Biomedical Letters ISSN 2410-955X DOI: https://doi.org/10.47262/BL/7.1.20210409

Research article
2021 | Volume 7 | issue 1 | Pages 42-47

ARTICLE INFO

**Received**
February 25, 2021
**Revised**
March 29, 2021
**Accepted**
April 30, 2021

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**Keywords**
Wilson's disease
ATP7B
ATOX1
Protein-Protein interactions

**How to Cite**
Aslam A, Answer A, Baloch SMA, Sajid M. Homology Modeling and Protein-Protein Molecular Docking analyses elucidate the Potential Binding Pockets of ATP7B: A Candidate Wilson's disease. Biomedical Letters 2021; 7(1):42-47.

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# Homology Modeling and Protein-Protein Molecular Docking analyses elucidate the Potential Binding Pockets of ATP7B: A Candidate Wilson's disease

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## Abstract

There has been progressive improvement in computational drug design from last decade. Numerous computer aided compounds have been reported against neurodegenerative disorders. Wilson's disease is a common neurodegenerative disease in humans associated with *ATP7B* that encodes a transmembrane copper-transporting ATPase which induces the copper export from hepatic cells into bile and supplies copper for the functional synthesis of Ceruloplasmin. Almost, 150 mutations of ATP7B have been identified lead to cause Wilson's disease having symptoms of cancers, loss of memory and postural instability. In this research article, 3D structure of ATP7B was predicted by using comparative modelling approaches. The predicted structures were evaluated by utilizing numerous evaluation tools and 98.50% of overall quality factor was observed for the final selected structure. ATOX1 was predicted as the interacting partner of ATP7B and molecular docking analyses of ATP7B and ATOX1 were conducted by using PatchDock. The least global energy of -35.45 Kcal/mol was observed having the interacting residues in the binding pocket. The reported interacting residues may help to target the specific drug development against ATP7B. This research article can be a major initiative to predict the therapeutic drug targets against Wilson's disease.

## Introduction

Wilson's disease (WD) is uncommon autosomal recessive and treatable disease caused due to copper metabolism characterized by copper accumulation [1]. Copper is a metal ion which serves as a reactive co-factor in proteins and play role in key metabolic processes including connective tissue formation, respiration, pigmentation, neurotransmitter synthesis and radical defense [1, 2] . Copper ions in free form tend to generate hydroxyl radicals which induce the damages to structures of proteins, nucleic acids, and lipids. Therefore, the production of free Cu radicals leads to disrupted the function of cell [2]. The excessive copper deposition in various organs results to damage the organs mainly hepatocytes associated to liver pathology and brain (particularly of the basal ganglia cerebellum are affected in it) which leads to neurological symptoms and psychiatric abnormalities [1]. WD is caused by mutations in *ATP7B* that encodes a transmembrane copper-transporting ATPase which induces the copper export from hepatic cells into bile and supplies copper for the functional synthesis of Ceruloplasmin (major copper containing protein in blood) [1, 3] .

Commonly, disease occurs in children and adults [4]. WD occurrence is higher in China as compared to western countries and it is also termed as Hepatolenticular Degeneration. In 1932, first two cases of WD were reported in China. Mostly, its symptoms present between the age of 5 to 35 years. In 1950, it was concluded that the neurological damage leads to clinical manifestations specifically in extrapyramidal system. These manifestations were characterized by disturbed associated movements and involuntary movements (such as tremor) [2] . WD is considered as a rare disease with the prevalence of symptomatic disease estimated to be 1 in 30,000 in 1984 [1, 3]. Epidemiological studies from Germany and Japan indicated the prevalence of WD as 29 per 1000,000 and 33 per 1000,000 respectively. Furthermore, the frequency of individuals carrying two mutant alleles of ATP7B was 1 per 7000 individuals. This data seems to be more reliable according to genetic WD. However, further studies and investigations are required for most devoted epidemiological results [1]. Primary symptoms of WD are pathological changes in tissues caused by toxicity of copper overload [1]. The disease can be managed successfully if diagnosed and treated at early stage, while becomes lethal with ignorance [3]. Zinc salts and Cu chelators are the existing therapies for WD [5]. It was first treatable neuro-metabolic disease, though the treatment remains critical which may lead to disability at considerable level. The clinical heterogeneity and disease rarity are some reasons due to which a practitioner finds it complex to develop significant expertise for the treatment of WD [6, 7].

ATP7A and ATP7B are the P-type ATPase's which play an essential role in the transport of copper across Golgi membranes making the use of the energy released from adenosine triphosphate (ATP) hydrolysis [4]. Copper is the part of some essential enzymes that maintain the normal cell functions. Copper transporting ATPase is also responsible for the removal of excess copper from the body.

ATP7B plays a critical role in maintenance of homeostasis [3] he ATP7B mutant results in copper overload in tissues particularly liver, brain and kidneys. These mutations can be homozygous (one disease-causing allele) or heterozygous (two disease-causing allele) and can affect almost complete number of exons [1]. ATP7A and ATP7B are homologues to one another (similarity of amino acids is 67%). However, ATP7A is mostly present and play key role in non-hepatic tissues including intestines, heart and brain. Whereas ATP7B is mostly expressed in kidneys and liver and also characterized in lower levels in lungs, brain and placenta [3].

The copper-transporting ATPase is located on short arm of chromosome 13 consists of 20 introns and 21 exons [1]. Within liver cells, copper-transporting ATPase is found in a structure called the Golgi apparatus, which modifies newly produced enzymes and other proteins. In Golgi apparatus of liver cells, copper transporting ATPase delivers copper to a protein called Ceruloplasmin, which transport copper to other parts of the body through blood. The copper level gets too high in the liver cells leads to copper transporting ATPase leave the Golgi and transfers copper to small sacs for elimination through bile. Bile is a substance produced by the liver that is important for the digestion and removal of waste products from the body.

Relatively, the direct sequencing analysis is the accurate method to identify the mutations in ATP7B. Mutations that incidence in ATP7B protein are point mutations (most common type), small insertions, deletions, gross deletions and splicing site mutations. The low frequency and compound-heterozygous nature of disease-causing mutations make the analysis of individual mutants critical [4]. According to Human Gene Mutation Database, more than 700 mutations have been identified. Further investigations have indicated the role of mutant genes and proteins

which can serve as pathological agent to induce WD phenotype. The particular example of such mutated agent is Patatin-like phospholipase domain-containing protein 3(PNPLA3) associated with non-alcoholic fatty liver disease (NAFLD) leads to severity of hepatic steatosis in WD patients [1].

Bioinformatics analyses have revealed that the sequence of Cu-ATPase's is based on N-terminal binding domain (differs in Copper ATPase's from various species) and C-terminal core structure (shared in all Cu-ATPase's). The N-terminal domain of both ATPase's (ATP7A and ATP7B) consists of six metal-binding domains (MBDs). NMR structures have revealed that each MBD is folded into a structure looks like ferredoxin associated with a copper-binding motif CxxC. The C-terminal composed of eight transmembrane helices associated with highly conserved domains such as A-, N- and P-domains [4]. Number of biological problems have been resolved by employing bioinformatics techniques [8] and reported various novel compounds against cancer [9] and neurological disorders [8]. Various *in silico* studies also help to develop the epitope-based vaccine against SARS-CoV-2 for corona virus [10, 11]. The 3D structure of ATP7B was not completely reported in Protein Data Bank (PDB). The struggles started with the 3D structure prediction of ATP7B by satisfying the available literature. The protein interacting partner was identified and protein-protein molecular docking analyses were performed for exploring the binding domain. To accomplish the aims of research work, computational analyses, comparative homology modeling and molecular docking studies were utilized. The observed findings confirmed that the followed methodology has the capability to identify the binding interacting residues.

## Materials and Methods

*ATP7B* transcribed to 26 different transcripts that form five isoforms and have eight PDB structures collectively. The accession number of ATP7B is P35670 and other isoforms also contain same accession number in UniProt KB (KnowledgeBase). 3D structure prediction and protein-protein molecular docking studies were achieved. The target sequence was retrieved from UniProtKB and length of canonical sequence of ATP7B was 1465 amino acids. 3D structures of copper transporting ATPase 2 isoforms were predicted through homology modelling The analyses available in literature and biological database declared the plentiful information about sequence of *ATP7B*. The ATP7B sequence was retrieved from UniProtKB revealed five isoforms of ATP7B with various sequence length of and threading approaches. The retrieved sequences of ATP7B isoform was subjected to BlastP for obtaining the appropriate templates from PDB [12] against ATP7B. The protein modelling automated program MODELLER 9.14 [13]. was implemented to predict the 3D structures of selected templates one by one. The threading approach was also applied for 3D structure prediction by using I-TASSER [14], SWISS-MODEL [15], phyre2 and HHPred. UCSF Chimera 1.10 tool was used for visualization of generated structures for ATP7B. The evaluation tools including Rampage [16], Anolea [17] and ERRAT [18] were employed for overall estimation of protein structures and model quality. Quality factor and Z-score values for all generated structures were calculated through ERRAT and Anolea respectively. The favoured region allowed region and outliers were studied for the predicted structures by using Rampage. The visualization and structural analyses of the predicted structures were done through UCSF Chimera.

Protein-protein molecular docking studies were performed by using STITCH (Search Tool for Interacting Chemicals) [19] and STRING (Search Tool for the Retrieval of Interacting Genes/proteins) databases to predict the interacting partner of ATP7B. The crystal structure of functional partner (PDB Identity. ATOX1) of ATP7B was retrieved from PDB database. PatchDock was used to determine the protein-protein interactions of the target protein and ATOX1.

## Results and Discussions

Research resources are devoted to develop understanding of WD by using structural bioinformatics [1]. WD is a rare metabolic genetic disorder caused by multiple mutations of *ATP7B*. The defective function of ATP7B enzyme prevents free copper ions from excretion out of the cell which results in impaired secretion of Cu ions in various organs and cause toxicity to liver and brain, mainly to cerebellum and basal ganglia [2, 4]. WD is also known as Hepatolenticular degeneration and is a type of neurodegenerative disease [3]. The sequence analyses of P-type ATPase have determined its similarity to MNK (Meknes gene) having six metal binding domains. The genetic region where ATP7B express in liver and kidney consists of 300kb [2].

1465,1258,1354,1447 and 239 amino acids respectively.

Homology modeling techniques and threading approaches were employed to predict the 3D structures of ATP7B. The amino acid sequence of ATP7B was subjected to BlastP for suitable templates against PDB. The top ranked aligned templates having maximum identity, query coverage, E values and maximum scores were selected for 3D structure prediction (**Table 1**). The observed templates were used to predict the 3D structures of ATP7B.

**Table 1:** The selected templates for ATP7B sorted by their query coverage, PDB ID, percentage identity and E-values.

| PDB ID | Query Coverage | E-value | Identity %age | Max score |
|---|---|---|---|---|
| 3J09 | 69% | 2e-154 | 38.55% | 486 |
| 3J08 | 47% | 1e-141 | 41.17% | 449 |
| 2R0P | 35% | 2e-135 | 99.50% | 416 |
| 3RFU | 46% | 8e-134 | 38.07% | 432 |
| 4BBJ | 46% | 1e-132 | 37.92% | 429 |
| 2ARF | 11% | 6e-106 | 99.39% | 334 |
| 2EW9 | 39% | 3e-96 | 99.33% | 306 |
| 2KOY | 11% | 7e-83 | 84.05% | 268 |
| 4UMV | 54% | 2e-70% | 29.91% | 253 |
| 3A1C | 22% | 6e-59 | 38.96% | 206 |

Number of models for ATP7B were predicted by employing numerous tools (M4t, I-TASSER, SWISS MODEL, Phyre2, HHpred and MODELLER 9.14) and *in silico* approaches (homology modeling and threading) by satisfying the sequence.

All the predicted structures were evaluated for reliability on the basis of overall quality factor, allowed region, favored region and outliers. The most reliable structure was selected for further analyses.

The evaluation tool ERRAT showed 98.964% overall quality factor for ATP7B depicting the reliability of the predicted structures. The energy minimization was performed on the selected structure of ATP7B to further improve the quality of the structure. The predicted structure was employed to UCSF Chimera 1.10 for minimization at 1000 steepest and 1000 conjugates gradients runs. The final predicted structure with minimization analyses (**Fig. 1**) showed potential for further analyses.

*In vivo* and *in vitro* studies of ATP7B revealed six-metal binding domains at N-terminal. Each binding domain can attach to copper ion as well as with domains of other ATP7B proteins and other proteins. The existence of these binding domains is remarkable to ATP7B homologs of mammals. In addition, metal binding domains have missense mutations cause WD [3]. The canonical sequence of target protein consists of greater length which may cause errors for protein-protein docking examination

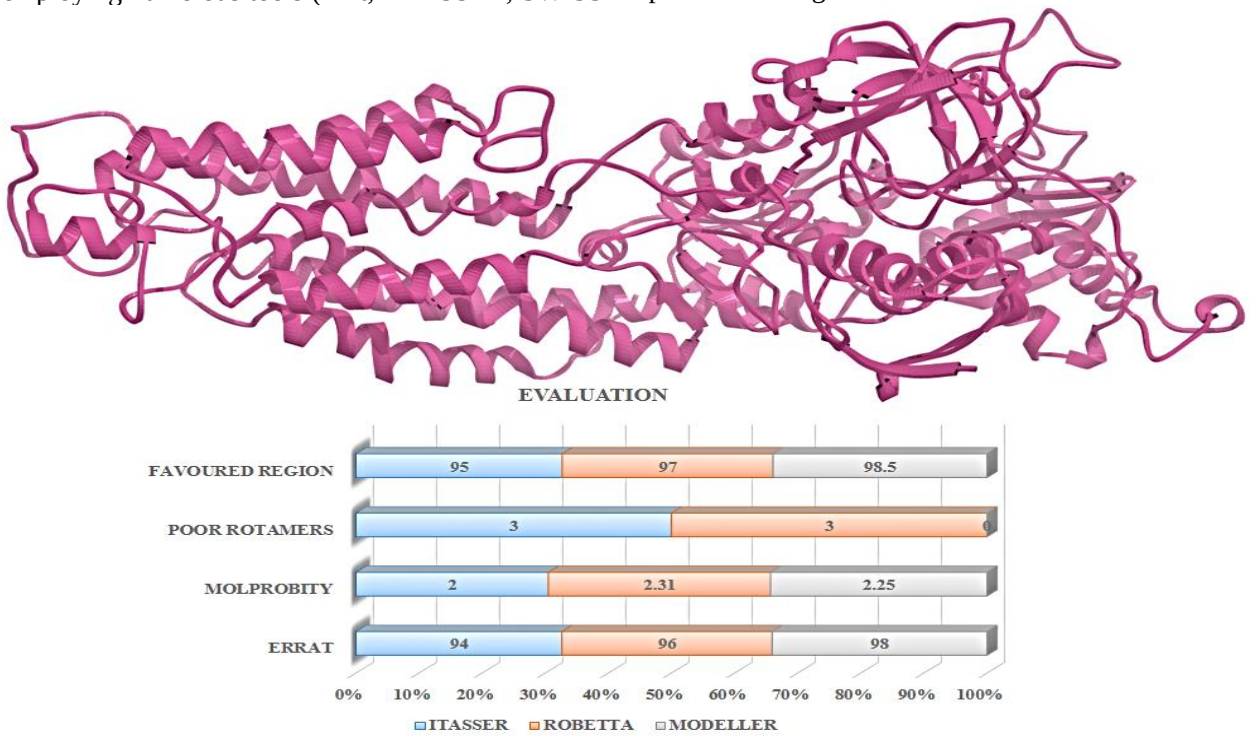


**Fig. 1:** 3D structure for ATP7B and its protein evaluation using ERRAT, MOLPROBITY, and RAMPAGE

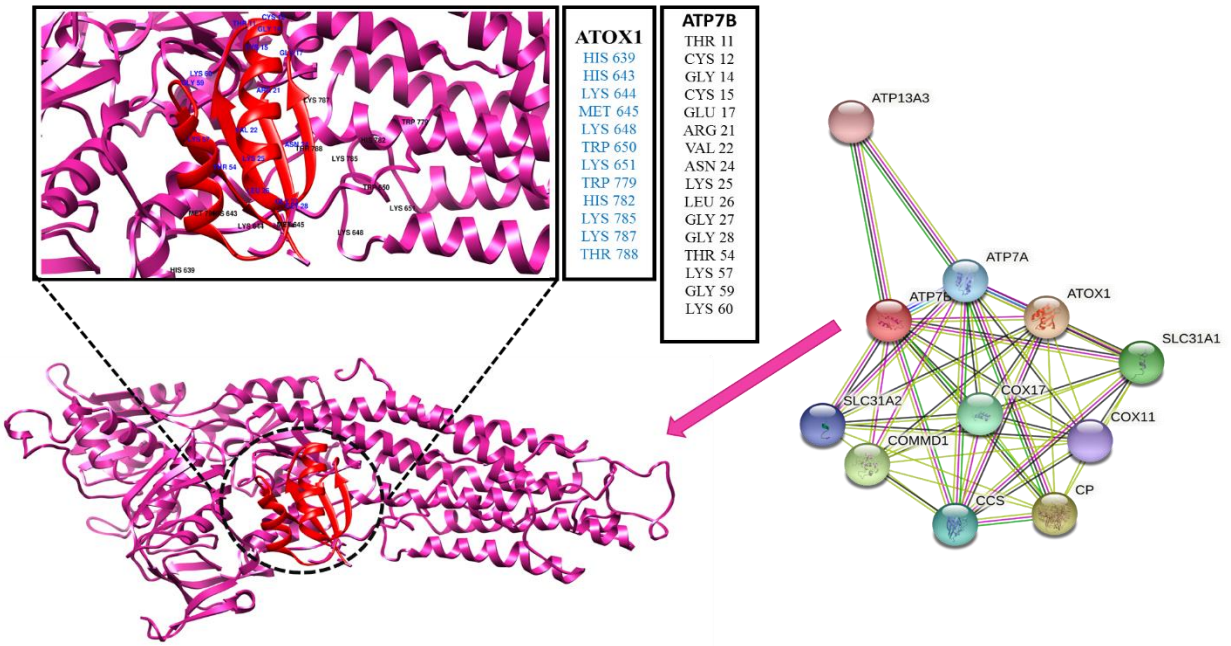


**Fig. 2:** Protein-protein interactions using string and molecular docking between ATP7B and its partner ATOX1.

due to which appropriate template 2LQB (with suitable sequence length) was used for this purpose. STRING database was utilized for prediction of interacting partner of 2LQB [20]. ATOX1 was observed as the interacting partner of 2LQB and subjected for protein-protein molecular docking analyses by using PatchDock.

ATOX1, the interacting partner of ATP7B was used for molecular protein-protein docking analyses. The ATOX1-ATP7B docked complexes were analyzed for interacting residues (**Fig. 2**). The protein-protein molecular docking studies were done, and the observed results were analyzed on the basis of approximate interface area of complex and Atomic Contact Energy (ACE) by using PatchDock. Numerous docked complexes were generated and analyzed on the basis of ACE and top 10 complexes having least ACE values were further selected for improvement by utilizing FireDock molecular docking software. All the generated docked complexes were visualized and analyzed on the basis of Attractive and Repulsive VdW, ACE and least binding global energy. The final docked complex showed the least global binding energy of −21.97. The least binding values suggested that ATP7B and ATOX1 showed potential binding affinity.

## Conclusion

In conclusion, the 3D structure of ATP7B was predicted by employing structure prediction approaches, evaluate the predicted structure through numerous evaluation tools and final selected structure was observed as most potent 3D structure. ATXO1 was identified as the interacting partner of ATP7B and molecular protein-protein docking analyses were performed to reveal the potential interacting residues. This current effort suggested that the predicted binding residues could be used for further studies. The predicted binding residues may lead to novel drug development.

## Acknowledgments

Authors are thankful to Mr. Muhammad Waqas Javed, Department of Bioinformatics and Biotechnology, Government College University Faisalabad, Faisalabad, Pakistan for guidance regarding computational analyses.

### Conflict of interest

The authors declare no conflict of interest.

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