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Detection of The Effect of CYP2C19*4 Mutation on Clopidogrel Response by *In Silico* Methods

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Abstract: Single nucleotide polymorphisms cause amino acid change, and protein structure and function are changed. Thus, the patient improves drug resistance and does not respond to therapy. Clopidogrel is an antiplatelet drug and is used for cardiovascular disease therapy such as heart failure, atherosclerosis, and myocardial infarction.CYP2C19 gene is a CYP450 enzyme and metabolizes clopidogrel. Polymorphism in the CYP2C19 gene causes clopidogrel resistance. A homology modeling study was carried out using the Swiss-Model database and the Chimera program. The selection of models was made with the evaluation of the QMEAN values of the three-dimensional structures. The physicochemical properties of the wild type and CYP2C19*4 mutant type were analyzed by the ExPASy-ProtParam Portal. The effects of the mutation on the protein structure were performed by the HOPE database. The HDock program was used to demonstrate interactions between clopidogrel and wild-type protein and, mutant type protein. Mutation of the residue might disturb this function. This mutation causes the loss of interactions and affects the drug response. In this study, it was shown that Clopidogrel drug interactions between mutant type protein by docking study. Possible drug conformation is designed for the effective treatment of patients carrying the common mutation.

Keywords: Heart failure, drug resistance, clopidogrel, homology modeling, single nucleotide polymorphism (SNP)

1 Introduction

Heart Failure (HF), is a complex syndrome that causes the heart to not be able to send oxygen to the tissues for its metabolic needs, due to insufficient cardiac filling and neurohormonal activation, resulting in shortness of breath, fatigue, and edema (Braunwald E., 2001). The European Society of Cardiology (ESC) defines HF as a cardiac structure and/or functional disorder that causes the heart to not deliver enough oxygen to the tissues for their metabolic needs, despite normal filling pressures (McMurray et al. 2012).

When we look at the frequency of HF seen in the adult population; It is seen that the average is 23 million worldwide, 15 million in European countries, and six million in the United States (Sari et al. 2016; Tokgözoğlu et al. 2018). The prevalence of HF in Turkey according to the results of the Survey on the Prevalence and Determinants of Heart Failure in Turkey (HAPPY) is over two million (Değertekin et al. 2012). Early diagnosis and treatments are the most important to increase the mortality ratio of heart failure patients. When Turkey is compared with other countries, the heart failure patient population is significantly high (McMurray et al. 2012). Heart failure improves at any age but, the incidence of heart failure increases in old age.

Its incidence is increasing in people younger than 65 years of age. HF is the most common cause of hospitalization in individuals over the age of 65. HF is a disease that is very costly to treat due to frequent hospitalizations, causes loss of workforce, and poses a problem for both the patient and society. Therefore, early diagnosis and treatment are important (Sari et al. 2016). The short-term goals of heart failure treatment are to control symptoms and improve quality of life.

Clopidogrel is among the antiplatelet drugs and shows its effect by preventing platelet aggregation (thrombus) that causes blood clotting. Clopidogrel is frequently used in patients with arteriosclerosis, a history of heart attack and stroke, and peripheral vascular disease to prevent thrombus formation in the vessels that cause death by causing conditions such as heart attacks and strokes (Simon et al. 2009). Clopidogrel is a pro-drug which absorbs in the intestine and activates in the liver (Sangkuhl et al.2010).

The CYP2 gene family consists of nine exons and eight introns (Hoffman et al. 2001). CYP2 genes are located on different chromosomes and are arranged in multigene clusters containing one or more subfamilies. The CYP2C19 gene, which is one of the CYP450 enzymes, is localized on the long arm of the 10th chromosome (10q23.3) and consists of 9 exons. CYP2C19 carries polymorphisms that affect the metabolism of drugs related to the treatment of cardiovascular diseases. It is a polymorphic gene (Gandhi et al. 2014). There are 25 different variants of the CYP2C19 allele (Bhat et al. 2015). The active allele (wild type) of the CYP2C19 gene is called CYP2C19*1 (Kim et al. 2010). The CYP2C19*4 key variant is the start codon changing variant A>G (rs28399504). CYP2C19*4 is a rare variant (Scott et al., 2012).

CYP2C19 metabolizes many drugs (Lee et al, 2013). Therefore, CYP2C19 polymorphisms affect the pharmacokinetics of CYP2C19 substrate drugs. Among the many factors that cause differences between individuals in the response to clopidogrel, the most important one is the differences in drug metabolism rates resulting from polymorphism of the CYP2C19 gene (Brown et al. 2018). CYP2C19 enzyme converted the Clopidogrel to its active form in the liver (Mega et al. 2009). Isoforms of hepatic cytochrome P450 (CYP) are responsible for the formation of the active metabolite of Clopidogrel. This process consists of two phases in which CYP2C19, CYP1A2, and CYP2B6 in the first line and CYP2C19, CYP2C9, CYP2B6, and CYP3A in the second line are responsible (Barnette et al. 2019). This active form inhibits a receptor protein which is called P2RY12. This receptor is located on the surface of platelets. The P2RY12 receptor protein provides platelet cluster formation to prevent blood loss. CYP1A2, CYP2B6, CYP2C9, CYP2C19 and CYP3A4 / 5 that are cytochrome P450 enzymes and play a role in clopidogrel metabolism. Studies have shown that CYP3A4, CYP2C9, CYP2C19, and CYP2B6 enzymes metabolize the 2-oxo-clopidogrel and form the active metabolites (Kurihara et al. 2005). Studies showed that a quarter of patients who are treated with clopidogrel have a less-than-normal response to treatment (Shuldiner et al. 2009).

2 Materials and Method

2.1 Homology Modeling Databases

Homology modeling of CYP2C19*4 (rs28399504) is created and Physico-chemical properties are obtained. FASTA format of an amino acid sequence of the CYP2C19 gene is obtained by Uniprot.org and the Gene bank database of National Center for Biotechnology Information, USA, NCBI (https://www.ncbi.nlm.nih.gov/ Accessed: 20.07.2022). Sequence information is arranged according to wild type and mutant type. Sequence with access number P33261 is used in our bioinformatics study for wild type. In the mutant type, Methionine changes to Valine at position 255. The amino acid sequence of the wild type and mutant type is shown in Table 1.

Table 1. The amino acid sequence of wild type and mutant type (M255V) (https://www.ncbi.nlm.nih.gov/ Accessed: 20.07.2022).

Wild Type

MDPFVVLVLCLSCLLLLSIWRQSSGRGKLPPGPTPLPVIGNILQIDIKDVS
KSLTNLSKIYGPVFTLYFGLERMVVLHGYEVVKEALIDLGEEFSGRGHF
PLAERANRGFGIVFSNGKRWKEIRRFSLMTLRNFGMGKRSIEDRVQEE
ARCLVEELRKTKASPCDPTFILGCAPCNVICSIIFQKRFDYKDQQFLNLM
EKLNENIRIVSTPWIQICNNFPTIIDYFPGTHNKLLKNLAFMESDILEKVK
EHQESMDINNPRDFIDCFLIKMEKEKQNQQSEFTIENLVITAADLLGAG
TETTSTTLRYALLLLLKHPEVTAKVQEEIERVIGRNRSPCMQDRGHMP
YTDAVVHEVQRYIDLIPTSLPHAVTCDVKFRNYLIPKGTTILTSLTSVLH
DNKEFPNPEMFDPRHFLDEGGNFKKSNYFMPFSAGKRICVGEGLARME
LFLFLTFILQNFNLKSLIDPKDLDTTPVVNGFASVPPFYQLCFIPV

Mutant Type

MDPFVVLVLCLSCLLLLSIWRQSSGRGKLPPGPTPLPVIGNILQIDIKDVS
KSLTNLSKIYGPVFTLYFGLERMVVLHGYEVVKEALIDLGEEFSGRGHF
PLAERANRGFGIVFSNGKRWKEIRRFSLMTLRNFGMGKRSIEDRVQEE
ARCLVEELRKTKASPCDPTFILGCAPCNVICSIIFQKRFDYKDQQFLNLM
EKLNENIRIVSTPWIQICNNFPTIIDYFPGTHNKLLKNLAFMESDILEKVK
EHQESVDINNPRDFIDCFLIKMEKEKQNQQSEFTIENLVITAADLLGAGT
ETTSTTLRYALLLLLKHPEVTAKVQEEIERVIGRNRSPCMQDRGHMPY
TDAVVHEVQRYIDLIPTSLPHAVTCDVKFRNYLIPKGTTILTSLTSVLHD
NKEFPNPEMFDPRHFLDEGGNFKKSNYFMPFSAGKRICVGEGLARMEL
FLFLTFILQNFNLKSLIDPKDLDTTPVVNGFASVPPFYQLCFIPV

Homology models of CYP2C19 (wild and mutant types) were created with the Swiss Model Program which is an internet-based bioinformatics tool (Waterhouse et al. 2018) and the obtained three-dimensional models were examined with the UCSF Chimera program, which is a visualization tool (Pettersen et al. 2004). A homology modeling study was carried out using the Swiss Model (www.swissmodel.expasy.org, Access date: 25.07.2022; Waterhouse et al, 2018) database and the Chimera program. Wild and mutant-type sequence sets were loaded into the system separately and their three-dimensional structures were obtained. The selection was made with the evaluation of the QMEAN values of the three-dimensional structures. By using UCSF Chimera program tools, the protein structures of the wild type and mutant type were visualized with ribbon display and structural differences of mutant protein were observed.

2.2 Bioinformatic Analysis Of Homology Models

The physicochemical properties of the wild type and CYP2C19*4 mutant type of the CYPC19 gene were analyzed by the ExPASy-ProtParam Portal (Walker 2005). Amino acid number, theoretical pI value, aliphatic index, molecular weight, amino acid composition, negatively and positively charged numbers, and hydrophobicity value were analyzed for each model.

HOPE is a web service that analyzes the structural effect of mutation on the protein sequence. HOPE shows the effects of the mutation on the protein and calculates probable damage of mutation on the protein structure (Venselaar et al. 2010). In this study, it was shown that the effect of CYP2C19*4 on the protein structure by the HOPE database (Venselaar et al. 2010).

2.3 Molecular Docking Study

Docking is a method that involves estimating the conformation and orientation of the ligand structure at a targeted binding site. Docking can also be defined as the determination of the binding mode for a ligand construct to the target protein. Molecular docking studies have an important role in drug discovery. Especially it is so important for drug-resistance therapies. Three-dimensional (3D) structures of ligands and proteins are necessary for docking studies. Docking programs use 3D structures and put in ligands the target region of the protein (Morris et al. 2009).

The HDock server is usually used for drug discovery studies (Yan et al. 2017). HDock which is an atom-based docking method uses a genetic algorithm. Genetic algorithms transfer the energy and geometry information of a newly formed conformation of the molecule to the next conformation and thus they are obtained optimal conformations. In this study, molecular docking of CYP2C19 and clopidogrel was made by HDock, and binding energies of wild and mutant types were calculated.

3 Results

The amino acid sequence format which is used as a basis in bioinformatics studies was created based on the NCBI-P33261 accession number sequence (https://www.ncbi.nlm.nih.gov/ Accessed: 20.07.2022). Three-dimensional structures were obtained by using CYP2C19 wild and mutant type sequence sets, for which homology modeling was performed using the Swiss-Model database and the Chimera program (Waterhouse et al. 2018; Pettersen et al. 2004). CYP2C19 wild and mutant-type ribbon structures were shown in Figure 1.

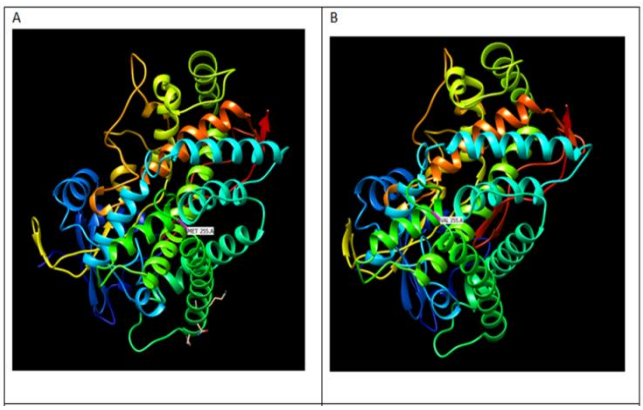

Figure 1. A: CYP2C19 wild-type ribbon structure, B: CYP2C19*4 mutant-type ribbon structure (Pettersen et al. 2004).

Each amino acid has its specific size, charge, and hydrophobicity value. CYP2C19 wild and mutant-type hydrophobicity were shown in Figure 2 as hydrophobic surfaces by the Chimera program (Pettersen et al. 2004).

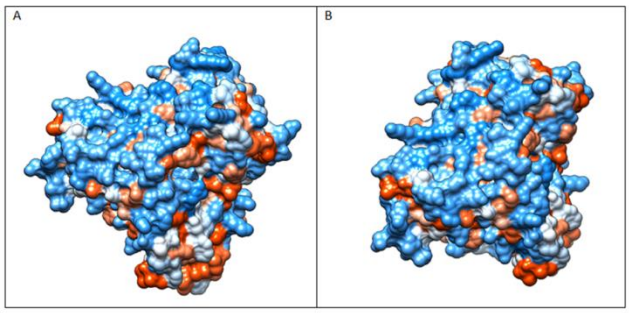

Figure 2. A: CYP2C19 gene wild type hydrophobic structure, B: CYP2C19 mutant type hydrophobic structure (Pettersen et al. 2004).

Schematic structures of the wild (left) and the mutant (right) amino acids were shown in Figure 3.

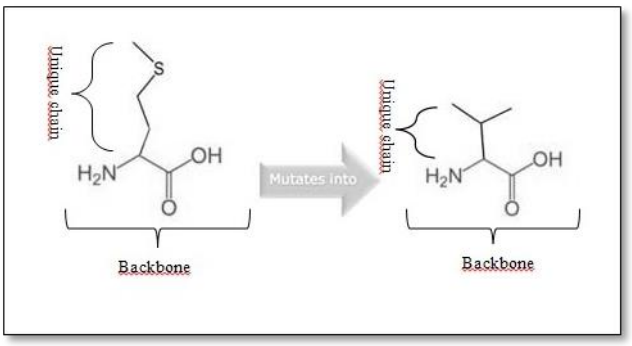

Figure 3. The wild (left) and the mutant (right) amino acid schematic structures (Venselaar et al. 2010).

The original wild-type residue and newly introduced mutant residue often differ in these properties. The mutant residue is smaller than the wild-type residue. The mutated residue is located in a domain that is important for the binding of other molecules. Mutation of the residue might disturb this function.

The amino acid change was shown in Figure 4. The side chains of both the wild-type and the mutant residue were shown in the figure with labels.

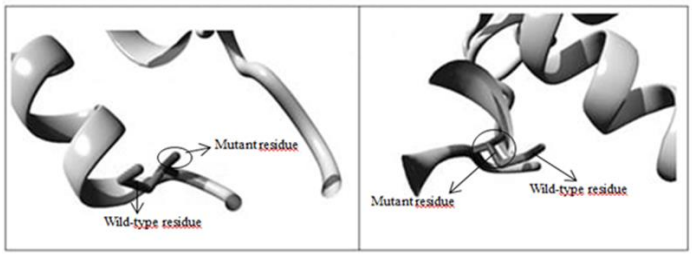

Figure 4. Close-up of the mutation (Venselaar et al. 2010).

We observed that the mutant residue is smaller, this situation might lead to a loss of interactions. It is possible that the mutant residue affects protein activity and function as it is located at the binding site.

The grand average of the hydropathicity (GRAVY) index shows the solubility of the proteins (Table 2). If it is a positive charge, it means that hydrophobic, and if it is negative, it means hydrophilic (Chang et al. 2013). While wild-type protein is hydrophilic, mutant-type is hydrophobic.

Table 2. Physicochemical properties of wild and mutant proteins (Walker 2005).

CYP2C19 Wild Type	**CY2C19*4 Mutant Type**
Number of amino acids: 490	**Number of amino acids:** 490
Molecular weight: 55931.06	**Molecular weight:** 55899.00
Theoretical pI: 7.11	**Theoretical pI:** 7.11
Amino acid composition:	Ala (A) 17 3.5%
Ala (A) 17 3.5%	Arg (R) 27 5.5%
Arg (R) 27 5.5%	Asn (N) 26 5.3%
Asn (N) 26 5.3%	Asp (D) 24 4.9%
Asp (D) 24 4.9%	Cys (C) 13 2.7%
Cys (C) 13 2.7%	Gln (Q) 16 3.3%
Gln (Q) 16 3.3%	Glu (E) 33 6.7%
Glu (E) 33 6.7%	Gly (G) 28 5.7%
Gly (G) 28 5.7%	His (H) 10 2.0%
His (H) 10 2.0%	Ile (I) 35 7.1%
Ile (I) 35 7.1%	Leu (L) 56 11.4%
Leu (L) 56 11.4%	Lys (K) 30 6.1%
Lys (K) 30 6.1%	Met (M) 12 2.4%
Met (M) 13 2.7%	Phe (F) 33 6.7%
Phe (F) 33 6.7%	Pro (P) 30 6.1%
Pro (P) 30 6.1%	Ser (S) 26 5.3%
Ser (S) 26 5.3%	Thr (T) 27 5.5%
Thr (T) 27 5.5%	Trp (W) 3 0.6%
Trp (W) 3 0.6%	Tyr (Y) 11 2.2%
Tyr (Y) 11 2.2%	Val (V) 33 6.7%
Val (V) 32 6.5%	Pyl (O) 0 0.0%
Pyl (O) 0 0.0%	Sec (U) 0 0.0%
Sec (U) 0 0.0%	(B) 0 0.0%
(B) 0 0.0%	(Z) 0 0.0%
(Z) 0 0.0%	(X) 0 0.0%
(X) 0 0.0%	**Total number of negatively charged residues (Asp + Glu):** 57
Total number of negatively charged residues (Asp + Glu): 57	**Total number of positively charged residues (Arg + Lys):** 57
Total number of positively charged residues (Arg + Lys): 57	**Atomic composition:**
Atomic composition:	Carbon C 2529
Carbon C 2529	Hydrogen H 3986
Hydrogen H 3986	Nitrogen N 666
Nitrogen N 666	Oxygen O 711
Oxygen O 711	Sulfur S 25
Sulfur S 26	**Formula:** C2529H3986N666O711S25
Formula: C2529H3986N666O711S26	**Total number of atoms:** 7917
Total number of atoms: 7918	**Instability index:**
Instability index:	The instability index (II) is computed to be 43.37
The instability index (II) is computed to be 43.68	This classifies the protein as unstable.
This classifies the protein as unstable.	**Aliphatic index:** 95.43
Aliphatic index: 94.84	**Grand average of hydropathicity (GRAVY):** -0.093
Grand average of hydropathicity (GRAVY): -0.098	

Molecular docking interaction of Clopidogrel and CYP2C19 wild and mutant types was carried out using the HDock server. HDock is used Bioinformatics-based methods to predict the structure and generate interactions (Yan et al. 2017). Clopidogrel docking results were shown in Figure 5.

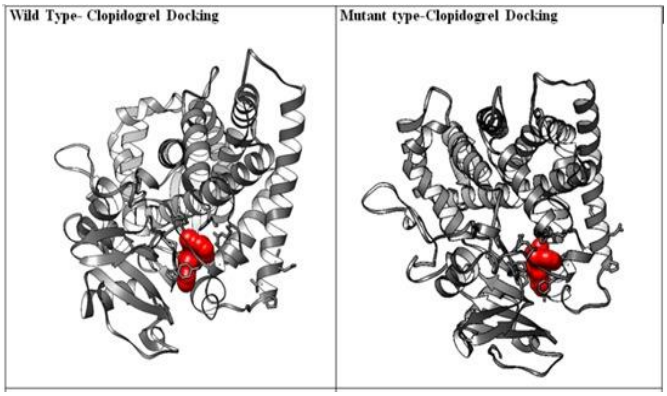

Figure 5. Molecular docking interaction of Clopidogrel and CYP2C19 wild and mutant type (Yan et al. 2017).

4 Discussion

The United States Food and Drug Administration (FDA) gave some warnings on clopidogrel metabolism and the CYP2C19 gene in 2017 (FDA, 2017). It was reported that some patients metabolized clopidogrel poorly and developed drug resistance. Clopidogrel activity would decrease in these patients. Shuldiner et al. determined the effect of 13 polymorphisms located near the CYP2C18-2C19-2C9-2C8 gene region on the response to clopidogrel treatment. The CYP2C19*2 genotype is detected in approximately 12% of individuals with variable responses to clopidogrel (Shuldiner et al. 2009).

Liu Y. et al. detected that the loss of function of the CYP2C19 gene causes cardiovascular system diseases in Chinese patients (Liu et al. 2013). Siller-Matula JM et al. explained that the loss of function alleles (*2, *3, *4, *5, *6, *7, and *8) were responsible for the cardiovascular diseases risk (Siller-Matula et al. 2013). Pettersen et al. detected that CYP2C19*2 carriers were observed to have higher platelet activity than non-carriers (Pettersen et al. 2004). Zhuo ZL et al. found that the CYP2C19 mutant allele was significantly associated with clopidogrel responsiveness and explained that CYP2C19*4 and CYP2C19*5 are associated with clopidogrel resistance in the Caucasian population (Zhuo et al. 2018). Jeong YH. et al. showed that CYP2C19*2 and *3 affects clopidogrel response, the pharmacodynamics of clopidogrel, and cardiovascular events (Jeong YH. et al. 2011). Holmes DR. et al. detected that CYP2C19*4, CYP2C19*5, CYP2C19*6, CYP2C19*7, and CYP2C19*8 have low clopidogrel metabolism (Holmes DR. et al. 2010).

In this study, it was observed that the CYP2C19*4 mutation is related to clopidogrel responsiveness, and this mutation might cause clopidogrel resistance. Interactions between clopidogrel and mutant protein and wild-type protein were shown by docking analysis.

5 Conclusion

In this study, it was detected that CYP2C19*4 (rs28399504) mutation might disturb protein function and activity. The interactions between clopidogrel and wild and mutant-type proteins were observed. It was thought that CYP2C19*4 (rs28399504) mutation might affect the development of clopidogrel resistance in heart failure patients and also it might reduce drug response and complicate the treatment.

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