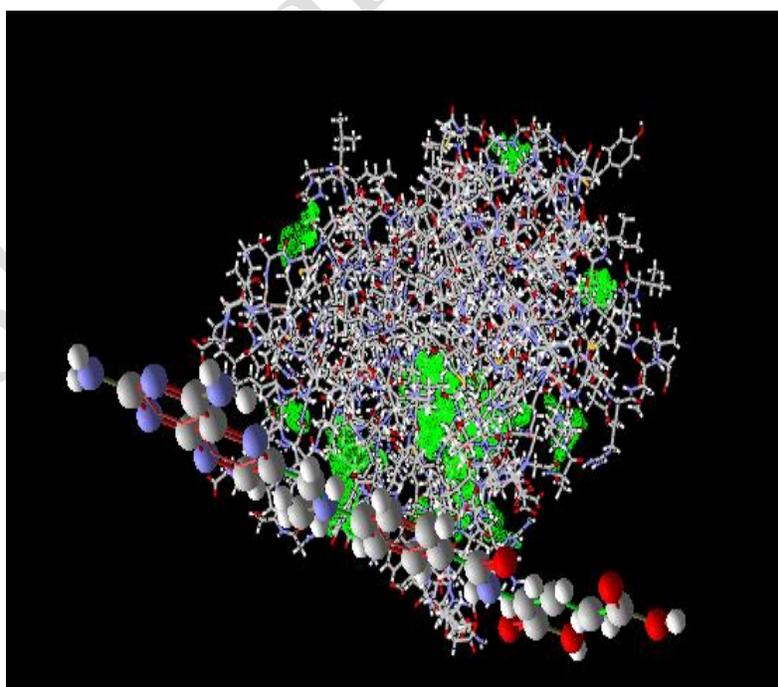
Table 2: Showing the binding energies with which 5-Fluorouracil binds to the MTHFR protein.

| Wild type                | Wild type       | Mutant type              | Mutant type     |
|--------------------------|-----------------|--------------------------|-----------------|
| Drug Name                | Energy Value    | Drug Name                | Energy Value    |
| 5- Fluorouracil Analog-1 | -19.9174        | 5- Fluorouracil Analog-1 | -19.8998        |
| 5- Fluorouracil Analog-2 | -18.3215        | 5- Fluorouracil Analog-2 | -18.5097        |
| 5- Fluorouracil Analog-3 | -13.8722        | 5- Fluorouracil Analog-3 | -11.4927        |
| 5- Fluorouracil Analog-4 | -14.3034        | 5- Fluorouracil Analog-4 | -8.96726        |
| 5- Fluorouracil Analog-5 | -14.5717        | 5- Fluorouracil Analog-5 | -16.2359        |
| <b>Average Energy</b>    | <b>-16.1972</b> | <b>Average Energy</b>    | <b>-15.0211</b> |

(iii) For Methotrexate, the binding energy was -16.1972 (for wild type MTHFR) and for the mutated structure (with A222V polymorphism) it was -15.0211 (Figure 6-A & 6-B, Table 3).



**Figure 6-A: Methotrexate binding on the active site (green colour indicates active sites) of wild type MTHFR.**



**Figure 6-B: Methotrexate binding on the active site of the mutated structure of MTHFR (green color indicates the active site of mutated structure).**

### III. Methotrexate Binding with MTHFR

**Table 3: Showing the binding energies with which Methotrexate binds to the MTHFR protein.**

| Wild type             | Wild type    | Mutant type           | Mutant type  |
|-----------------------|--------------|-----------------------|--------------|
| Drug Name             | Energy Value | Drug Name             | Energy Value |
| Methotrexate Analog-1 | -172.729     | Methotrexate Analog-1 | -173.729     |
| Methotrexate Analog-2 | -179.797     | Methotrexate Analog-2 | -170.334     |
| Methotrexate Analog-3 | -164.725     | Methotrexate Analog-3 | -179.506     |
| Methotrexate Analog-4 | -174.181     | Methotrexate Analog-4 | -163.026     |
| Methotrexate Analog-5 | -164.583     | Methotrexate Analog-5 | -158.545     |
| <b>Average Energy</b> | -171.203     | <b>Average Energy</b> | -169.028     |

This study reveals that because of the changes in structure due to the polymorphism (SNP changes) in the structure of MTHFR, the drug binding capacities changed and due to this the response to these chemotherapeutic agents varies in the various cases.

#### Discussion

Worldwide, breast cancer is the second most common type of cancer after lung cancer (10.4% of all cancer incidence, both sexes included) and the fifth most common cause of cancer death. In 2005, breast cancer caused 502,000 deaths worldwide (7% of cancer deaths; almost 1% of all deaths) (WHO, 2006; Mathew and Raj, 2009). Various studies have identified various genes like BRCA1 and BRCA2 and a whole list of markers associated with the disease like breast cancer. Although all humans have many similar genetic characteristics, the fact remains that each individuals' genome differs slightly. Even this slight genetic difference can have a significant impact on an individual's susceptibility to disease and response to treatment. Many of the chemotherapeutic agents currently employed for cancer treatments are discovered by screening large collections of chemical compounds or complex natural products for cytotoxic activity on cancer cell lines. As a result, most of these drugs are relatively non-specific, toxic, and cause significant side effects on cancer patients. Pharmacogenomics is the study of how an individual's genetic makeup affects the body's response to drugs. Some drugs are safe for 95% or more of the population, but fail to make it through clinical trials due to genetic differences in 5% or less of the population that

experience adverse effects. As a result, the vast majority of the population is denied the benefits of these drugs, to protect a relatively small percentage of non-responders. The SNP at the genome level that encodes for the substrate binding domain may alter the amino acid which effects KI. The KI is the inhibitor constant, which in turn affects Km and thus Vo of the reaction, this results in the varied rates of response. So we can visualize that the drug enzyme affinities have a role in rate of reactions (Sankar *et al.*, 2007).

Tumor resistance to chemotherapeutic drugs has been traced to molecular mechanisms that enhance individual cell survival, such as DNA damage repair, alterations in drug target, drug metabolism and cell cycle checkpoint mediators, decreased drug uptake, increased drug efflux, and diminished apoptosis. Experiments studying cells in spheroids show that tumor mass-induced changes in protein expression, localization, and altered gene expression, such as mutation due to SNP changes or amplification of genes encoding multidrug resistance protein (MDR1) efflux pumps, could favor drug resistance. Drugs may be sequestered in outer tumor regions, although active efflux by cells in inner layers may minimize tissue penetration barriers. It is reasonable to assume that resistance introduced by tumor tissues in 3-D, stems from substrate gradients in the cellular microenvironment causing hypoxia, hypoglycemia, and acidosis, which may alter cell cycle kinetics, reduce cell proliferation, decrease drug sensitivity, favors a toxic cellular environment by favoring up regulation of efflux mechanisms and selecting more resistant phenotypes.

Hence, studies using the docking program could determine the best fit between the ligand and the target, the binding site can be seen in a variety of possible ways. In other words, we are using it as a conformation generator for conformations that fit into the active site. The docking program allows us to focus on a set of conformations for each molecule that fit into the binding site. Additionally, the docking program performs the alignment of all the conformations to one another, which is much simpler than dealing with the combinatorial explosion of ways in which the active compounds can be aligned and the number of features which are required. The drug-gene interaction plays a significant role in safe therapy regime for cancer patients. In this work, molecular docking studies were carried out to explore the binding mechanism of chemo drugs to the target protein of MTHFR, these chemotherapeutic agents are commonly used in breast cancer. This study determined not only the conformation of the protein but also their free binding energies by molecular docking. This study also revealed that energy for binding to the polymorphic structure of MTHFR was greater than that of the wild type structure. This model study can be extended to several neoplastic drugs in order to determine their reactions to predict their drug binding capacities for their effectiveness in the cancer patients, as we have seen that the drug action varies in various cases. Further studies can be done on pharmacophore analysis, pharmacodynamics, and molecular descriptors of drugs for the various genes.

### Conclusion

The commonly used Chemotherapy drugs for breast cancer in these studies included, Cyclophosphamide, 5-Fluorouracil, Methotrexate, among several others. The mechanism of action of these chemotherapeutic agents is to inhibit DNA synthesis, increase cyto-toxicity at the cell division stage and induce apoptosis in cancer cells (and other rapidly dividing cells). These drugs interact with, and are substrates of some gene products like Methylenetetra hydrofolate reductase (MTHFR). Hence, we modeled the gene MTHFR to determine the binding capacity of the chemotherapeutic agents to the targets; thus we could study the drug-gene interactions. We used several bioinformatics tools to proceed with in silico studies and the results showed that the

variations in the structure of the targets due to SNP changes influences the drug-binding capacity and therefore affects drug toxicity. This is the first computational report for these drug-gene interactions, which opens up the avenues for personalized medicine, since the differences in structure (due to mutations or SNPs) of the targets influences the dose response of the therapeutic agents.

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