Effects of CYP2D6*10 Mutation on Binding Affinity on Common Antidepressants in Japan

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Abstract – In Japan, there is an apparent lack of development and stigma associated with mental health treatment, including the use of antidepressants. Additionally, there is a large portion of the population possessing the allelic variant CYP2D6*10, which is part of the Cytochrome P450 superfamily responsible for metabolizing a wide variety of pharmaceuticals. Through this study, we aim to clarify the effects of genetic variation of CYP2D6 on adverse effects when using different antidepressants. We conducted an *in silico* study of binding affinities of different ligands to two CYP2D6 wild-type models, 4WNW and 2F9Q, and their respective threaded *10 models. Using SwissDock, the 4WNW model showed that the *10 variant had a lower binding affinity as compared to the wild-type. When using the 2F9Q model or X-Score, we had inconsistent results, leading us to question the reliability of these resources. Our results from SwissDock suggest that the *10 variant has a lower binding affinity, and therefore leading to a lower rate of metabolism. This suggests that people with this genetic mutation may be more susceptible to adverse effects when using antidepressants. Furthermore, future experiments regarding the correlation between CYP2D6*10 and drug metabolism should be conducted to advance mental health treatment and personalized medicine in Japan.

Key Words – binding affinity, CYP2D6, Cytochrome P450, mutation

INTRODUCTION

Mental health has, over time, presented itself as a major problem in Japan. Not recognized until the 90's, depression, anxiety, and other mental illnesses are still widely stigmatized and increasingly prevalent in Japanese youth (Nishi et al. 2019). Symptoms oftentimes are difficult to recognize, making it troublesome for the patient and clinicians to notice. Treatment methods include therapy, prescription medicine, or a combination of both. Among many, two major antidepressants sold to treat depressive disorders are fluoxetine and paroxetine (otherwise known as Prozac and Paxil, respectively), which are both selective serotonin reuptake inhibitors (SSRI).

The majority of medicines are metabolized by Cytochrome P450, crucial to the oxidation of xenobiotics and steroids (Guengerich 2007). Within the superfamily, one specific enzyme metabolizing a wide variety of antidepressants and antipsychotics is CYP2D6, primarily expressed in the liver (McConnachie et al. 2004). The CYP2D6 gene has been extensively researched, with many studies showing a strong correlation between allelic variation, drug metabolism and overall efficacy.

Gaedigk et al. (2017) paper gathered CYP2D6 allele-frequency data from various studies and sources to estimate frequencies of phenotypes across major world populations. Subjects categorized in the poor metabolizer status were predicted to make up about 0.4 to 5.4% of the population, intermediate metabolizers (decreased function) between 0.4 and 11%, normal metabolizers between 67 and 90%, and finally ultrarapid metabolizers between 1 and 21% of the population. Certain function and non-functional allelic variants were extracted from the data, as they are only specific to some populations; for example, the CYP2D6*10 decreased-function allele is very high in frequency in East Asia, as 45% of the population is reported to possess the gene (Gaedigk et al. 2017).

Studies conducted show the CYP2D6 wild-type allele and CYP2D6*10 differ in their amino acid sequences; in the *10 variant, the 34th amino acid is changed to a Serine from a Proline, and the 486th position is a Threonine instead of a Serine (Yokota et al. 1993, Dai et al. 2015). The CYP2D6*10 variant is also known to have a decreased metabolism, as discussed above, which suggests an effect on drug efficacy (Del Tredici et al. 2018). Given the association between ethnicity and CYP2D6 genotypes, it would be greatly beneficial to develop phenotype- or allele-specific drugs, utilized differently for various populations.

In a 2018 paper examining frequencies of CYP2D6 alleles, it was observed that 93% of all alleles were single copy alleles, with the majority (62%) functioning normally; of the 7% of structural variants, the majority (68%) had no function. As such, the proportion of structural variants with no or decreased function (72%) were substantially higher than the corresponding proportion of single copy variants (38%), suggesting a strong correlation between structural variants and gene function. Additionally, the proportion of structural variants was higher in Asians (30%) compared to other ethnicities (6-11%), suggesting an overall decrease of CYP2D6 enzyme activity in Asians. There are clear, demonstrated connections between ethnicity and genotype, and an association between structural variants and metabolism type (Del Tredici et al. 2018).

Here, we aim to characterize the interaction between the substrates and the CYP2D6*10 variant. Through

computational methods, we modelled the binding affinity of the three substrates to CYP2D6 wild-type, *10, and Aldehyde Reductase.

METHODS

The 4WNW protein was reported to be the most similar to the CYP2D6*10 enzyme when bound to a ligand (Don et al. 2020). We retrieved the amino acid sequences of both the wild-type CYP2D6 as well as the *10 variant from UniProt in order to model a structure for the latter using SwissModel (Waterhouse et al. 2018). To model the structures for our ligand, we used the application ChemSketch and converted the models to PDB files suitable for Chimera. We then proceeded to remove the remaining substrates and non-integral charges from the retrieved 4WNW crystal structure, leaving just the enzyme itself. Our selected negative control enzyme, Aldehyde Reductase, is not from the Cytochrome P450 superfamily, therefore not metabolizing fluoxetine, paroxetine, venlafaxine or nortriptyline. Glucose is a known substrate of Aldehyde Reductase, but not metabolized by any Cytochrome P450 protein, therefore serving as a positive control for Aldehyde Reductase and a negative control.

In order to prepare the structures for docking, we used the Dock Prep plugin within Chimera; first removing non-complexed ions from the structure (Shapovalov et al. 2011), then determining the residue charges by the Gasteiger method (Wang et al. 2006). We converted the enzyme structures into PDB files in order to make it compatible with SwissDock when submitting the protein-ligand pairs (Grosdidier et al. 2011).

After we received the results, we analyzed the structures in order to determine whether or not the substrates were binding in their correct binding sites. For some of our negative controls, while we did get a full fitness value, we decided to disregard the number as the ligand was not binding in the correct active site. Full fitness value (kcal/mol) refers to the binding affinity of the ligand and the protein; the lower the full fitness value, the better the binding affinity. The Delta G value (Gibbs free energy) also refers to the binding affinity; similarly, the lower the Delta G value, the better the binding affinity.

Additionally, we decided to confirm our data using another CYP2D6 wild-type (PDB code: 2F9Q), as the structure was used in a study conducted in 2008 (Ito et al. 2008). We submitted this structure and the *10 amino acid sequence for threading using SwissModel (Waterhouse et al. 2018). We repeated the preparation process in Chimera and submission to SwissDock.

In order to further validate the SwissDock results, we used X-Score, a program designed to compute binding affinity by Dr. Shaomeng Wang's group at the University of Michigan Medical School (Wang et al. 2002). In order to run the program successfully, we were required to split the PDB files downloaded from SwissDock into the protein and the ligand, a PDB file and a Mol2 file respectively.

RESULTS

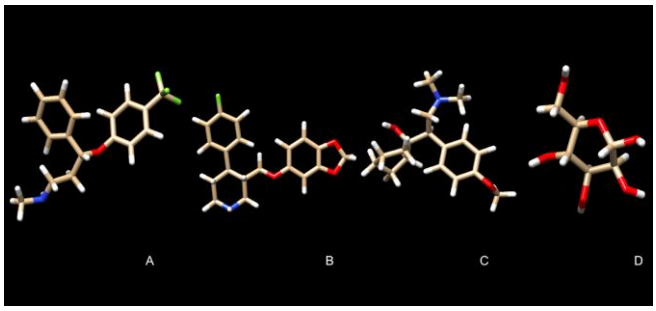

FIGURE 1: comparison of fluoxetine (A), paroxetine (B), venlafaxine (C), and glucose (D) chemical structure.

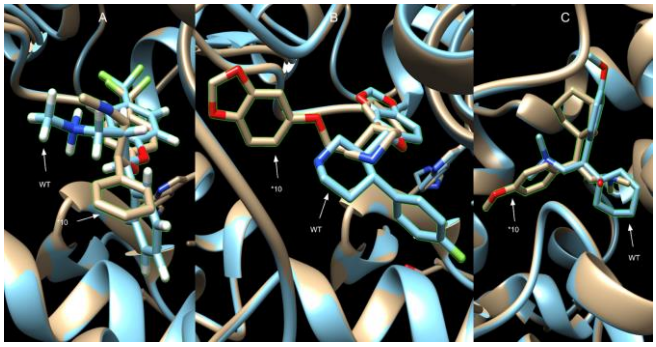

FIGURE 2: Comparison of the WT and *10 binding sites with fluoxetine (A), paroxetine (B), and venlafaxine (C) (blue shows WT, tan shows *10).

After inspection of the amino acid sequences, the mutation in the 486th position of the *10 allele from a Serine to a Threonine is only three amino acids away from the active site. This change seems to have a great effect on the binding affinity, and therefore causes substantially lower metabolism. As shown in Figure 2, CYP2D6 WT and *10 have minimal differences in their structures. One noticeable difference is the orientation of the ligand within the binding site; for example, paroxetine binds completely flipped within the binding pocket. Since there are not many obvious differences between the structures as a whole, this suggests that the orientation of the ligand in the active site immensely affects binding affinity.

TABLE 1: Full fitness (kcal/mol) of each enzyme and substrate. NB indicates SwissDock did not predict binding in the correct binding site.

	CYP2D6 WT (4WNW)	CYP2D6*10 (4WNW)	CYP2D6 WT (2F9Q)	CYP2D6*10 (2F9Q)	Aldehyde Reductase
Fluoxetine	-2104.54	-2006.49	-2277.55	NB	NB
Paroxetine	-2133.69	-2038.27	NB	NB	NB
Venlafaxine	-2106.40	-2005.68	NB	NB	NB
Nortriptyline	-2073.38	-1981.23	NB	NB	NB
Glucose	NB	NB	NB	NB	-1405.39

TABLE 2: Estimated ΔG (kcal/mol) of each enzyme and substrate. NB indicates SwissDock did not predict binding in the correct binding site.

	CYP2D6 WT (4WNW)		CYP2D6*10 (4WNW)		CYP2D6 WT (2F9Q)		CYP2D6*10 (2F9Q)		Aldehyde Reductase	
	Swiss Dock	X-Score	Swiss Dock	X-Score	Swiss Dock	X-Score	Swiss Dock	X-Score	Swiss Dock	X-Score
Fluoxetine	-9.225	-8.10	-9.06	-8.10	-8.01	-8.40	NB	NB	NB	NB
Paroxetine	-9.17	-8.11	-9.13	-8.37	NB	NB	NB	NB	NB	NB
Venlafaxine	-8.63	-7.75	-8.62	-7.87	NB	NB	NB	NB	NB	NB
Nortriptyline	-7.02	-8.32	-8.29	-8.35	NB	NB	NB	NB	NB	NB
Glucose	NB	NB	NB	NB	NB	NB	NB	NB	-6.44	-6.05

As predicted and shown in Table 1, our negative controls showed no binding to either WT nor *10 structures. Our positive control, aldehyde reductase, also bound to glucose. For all substrates except glucose, the wild-type protein was predicted to have substantially lower full fitness compared to the *10 variant when using SwissDock. Out of the four given substrates, paroxetine showed the best binding affinity as compared to the other two for both the WT and *10 CYP2D6 enzyme.

Ito et al. (2008) paper found that the ligand nortriptyline bound to the CYP2D6 wild-type (PDB model 2F9Q) had a ΔG value of -8.89 kcal/mol when modelled, and -9.00 kcal/mol when observed experimentally. It was also predicted that the CYP2D6*10 had a more positive ΔG value as compared to the wild-type protein, suggesting lower binding affinity. In contrast to their findings, none of our ligands bound correctly in the active site to the CYP2D6 wild-type or *10 threaded using the 2F9Q wild-type, with the exception of fluoxetine with the 2F9Q wild-type.

When X-Score was used, the ΔG values of the *10 variant were lower than that of the wild-type, which indicates better binding affinity. These results directly opposed our previous outcomes from SwissDock, as SwissDock had consistently, for all our substrates, predicted the wild-type ΔG value to be lower as compared to *10.

Ultimately, it was difficult to find previous studies with comparable results and data, and to run software that suited our study. Additionally, there was an inconsistency with our data from SwissDock; for example, when looking at the binding affinity of the CYP2D6 WT (PDB code: 2F9Q) with our ligands, only fluoxetine bound to the protein in the correct binding site, though paroxetine, venlafaxine, and nortriptyline are known substrates of CYP2D6.

DISCUSSION

Our results suggest altered docking and lower binding affinity of fluoxetine, paroxetine, venlafaxine, and nortriptyline to CYP2D6*10 when using SwissDock to predict binding affinity by full fitness value. To our surprise, none of the substrates except for fluoxetine bound correctly to the CYP2D6 wild-type 2F9Q model. Moreover, none of our ligands bound with the CYP2D6*10 in the actual active site when the 2F9Q model was used for threading.

From our results, it is probable that possessing the *10 variant causes lower binding affinity, leading to a drop in metabolism. This supports our initial hypothesis gathered from our literature review, as CYP2D6*10 is named as an "intermediate metabolizer". While this negative trend could be seen in the 4WNW wild-type and *10 when using SwissDock, the trend was lost when using X-Score or another model (PDB code: 2F9Q).

Our findings and results were fairly limited due to the lack of availability in software and previous research or resources. Our data was limited to an *in silico* experiment, only using relatively outdated freely available resources (such as X-Score from 2003). There was an apparent lack of possible software that could be used, alongside a frequent need for troubleshooting. Additionally, there was a lack of public available data and studies on the *10 allele and gene-based medication in Japan to use for comparison; we were unable to find reliable data for comparison.

Studies have shown that the *10 allele is most prevalent in East Asians, the gene first being discovered in Japanese subjects. Within a study conducted in 1993, CYP2D6*10 is found to have a lower metabolic rate than a regularly functioning enzyme (Yokota et al. 1993). In the future, we strongly believe the need for an in vivo experiment within a lab to produce accurate results to confirm this study; this would be greatly beneficial towards Japanese health programs and pharmaceutical development. We also suggest, if possible, a population study to view and scrutinize the direct correlation and relationship between allelic variant or genotype and the binding affinity or rate of metabolism. Given the prevalence of the *10 variant within the Japanese population, we hope that this study contributes to the limited body of research on Japanese mental health, and may in the future be applied clinically to lower dosages for Japanese patients as a "regular" dose may put the patient at a higher risk for adverse effects. This further suggests development in personalized medication, especially in Japan, since patients may be more prone to experiencing adverse effects.

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