Tertiary structural prediction and drug binding studies on mutated gene (HMBS) in human porphyria

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Abstract

Acute intermittent porphyria (AIP) is a rare autosomal dominant hepatic porphyria due to deficiency of hydroxymethylbilane synthase (HMBS), also known as porphobilinogen deaminase leading to accumulation of porphyrin precursors. The current work focuses on structure based drug designing against potential targets of porphyria disease. The advanced software tools and database are used to perform molecular modeling and drug docking studies. Disease caused gene (HMBS) sequence for porphyria was retrieved from National Centre for Biotechnology Information in FASTA Format. Tertiary structure prediction was performed using an automated knowledge based homology modeling server PHYRE and viewed through Discovery studio. Functional and Domain analysis were carried out through using Helix-turn-Helix server. Patch Dock Server was used to analyze the docking mechanisms of the Hematin and HMBS protein and designed ligand molecules. The results obtained from the investigation would have a scope for drug designing in the future.

Key Words: Porphyria, HMBS, Phyre, Helix-turn-Helix server, Hematin

INTRODUCTION

The most common acute porphyria, acute intermittent porphyria, has a prevalence of approximately 1 in 20,000 [1]. Acute intermittent porphyria (AIP; also called Swedish porphyria, pyrroloporphyria, intermittent acute porphyria) is an acute neurovisceral porphyria resulting from a partial deficiency of the heme biosynthetic enzyme porphobilinogen deaminase (PBGD) also known as hydroxymethylbilane synthase (HMBS). It is an autosomal dominant disorder with low penetrance; development of symptoms is affected by a variety of exacerbating factors [2]. Despite its well characterized molecular genetics, the diagnosis of AIP is challenging. Symptoms are often vague and nonspecific; the other possible causes of neurologic findings and abdominal pain are numerous; and acute porphyria is often not considered because it is rare [3]. Clues from the family history may be absent, because penetrance of AIP is low and symptoms may not manifest in the majority of family members with a disease-causing mutation. Even if acute porphyria is considered, many clinicians are unfamiliar with typical findings of the disease, appropriate testing, and interpretation of test results [4]. As a consequence, diagnosis and life-saving treatment are often delayed. Defects in HMBS cause acute intermittent porphyria (AIP), with over 300 such mutations apparently identified. Many of the mutations are unique and are not listed in public databases [3].

MATERIALS AND METHODS

The target gene sequence was retrieved from NCBI database in Fasta format, the functional analysis of target protein was done with gym motif server. The target sequence was studied for the purpose of structural prediction using Phyre server [5]. Modeled HMBS protein was viewed through advanced molecular visualization tool DISCOVERY STUDIO SOFTWARE. The validation was done with rapper server [6]. The docking analysis between the drug and protein was performed by using patch dock server. The pocket structure of HMBS protein was found by using PEB-FOLD server [7].

RESULTS AND DISCUSSION

The target protein sequence of Hydroxymethylbilane synthase (HMBS) shows their best motif from GYM-motif server (Fig 1 & 2). From the result we found two highlighted HTH motif
To model 3D structure of HMBS protein phyre server was used and viewed with the help of molecular visualization tool such as Discovery studio software figure(3) shows , atom-ball and stick: figure (4 )shows protein ribbon model: also the drug of acute intermittent porphyria for recently used hematin [9]. The structure of hematin shows in figure (5). The protein structure was validated using Rapper server figure (6&7) represents the assessment of the Ramachandran plot and also indicates the overall quality of the 3Dimensional structure of HMBS protein [10]. The pockets structure of target protein was obtained by pebfold server figure (8). The protein was applied for the docking analysis by using patch dock server (9).

**Fig 1. FASTA sequence**

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>EA67450.1 hydroxymethylbilan synthase, isoform CRA_d [Homo sapiens]
MSGNGNAAATAEENS PKMVRGTRSKQLARIQTDSVATL KAYSYPQFEIIAMSTTGDK I LTA SK
IGEKSLFTKELEHALEKNEVDLVVHSLKDLPVTLPVP GFTIGAICKRENPHDAVVFHPFKFVGTLELPK
SVVGTSSLRRAAQLQRKFPHELFRSRGNLNLRLRLKLDDEQQEFSAILATAGLQRMGWHNVRGQILHPEE
CMYAVGQEGGCSVPVAHTAMKDGQLYLTVGVWSDLGDSIQTENMQATIHVPAQHEDGEPDPQLVGITA
RNIPRGQPQLAQQNLGISLANLLLSKGAKNILDVARQLNDAH
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**Fig 2. Motif identification – HMBS Gene**

<table>
<thead>
<tr>
<th>Pick</th>
<th>Loc</th>
<th>LP</th>
<th>NPM</th>
<th>Score</th>
<th>Detected?</th>
<th>Motif</th>
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<tbody>
<tr>
<td>Best</td>
<td>365</td>
<td>4</td>
<td>4</td>
<td>30</td>
<td>+</td>
<td>RGPQLAAQNLGISLANLLLSKG</td>
</tr>
<tr>
<td>2nd</td>
<td>255</td>
<td>4</td>
<td>2</td>
<td>29</td>
<td>+</td>
<td>EQQEFSAILATAGLQRMGWHN</td>
</tr>
</tbody>
</table>

**Fig 3 & 4 3D structure of HMBS target**
Lavanya G and Shoba K, Tertiary structural prediction and drug binding studies on mutated gene (HMBS) in human porphyria

Fig 5. 3D structure of Drug Compound

Fig 6 & 7. Validation results of HMBS Protein

Evaluation of residues
Residue [ 75 :SER] ( 80.24, 33.68) in Allowed region
Residue [ 88 :ASN] ( 87.40, 17.72) in Allowed region
Residue [ 110 :ILE] (-128.95, 80.42) in Allowed region
Residue [ 140 :LYS] ( 85.49, -4.99) in Allowed region
Residue [ 181 :GLN] ( 80.81, 19.76) in Allowed region
Residue [ 218 :GLU] ( 85.06, -1.03) in Allowed region
Residue [ 269 :PRO] ( -97.19, 127.88) in Allowed region
Residue [ 276 :VAL] ( 81.20, -3.03) in Allowed region
Residue [ 73 :GLU] ( 97.66, 169.37) in Outlier region
Residue [ 126 :HIS] (-91.52, -14.66) in Outlier region
Residue [ 127 :PRO] (-125.17, -28.77) in Outlier region
Residue [ 224 :PRO] (-143.18, 173.96) in Outlier region
Residue [ 284 :PRO] (-140.91, 152.10) in Outlier region

Number of residues in favoured region (~98.0% expected) : 258 (95.2%)
Number of residues in allowed region (~2.0% expected) : 8 (3.0%)
Number of residues in outlier region : 5 (1.8%)
Lavanya G and Shoba K, Tertiary structural prediction and drug binding studies on mutated gene (HMBS) in human porphyria

Fig 8. Pockets Structure Analysis HMBS Protein

Fig 9. Docking Analysis HMBS Protein with Drug Molecule

CONCLUSION
This gene encodes a member of the hydroxymethylbilane synthase superfamily. The encoded protein is the third enzyme of the heme biosynthetic pathway and catalyzes the head to tail condensation of four porphobilinogen molecules into the linear hydroxymethylbilane. Mutations in this gene are associated with the autosomal dominant disease acute intermittent porphyria [11]. The molecular docking simulation are commonly used in modern drug design process to take a closer look into ligand-protein receptor binding mode and prove guidance for future studies [12]. The 3D structure clearly explains the drug molecular binding sites which helps in future drug designing, drug docking studies, experimental modeling, and pharmaceutical applications.

ACKNOWLEDGEMENT
None

CONFLICT OF INTEREST
No conflict of interest.
REFERENCE


