INTERNATIONAL JOURNAL OF APPLIED AND CLINICAL RESEARCH

IJACR

Original article

Computational Drug Repurposing for The Management of Pathogenicity of Beta-Thalassemia

Samrina Muskan[1], Sajila Mustafa[2], Usama[1], Uzair Malik[1]*

1 Center of Biotechnology and Microbiology, University of Peshawar, 25120, Khyber Pakhtunkhwa, Pakistan
2 Department of microbiology, Quaid-e-Azam university, Islamabad, Pakistan
* Correspondence: um903307@gmail.com

Abstract: Beta-Thalassemia is an inherited disorder caused by mutation in the HBB gene, resulting in the abnormal synthesis of beta globin protein. The c.92G>C (p.Arg31Thr) mutation of HBB gene is prevalent in South Asian population and results unstable protein. The current study uses computational approaches to analyze the pathogenicity of this mutation and the ability of three ligands, 4-Phenylbutyric Acid (4-PBA), Voxelotor, and Heminin stabilizing mutant beta-globin protein. Bioinformatics tools (MutationTaster, SIFT, PolyPhen-2, FATHMM) confirmed that the mutation is deleterious and impact the function of protein. I-Mutant and Mu-Pro suggested that the stability of protein decreases due to p.Arg31Thr mutation. Molecular docking was carried out using AutoDock Vina revealed that 4-PBA and Hemin show stronger binding affinity towards the mutant protein (−6.5 kcal/mol and −6.8 kcal/mol, respectively) as compared to wild-type protein, suggesting a stabilizing effect. Post-dock analysis employing Protein-Ligand Interaction Profiler (PLIP) showed that enhanced hydrophobic interactions and hydrogen bonding were observed in mutant-ligand complexes. In contrast, Voxelotor represented a reduction in the binding affinity to mutants, indicating that conformational changes have affected the binding. These findings indicate that 4-PBA and Hemin can be potentially used to reduce the severity of beta-thalassemia by stabilizing the mutant beta-globin. Further in vitro and in vivo validation is needed to confirm the results since in silico approaches alone cannot be relied upon. This study underlines computational drug repurpose as a promising strategy for treatment of Beta-Thalassemia.

Academic Editor: Bibi Zainab [Gold medalist]

Volume No 3, Issue 2

Keywords: Beta-Thalassemia, 4-Phenylbutyric Acid (4-PBA), Voxelotor, MutationTaster, SIFT, PolyPhen-2, FATHMM.

1. Introduction

Beta thalassemia is a monogenic autosomal recessive disorder, characterized by microcytic hypochromic anemia, ineffective erythropoiesis, and hepatosplenomegaly due to extramedullary hematopoiesis (Drew C Baird, 2022) (Luca Melchiori, 2010). This disorder arises due to the deficiency (β^+-thalassemia) or complete absence (β^0-thalassemia) of beta-globin chains, caused by mutation in the HBB gene located on the short arm of chromosome 11 (Renzo Galanello, 2010) (Thein, 2013). More than 350 mutations have been reported to be responsible for beta thalassemia, the majority of which are point mutations (single nucleotide substitution, deletions, or insertions) (Vincenzo De Sanctis, 2017). These mutations can occur anywhere in the exons, introns, or regulatory regions affecting the transcription, mRNA splicing and translation of β-globin. (Renzo Galanello, 2010). The results can appear in the form of protein misfolding, aggregation, and altered oxygen-binding affinity. (Arthur W Nienhuis, 2012). Approximately 1.5% of the world's population (80-90 million people) carry the β-thalassemia trait, and 60,000 infants are born with β-thalassemia major annually. In Pakistan, β-thalassemia is the most prevalent genetic disorder, with a carrier rate of 5–7% (9.8 million people). Each year, 5000 to 9000 children are born with β-thalassemia

major in the country. (Naghmi Asif, 2014). Computational analysis of β-thalassemia provides a powerful platform to study the functional and structural implications of mutations in the HBB gene and identify potential therapeutic candidates to regulate the pathogenicity of the disease-causing gene. In this study, we investigated the HBB missense mutation c.92G>C (p.Arg31Thr) prevalent in South Asian populations using computational tools. Three ligands-4 Phenyl Butyric Acid, Voxelotor, and Hemin were docked with the wild-type and mutant HBB proteins, and their molecular interactions were studied. The resulting binding affinities and interaction profiles were compared to identify promising potential drug candidate that can be repurposed for stabilizing the mutant β-globin and mitigating the severity of Beta-Thalassemia.

2. Methodology

Gene selection

Different bioinformatics databases were used to identify the gene associated with beta-thalassemia. DisGeNET was employed to obtain a list of known disease-causing mutations in the HBB gene in the Excel format. MalaCards was used to extract information on beta-thalassemia-related genes in the CSV format. To select the disease-associated genes, ShinyGO was utilized. Genes were ranked based on p-values, and HBB was selected as the top gene associated with beta-thalassemia, having the smallest p-value below the threshold of 0.05, indicating strong statistical significance in disease association.

Mutation Selection and Pathogenicity Prediction

GnomAD database was accessed to explore variants of the HBB gene. A CSV file containing all the variants was downloaded and opened in Microsoft Excel. Filters were applied to collect mutations that were exonic, single nucleotide, missense and had a highest allele frequency in south Asian population. For the current study mutation (c.92G>C) was chosen for further analysis of pathogenicity. Various online tools were employed for this purpose. Mutation tester is an online tool that was used to predict the disease-causing potential of the said nucleotide change. Further, this tool provided the amino acid sequence of the wild-type and mutant HBB protein. SIFT, PolyPhen2 and FATHMM were employed to suggest the functional implications of the mutation. The stability of protein after change in amino acid sequence was analyzed using I-mutant 2.0 and Mu-pro.

Structure acquisition of HBB and Ligands

The structure of amino acid sequence for both wild-type and mutant HBB proteins from MutationTaster were constructed using AlphaFold Server. The output was downloaded as ZIP archive with many models in .cif format. The 0th model was selected and converted into .pdb format using PyMol. The pdb versions were then transformed into .pdbqt, to make them compatible for docking.

Three ligands were selected based on their potential to stabilize the mutant protein. 4-Phenyl butyrate is a chemical chaperone which can assist in preventing protein misfolding and aggregation. Voxelotor is an established drug for treating sickle cell anemia and acts as hemoglobin oxygen-affinity modulator. It may potentially stabilize mutant HBB protein. Hemin has an important role in regulation of hemoglobin synthesis and may enhance the stability of mutant protein. The Ligands were downloaded from PubChem in .sdf format, converted into .pdb using PyMol, followed by their conversion into .pdbqt using AutoDock Tools.

Molecular docking

AutoDock Vina was used for docking wild-type and mutant HBB protein with each of the three chosen ligands. Before docking, the protein along with its ligand was imported into PyMol to determine the coordinates of the active site, which were then used to define the dimensions of the grid box for docking. In AutoDock Tools, .pdbqt of ligand as well as protein were allowed to interact. Grid box was chosen and adjusted using active site information from PyMol. AutoDock Vina was run to perform the actual docking. Seed 12345678 was used to avoid randomized results. The results were in the form of a log file and a

.pdbqt output file. The log file contained different possible values of binding energies for the interaction. The top binding affinities were picked for further analysis. The output file contained different binding poses ranked according to their stability. This file along with the original protein in .pdb format were imported into PyMol. The top pose was exported in .pdb format as the dock complex.

Post Dock Analysis

The dock complexes from each run of docking were uploaded to PLIP (Protein-Ligand Interaction Profiler) to understand the interaction between ligand and protein. PLIP identified different types of bonding like hydrogen bonds, hydrophobic contacts, π-stacking, salt bridges, and water bridges.

3. Results

Pathogenicity Prediction of c.92G>C Mutation

Various bioinformatics tools determined the functional and stability related implications of the nucleotide replacement in the HBB gene. MutationTaster predicted genetic mutation to be disease-causing. Likewise, SIFT, PolyPhen2, and FATHMM detected a deleterious impact on protein function. Similarly, I-mutant and mu-pro tools for protein stability, predicted that the mutation decreases stability. Combining these results imply that the mutation is structurally and functionally pathogenic.

Database/Tool	Scores	Range	Prediction
Mutation tester	-	-	Disease causing
SIFT	0.00	0–1; ≤0.05 damaging	Effect of protein function
PolyPhen2	1.000	0 (benign) to 1 (pathogenic)	Probably damaging
FATHMM	-5.09	≤-1.5 damaging	Damaging
I-Mutant	-	-	Decrease stability
Mu-Pro	-	-	Decrease stability

Table 1: Pathogenicity prediction given by various tools

Structural Modeling of Wild-Type and Mutant HBB Proteins

The structures of both wild-type and mutant HBB were successfully constructed using AlphaFold server. When the structures of the top models were compared in Pymol, no major structural distortion was observed in the mutant protein. The mutant and wild-type appeared identical in terms of structure.

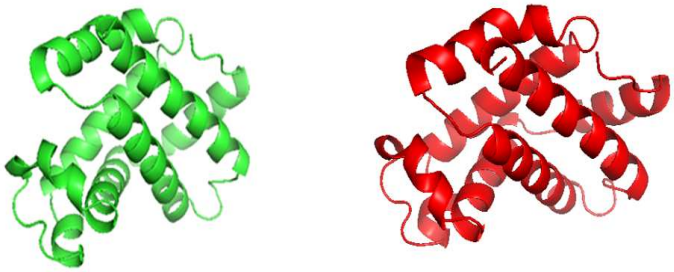


Figure 1: Structural Models of Wild-Type (: green illustration) and Mutant HBB (red illustration) Proteins (AlphaFold)

Ligand Preparation

4-PBA, Voxelotor, and Hemin were selected based on their known role in protein stabilization and hemoglobin related functions. 4-PBA is a chemical chaperone that stabilizes misfolded proteins. Voxelotor is an FDA approved drug for sickle cell anemia which interacts with hemoglobin and increases its oxygen binding affinity. Hemin is involved in regulating hemoglobin synthesis. All ligands were downloaded from PubChem in the .sdf format and converted to into .pdb format. Pdb files were converted into .pdbqt format using AutoDock Tools. All three ligands were successfully prepared for molecular docking.

Ligands	PubChem ID	Role
4-Phenyl Butyric Acid	4775	chemical chaperone; stabilize misfolded proteins
Voxelotor	71602803	Hemoglobin oxygen-affinity modulator
Hemin	455658	Hemoglobin synthesis regulator

Table 2: Ligands used for molecular docking along with PubChem IDs

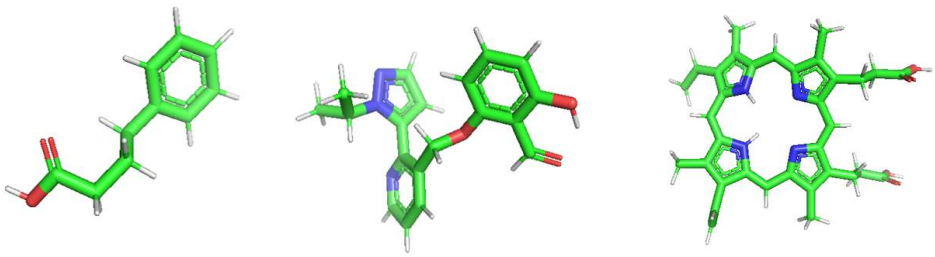


Figure 2: Ligand Structures Used for Docking (4-Phenyl Butyric Acid, Voxelotor, Hemin)

Molecular Docking

Molecular docking was performed for each ligand against both proteins using AutoDock Vina. The top binding energies were observed for each ligand-protein pair.

Ligands	Wild-Type	Mutant	Δ Binding Energy (Mutant – Wild-Type)
4-Phenyl butyrate	-6.1	-6.5	-0.4
Voxelotor	-6.5	-5.6	0.9
Hemin	-5.9	-6.8	-0.9

Table 2: Binding Affinities (kcal/mol) of Ligands with Wild-Type and Mutant HBB Proteins

Interpretation

The energy difference of (-0.4 kcal/mol) suggests that 4-phenyl butyric acid binds more strongly to the mutant as compared to the wild-type and may potentially stabilize the mutant HBB protein. Voxelotor's affinity towards wild-type was greater suggesting the mutation may have altered its binding capacity towards the said ligand. Hemin showed the greatest binding energy difference between mutants and

wild-type proteins. A significant binding of mutant compared to wild-type suggests a stronger stabilization of hemin. These results suggest that 4PBA and hemin demonstrate strong binding affinity towards mutants as compared to the wild-type. Voxelotor is less effective in binding with mutants.

Post dock analysis interaction

The docked complexes were analyzed using PLIP, for molecular interactions. The important findings included:

4-PBA-HBB

The high interaction counts of Val138A in wild- type, and Leu107A and Leu142A in mutant HBB shows strong hydrophobic binding of these residues with the ligand. Mutant-type gains new hydrophobic interactions at Ala71A and Leu89A, which were not previously present in the wild-type-HBB complex. The wild-type forms salt bridge using its His93A residue while no such interaction is found in the mutant. However, a new hydrogen bond forms at Phe104A residue in the mutant.

Voxelotor-HBB

The wild-type shows hydrophobic interactions via the Leu, Val, and Phen residues while mutant binds charged residues Asp, Glu, and Lys. Leu142A has the highest number of interactions to the ligand in wild-type, on the contrary Asp22A has strongest interaction to the mutant. Wild type shows π-Stacking at His93A. Mutant has π-Cation at Lys62A and weak H-bonds with Pro59A/Lys62A

Hemin-HBB

Mutant's Trp38A with 40 interactions hold hemin tighter than wild-type's Val21A. Wild type has no hydrogen bonding, but the mutant has 3 H-bonds. Weak π-Stacking are present in the wild type and totally absent in mutant form.

4. Discussion

The purpose of this study was to identify a pathogenic exonic missense mutation in the HBB gene which results in the formation of mutant beta-globin protein giving rise to beta- thalassemia and evaluate the stabilization potential of 3 ligands such as 4-PBA, Voxelotor, and hemin– on the mutant protein. We compared the binding affinities of the ligands when complexed with wild-type and mutant β-globin. The aim was to identify ligands that bind strongly to the mutant protein, enhancing its stability and potentially mitigating the pathogenicity.

Pathogenicity of the c.92G>C Mutation

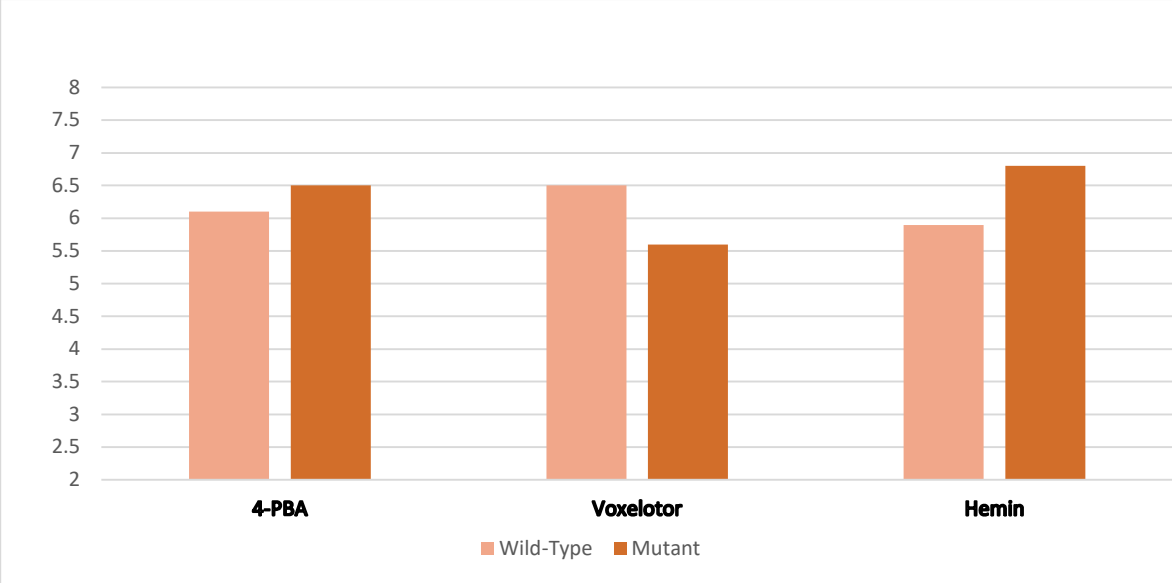
c.92G>C was evaluated using different bioinformatics tools (Mutation Tester, SIFT, I-mutant etc.) All of them suggested that the mutation was pathogenic and impacted on the function and stability of HBB protein. c.92G>C mutation corresponds to R31T in the polypeptide sequence, where Arginine residue is replaced by threonine. Arginine (Arg) is a basic amino acid because its side chain contains a positively charged guanidinium group which readily accepts protons. Threonine is polar in nature due to its R group, which contains a side chain with a hydroxyl group. The two amino acids are distinct from each other, replacing one with another could disrupt electrostatic interactions and hydrogen-bonding protein folding, thus contribute to pathogenesis.

Structural Comparison of Wild-Type and Mutant HBB

The structures of wild-type and mutant HBB protein were visualized showed no apparent difference. The global structure was intact in the mutant and no distortion was seen. However, the difference in binding energies of wild-type and mutants suggests that the mutation has caused subtle local conformational changes which are responsible for protein dysfunction.

Ligand Binding Affinity and Stability

The binding of a ligand molecule can influence the stability of a protein. When a ligand binds to a protein it often causes conformational changes that can stabilize the protein. In beta-thalassemia some mutations result in unstable, misfolded proteins that degrade prematurely. If a ligand binds to this mutant protein with high affinity, it could prevent misfolding and degradation of β-globin. Molecular docking studies revealed that Hemin and 4PBA demonstrate high binding affinity and suggests stabilizing effect to the mutant. In contrast, voxelotor shows strong binding towards wild-type protein suggesting that R31T mutation has impacted binding site or protein conformation in such a way that its affinity has decreased towards the ligand. The stronger binding of 4-PBA and hemin to the mutant protein suggests their potential therapeutic significance. These known chemicals can be potentially repurposed to mitigate the effects of damaging mutation in the HBB protein.



Graph 1: Comparison of binding affinities of different ligands with mutant and wild-type proteins

Molecular Interaction Analysis

The molecular interactions were notably different when mutants and wild type were compared: 4PBA-mutant complex gains new hydrophobic interactions at Ala71A and Leu89A, and a new hydrogen bond forms at Phe104A residue. These bonds likely account for the improved binding of ligand with mutant protein. In voxelotor, the complex exhibits aromatic/hydrophobic interactions in the wild type and charged residues in the mutant. This change in interaction may explain the weaker interaction between voxelator and mutant protein. The hemin-mutant complex exhibited an unusually high number of interactions at the Trp38A and also forms 3 hydrogen bonds, in contrast to the wild-type. This reinforces hemins ability to form strong interaction with mutant protein imparting stabilizing effect.

Therapeutic Potential

Among the studied ligands, 4PBA and hemin appear to be potential candidates for stabilizing mutant HBB protein. They may prevent misfolding and aggregation of beta-globin protein. Therefore, it could potentially serve as therapeutic agent for treating Beta-thalassemia, particularly caused by mutations that result in protein instability. However, computational analysis results alone are not sufficient, and the study requires further in vivo and in vitro validation, repurposing the mentioned ligands as a treatment for Beta-Thalassemia.

5. Conclusion

In this research, the therapeutic potential of three ligands 4PBA, Voxelotor, and Hemin was studied to mitigate the effect of mutant beta-globin protein. Using molecular docking we analyzed the interaction of ligands with wild-type and mutant protein. The ligands that showed stronger affinity towards the protein, likely stabilizes it more. Also, if the ligand forms stronger connections with the protein (like hydrogen bonds or hydrophobic interactions), it's even more likely to keep the protein stable. The results of the study showed that 4-PBA and hemin have strong binding affinities towards mutant HBB. This suggests that they can be potentially used to stabilize misfolded beta-globin proteins and therefore can be repurposed to treat beta-thalassmia. However, in vitro and in vivo validation is required to confirm these results.

References

1. Arthur W Nienhuis, D. G. (2012). Pathophysiology and Clinical Manifestations of the β-Thalassemias. PubMed Central.
2. Drew C Baird, S. H. (2022). Alpha- and Beta-thalassemia: Rapid Evidence Review. PubMed, 272-280.
3. Luca Melchiori, S. G. (2010). β-Thalassemia: HiJAKing Ineffective Erythropoiesis and Iron Overload. PubMed Central.
4. Naghmi Asif, K. H. (2014). Prevention of Beta Thalassemia in Pakistan. Journal of Islamabad Medical & Dental College, 46-47.
5. Renzo Galanello, R. O. (2010). Beta-thalassemia. PubMed.
6. Thein, S. L. (2013). The Molecular Basis of β-Thalassemia. PubMed.
7. Vincenzo De Sanctis, C. K. (2017). β-Thalassemia Distribution in the Old World: an Ancient Disease Seen from a Historical Standpoint. PubMed.