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Structural analysis of misfolding equilibrative nucleoside transporter 3in H-syndrome

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ABSTRACT

Recent findings interpret that misfolding, aggregation and accumulation of proteins consequence in some disease. Evidence from different practices intensely support this hypothesis and demonstrate that a common therapy for these pernicious disorders might be possible. However, H syndrome is related to the recessive mutations in *SLC29A3*, encoding the equilibrative nucleoside transporter Equilibrative nucleoside transporter 3 (hENT3) which is expressed in mitochondria. The aim of this study is to investigate the misfolding and aggregation of ENT3 protein regarding in H syndrome, which can be a potential target for therapeutic interference in this disorder. Therefore, the 3D structure of the ENT3 protein was assumed using the I-TASSER online server and the best-predicted structure with the maximum confidence score (C-Score) was selected. The reliability and quality of the structure were evaluated by Z-score. However, the structural model of the selected mutant was accomplished using the I-TASSER server. The comparison with the corresponding of non-mutated structure and structural difference between mutant and wild-type structures was demonstrated by PyMOL.

In the present study, computational models combined with experimental approaches provide new insights into the ENT3 which could lead to expanding new classes of nucleobase analogs for treatment and quintessential therapeutic strategies for H syndrome and some other diseases like cancer and viral infection.

1. Introduction

Proteins are molecules that control the most vital cellular functions. Thus, a protein must first fold into its correct three-dimensional structure, in addition to assuming complex tertiary and even quaternary conformations. However, the process is quite complicated and susceptible to errors but many aspects of folding being distinct to the biophysical properties of the protein itself [1]. Proteins consist of the perplexing arrangement of interior folds that collapse into a final stable structure and, for many proteins, only a modest free energy gain (generally only −3 to −7 kcal/mol) [2] is associated with the relevant folding of a protein compared with its numerous potential misfolded states. Therefore, a number of misfolded proteins complicated in disease contain mutations that destabilize the correct fold and/or stabilize a misfolded state. Different chemical natures of protein compartments cause various protein-folding problems. For instance, in eukaryotic cells, protein folding must occur in many different organelles such as peroxisomes and mitochondria [3].

Retaining protein homeostasis is required for normal cell function and its viability.

Protein misfolded has occurred in many diseases of human beings and all other organisms. During diseases, the normally folded proteins, misfold and gain functions such as infectivity, toxicity and loses function. In designing towards conventional therapeutics it is necessary to know more on the miss functions of proteins based on their structures [4].

One of the most credible ways to characterize proteins with an unknown activity is experimental determination and a number of computational approaches have been developed for prediction of protein function [5]. Web portals have been used to contribute information about protein structures [6].

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Previous studies demonstrated that mutations in hENT3 make a wide range of human genetic disorders. Vered Molho-Pessach et al. [34] reported five mutations in H syndrome. On the other hand [7], consider that mutations in *SLC29A3* perceive in Familial Histiocytosis Syndrome and Familial Rosai-Dorfman Disease. Therefore, it's assumed that hENT3 related disorders have some overlapping clinical symptoms.

Accumulating, the assessment of SLC29A3 mutations as the molecular basis in H syndrome, Familial Histiocytosis Syndrome and Familial Rosai-Dorfman certify a direct link between links these disorders to SLC29A3-associated phenotypes.

Mutations on membrane proteins may lead to small structural variations. Prediction of such structural variations can help to further understand the related bio-activities of membrane proteins [8]. It was investigated that, hENT3 is involved in protein sorting at the multivesicular body; Epsin-3 has a role in the trafficking of clathrin between the Golgi network and endosomes. It is involved in the recruitment of clathrin to the Golgi network and endosomes to form clathrin-coated vesicles. The beginning and the end of transmembrane helices play an important role in transferring through the membrane and intracellular activation pathways. hENT3 is involved in protein sorting at the multivesicular body because it binds to membranes and, in association with vacuolar protein sorting-associated protein 27.

H syndrome caused by mutations in the *SLC29A3* gene. That is an autosomal recessive genodermatosis disease with multisystem involvement. H syndrome is identified by hyperpigmentation, cutaneous changes of progressive sclerosis and hypertrichosis that follow a specific pattern with multiple systemic demonstrations. H syndrome as an anomaly discloses to the major clinical findings of hyperpigmentation, hypertrichosis, hepatosplenomegaly, heart anomalies, hearing loss, hypogonadism. Additionally, laboratory test results showed systemic manifestations, with characteristic cutaneous findings accompanying systemic inherited histiocytosis.

The human SLC29 family of proteins is recognized as equilibrative nucleoside transporters (ENTs). They belong to the eukaryotic ENT family of equilibrative and concentrative nucleoside and nucleobase transporters. ENTs are polytopic imperative membrane proteins which transport the nucleosides and some therapeutic analogs. Equilibrative nucleoside transporter 3 protein is one of ENTs members of equilibrative transporter which is arbitrated both efflux and influx of nucleosides across the membrane. Equilibrative nucleoside transporters act in the extracellular space by ecto-ATPases and nucleotidases uses [9].

Our aim is to understand the structure of ENT3 leading to H syndrome, relevant to the development of new therapeutic strategies in the future.

Our model seems to show well the differences between the wild-type and mutant form of the protein existing beside it consents with the laboratory results of patients. Based on our computational results and good agreement with all available information on this protein structure, we hope that experimentalists will find this problem challenging in H syndrome and will eventually confirm our findings.

2. Materials and methods

[10] illustrated that SLC29A3 had a heterozygous point mutation in exon 6, (at positions 53) which was a nucleotide transition c.1309G > A resulting in the missense amino acid substitution P.Glycine 437 Arginine. On the other hand, they revealed, there was a mutation in exon 3 (at positions 437) G155 > A mutation, changing Threonine to Alanine.

After detection the mutations in the eligible sequence, to study whether the mutations in the gene can either have an effect or modify the structure of the product of a gene, the amino acid sequences of the proteins were retrieved from National Centre for Biotechnology Information database (NCBI) and aligned using ClustalW software [11] to determine the appropriate sequence for protein structure prediction. By using the sequence similarity model, the structural homologs for retrieved sequences was documented from the available structures present in the protein data bank (PDB). Additionally, There was no protein structure (PDB) encoded by the SLC29A3 gene in PDB.

However, the structure of the protein was auspicated by fold recognition methodology using i-TASSER prediction server. The structures of the protein accomplished using I-TASSER servers were then approved by SAVes server [12].

I-TASSER simulations develop tens of thousands conformations that denominated decoys, for each target sequence. SPICKER program [13] is used to cluster all the decoys based on the pair-wise structure similarity, and indicate up to five models which correspond to the five largest structure clusters in I-TASSER. Based on the Monte Carlo theory, the largest clusters correspond to the states of the lowest free energy or largest partition function and thus have the highest confidence. In I-TASSER the confidence score for estimating the quality of the predicted model was quantitatively measured by C-score.

The C-score is computed based on the significance of threading template alignments and the convergence parameters of the structure assembly simulations. It is typically in the range of −5 to 2, where a C-score of higher value signifies a model with a high confidence and vice-versa.

The C-score of the I-TASSER models is described as

$$\mathrm{C-score} = \ln\left(\frac{M}{M_{tot}}\cdot\frac{1}{RMSD}\cdot\frac{\prod_{i=1}^{4} z(i)}{\prod_{i=1}^{4} z_{0(i)}}\right)$$

where M is the multiplicity of structures in the SPICKER cluster; M_{tot} is the total number of the I-TASSER structure decoys used in the clustering; RMSD is the average RMSD of the decoys to the cluster centroid; Z(i) is the highest Z-score (the energy to mean in the unit of standard deviation) of the templates by the ith PPA threading program and Z0(i) is a program-specified Z-score cutoff for distinguishing between good and bad templates [14].

Z(i)/Z0(i) is the normalized Z -score of the best template gene and I-TASSER adjusts a normalized B-factor with the Z-score-based transformation. C-score defined in Equation 1 is correlated with the quality of the assumed models (with a Pearson correlation coefficient >0.9 to the TM-score relative to the native) [15]. TM-score is a sequence length-independent metric for adjusting structure similarity with a value in the range (0, 1).

In the top 5 models which were provided by I-TASSER, the first model had a higher C-score and a better quality.

However, the C-score has a strong correlation with the quality of the final models, which has been used to quantitatively estimate the RMSD of the final models. Therefore, the C-score of the first model was used for further analysis.

A typical secondary structure prediction using I-TASSER accommodated three states: alpha helix (H), beta strand (S) and coil (C), with confidence scores for each residue. The predicted secondary structure was used for estimating the secondary structure of the protein.

Normalized B-factor with the *Z*-score-based transformation was computed using I-TASSER. The normalized B-factor is predicted by ResQ by the combination of template-based assignment and

machine-learning-based prediction [9].

On the other hand, the Consensus Constrained TOPology prediction (CCTOP) method was utilized to anticipate the human transmembrane proteins, which are collected in the HTP database [16]. To examine the accuracies of transmembrane topology of human proteins, a particular benchmark set was arranged, and on this set, CCTOP demonstrated to be preferable in topology prediction accuracy.

It was described the CCTOP analysis as a novel consensus topology predictor for ENT3.

The CCTOP server automatically compounds information from certain experimental, structural and bioinformatics studies which are collected in other databases (TOPDB, PDBTM, and TOPDOM). Transmembrane protein filtering and signal peptide prediction is also available on CCTOP server.

PyMOL software was used to visualize the selected model and a folded globular anti-parallel beta sheet and unstructured helix were shown [17].

The levels of energy were minimized, and the structures were reformed based on the generated Ramachandran plot. At last, the modeled structures were deliberated using PyMOL v1.7.4.5. The active sites present of the protein were visualized using the computed atlas of surface topography of proteins (cPORT) server [18] as an online resource for measuring and locating concave surface regions of the constructed 3D model of proteins.

3. Result and discussion

During recent years computational methods have been exerted to study the protein misfolding and aggregation. This attitude is convenient whenever the computational models, especially combined with experimental approaches. Computational methods are more and more practical for characterization of the identification of molecular events and changes in protein dynamics which convince understanding and predicting protein misfolding.

However, human ENT3 (hENT3) is a 475-residue protein. The sequence analysis was showed the physiochemical properties of the protein determined the length of the protein to be 475 amino acids (Fig. 1).

It was offered to anticipate the 3D model of wild-type and mutant ENT3 using I-TASSER (Fig. 2). In the next step, it was attempted to predict the model based on multiple-threading alignments using I-TASSER. The 3D model was created using I-TASSER multiple templates and refined with energy minimization. However, to amend the quality of the full-length of the model, it was used to combine 3D modeling methods and molecular visualization using PyMOL.

Three approaches of ResQ were tested to generate B-factor predictions. The template-based prediction is achieved by transferring the B-factors of the template proteins as reached by threading. The models predicted by I-TASSER, with global and local accuracy estimations. Since the C-score was −1.33, the model was

Wild-Type

MAVVSEDDFQHSSNSTYRTTSSSLRADQEALLEKLLDRPPPGLQRPEDRFCG**T**YIIFFSL
GIGSLLPWNFFITAKEYWMFKLRNSSSPATGEDPEGSDILNYFESYLAVASTVPSMLCLV
ANFLLVNRVAVHIRVLASLTVILAIFMVITALVKVDTSSWTRGFFAVTIVCMVILSGAST
VFSSSIYGMTGSFPMRNSQALISGGAMGGTVSAVASLVDLAASSDVRNSALAFFLTATVF
LVLCMGLYLLLSRLEYARYYMRPVLAAHVFSGEEELPQDSLSAPSVASRFIDSHTPPLRP
ILKKTASLGFCVTYVFFITSLIYPAICTNIESLNKGSGSLWTTKFFIPLTTFLLYNFADL
CGRQLTAWIQVPGPNSKALPGFVLLRTCLIPLFVLCNYQPRVHLKTVVFQSDVYPALLSS
LLGLSNGYLSTLALLY**G**PKIVPRELAEATGVVMSFYVCLGLTLGSACSTLLVHLI

MUTANT

MAVVSEDDFQHSSNSTYRTTSSSLRADQEALLEKLLDRPPPGLQRPEDRFCG**A**YIIFFSL
GIGSLLPWNFFITAKEYWMFKLRNSSSPATGEDPEGSDILNYFESYLAVASTVPSMLCLV
ANFLLVNRVAVHIRVLASLTVILAIFMVITALVKVDTSSWTRGFFAVTIVCMVILSGAST
VFSSSIYGMTGSFPMRNSQALISGGAMGGTVSAVASLVDLAASSDVRNSALAFFLTATVF
LVLCMGLYLLLSRLEYARYYMRPVLAAHVFSGEEELPQDSLSAPSVASRFIDSHTPPLRP
ILKKTASLGFCVTYVFFITSLIYPAICTNIESLNKGSGSLWTTKFFIPLTTFLLYNFADL
CGRQLTAWIQVPGPNSKALPGFVLLRTCLIPLFVLCNYQPRVHLKTVVFQSDVYPALLSS
LLGLSNGYLSTLALLY**R**PKIVPRELAEATGVVMSFYVCLGLTLGSACSTLLVHLI

Fig. 1. Amino acid sequence alignment of wild-type and mutant form of hENT3 protein.

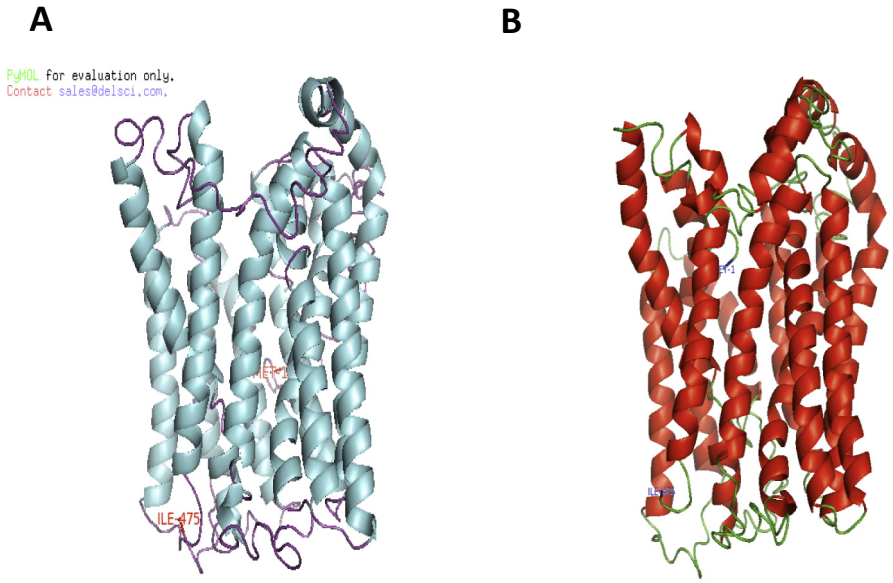

Fig. 2. Overview of the distribution of mutations within the human ENT3 protein and schematic representation of the domain structure of hENT3. The models predicted by I-TASSER for wild-type (A) and mutant (B) form of ENT3.

anticipated to have good quality, with an estimated RMSD = 13.0 ± 4.2 Å and the estimated TM = 0.44 ± 0.14. Additionally, the residue-specific accuracy estimation for each model apperceived and the local structure accuracy profile of wild-type and the mutant form of the protein was shown.

However, I-TASSER was used to determine the highest Z-score (Z-score = 2.46). The quality of the mutant model was assured using Z - scores, which is expressive of model quality and to avouch which the predicted structure is within range of score as established in the native protein.

Estimating of the quality of the predicted models was used *C*-score, and it was computed based on the significance of the threading alignments in LOMETS [19] and the convergence of the I-TASSER simulations. The higher *C*-score coordinated with a model of better quality. In general, models with the *C*-score > − 1.5 have a correct fold. Here, the *C*-score was −1.33 and it was an estimate of the confidence of structure prediction.

Prediction on the normalized B-factor was done (Fig. 3). The regions of the N- and C-terminals and most of the loop regions are anticipated with positive normalized B-factors, announcing that these regions are structurally more flexible than other regions. On the other hand, the predicted normalized B-factors for the alpha and beta regions are negative or close to zero, implying these regions are structurally more stable.

The cPORT software was used for predicting the active site and the area around the active site of the protein for ENT3 protein models.

As the structural figures were generated by PyMOL, different types of atoms in the stick or sphere representations were specified by different colors in mutant and non-mutant forms of hENT3 in order to study the amino acids in this study. It should be noted that the disordered region was placed between Thr53 and Gly437 (Fig. 4) (Fig. 5).

Fig. 5 is a schematic of a protein structure showing the binding site of the pyrimidine nucleotide in the internal cavity of protein, where these two mutations are located on both sides of that. These two mutations may affect the binding of the pyrimidine nucleotide in ENT3 protein which functions as a pyrimidine nucleoside carrier in all organs.

To check the accuracies of ENT3 protein as a transmembrane human protein, CCTOP was used as superior software in topology prediction accuracy.

The program CCTOP was used to identify the transmembrane region of the ENT3 protein (Fig. 6) and therefore the extracellular and intracellular regions of the protein to be identified. The mutations were examined in this study were located in the transmembrane region of the protein. The results were compared with I-TASSER results which are contained stands and helixes. Since the results were completely consistent thus CCTOP analysis was confirmed.

These two mutations didn't change the overall conformation of the protein, since; changes might impair nucleoside binding, because, Threonine to Alanine and Glycine to Arginine.

Threonine is generally noticed a somewhat polar amino acid, though it is justly neutral with regard to mutation. It is a hydroxylic amino acid which is used to form active sites of proteins, while

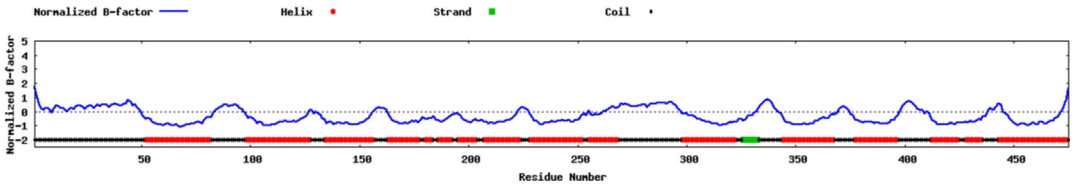

Fig. 3. Prediction on the normalized B-factor. The regions of the N- and C-terminals and most of the loop regions are predicted with positive normalized B-factors in normal ENT3 protein.

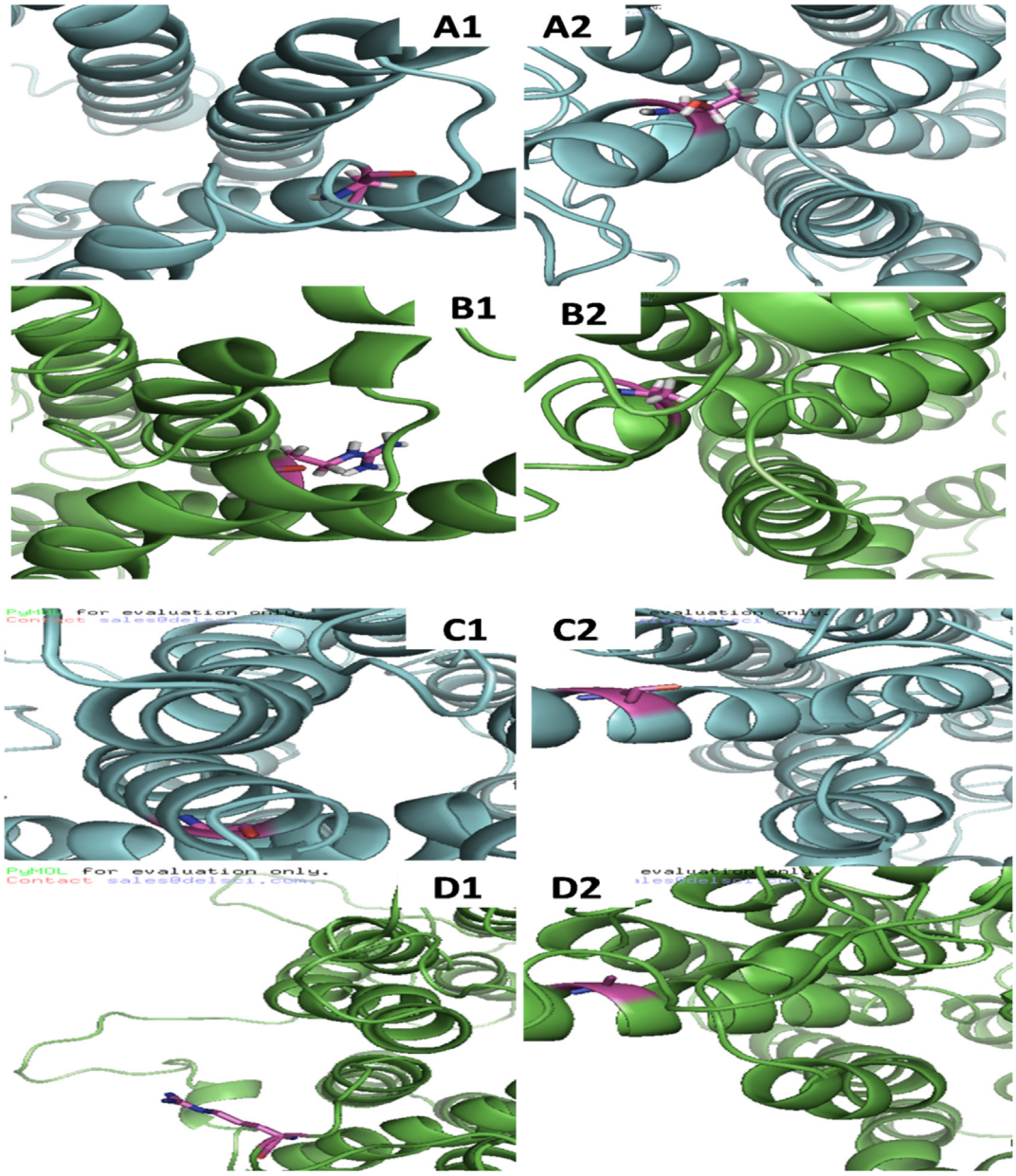

Fig. 4. α-helix-related mutation in hENT3. Conformational changes in position 437 and 53 in wild-type (A1, A2) and position 437 and 53 in mutant (B1, B2) form of ENT3 by I-Tasser server. Conformational changes in position 437 and 53 in wild-type (C1, C2) and position 437 and 53 in mutant (D1, D2) form of ENT3 by Medeller and memoir servers. (In side chains; N:blue, C:magentas, H:grays, O:red and S:orange).

Alanine is an aliphatic amino acid which is most easily used as an energy source for the synthesis of glucose in the body.

On the other hand, Glycine is an aliphatic amino acid and Arginine is a basic one that their substitution may change the function of the protein.

[20] reported that coding genes of transmembrane protein are often related to the human disorders. They showed that characteristic differences in amino acid changes within transmembrane regions: in the case of disease related to the mutations of non-polar to non-polar and non-polar to charged amino acid exchanges are equally frequent. Substitutions of Glycine to arginine and leucine to proline find in many diseases.

The arginine residue makes the further charge in the lipid bilayer that can change the function and structure of transmembrane proteins. They demonstrated that Arginine incorporated into the membrane-spanning segment by the Glycine to arginine amino acid variations stack in the interior of the lipid bilayer. Analysis of the literature showed that three of these changes eventuate in altered phenotypes (two being disorder generating, the rs34059508 and rs36209700 variants in the SLC22A1 [21] and ABCG8 [22] proteins respectively.

Misfolding of proteins is being recognized as the cause of a growing number of diseases. On the other hand, molecular modeling showed the prediction of the conformational changes expanded for detecting the selected amino acid substitutions and their correlation with the patients' phenotypes.

The different structure in α-helix is highlighted in purple in the backbone of the peptide which it showed the residue number 437 was mutated.

These results are consistent with a cPORT model in which the

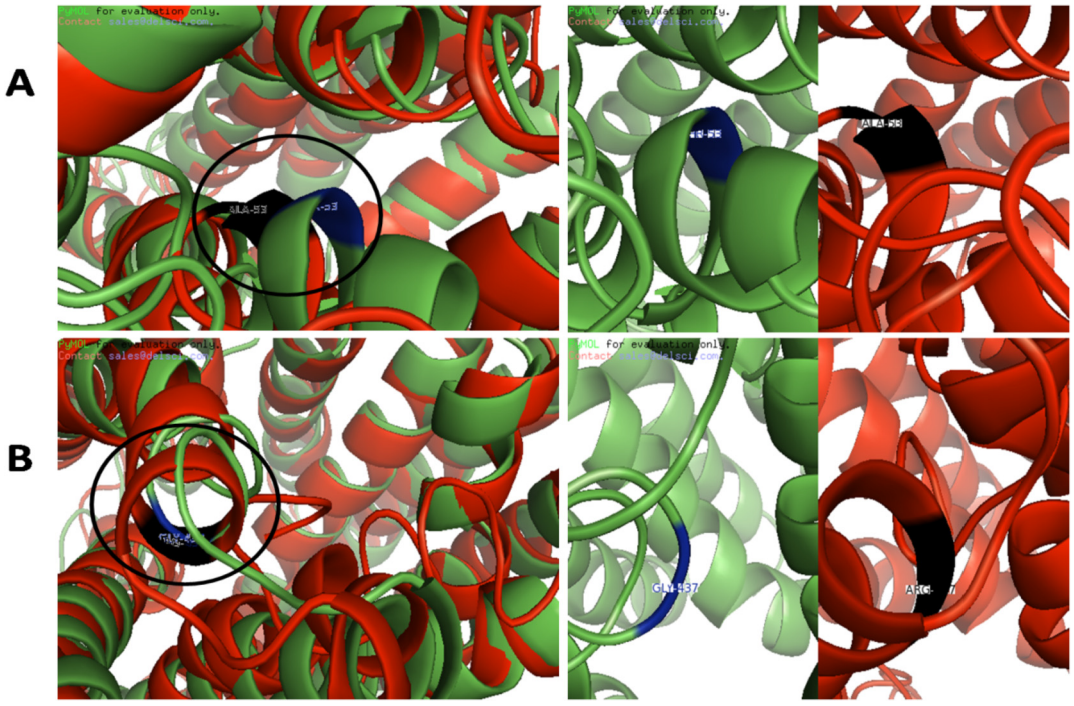

Fig. 5. Conformational changes in position 53 in wild-type (green color) mutant (red color) (A) and position 437 in wild-type (green color) mutant (red color) (B) form of ENT3.

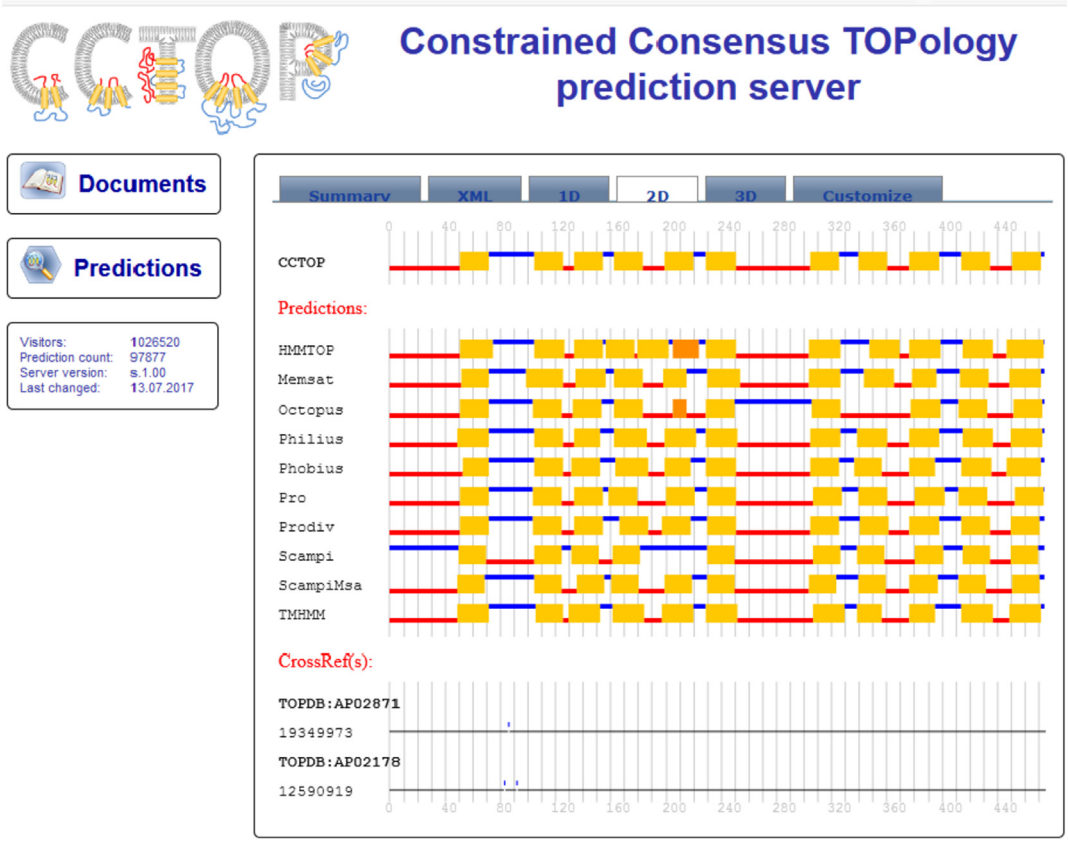

Fig. 6. The layout of the result 2D tab of the CCTOP web server.

active site and the area around of the active site are attainable to large peptide substrates only when the N-terminus is in the non-structured region. However, small molecules may penetrate the ENT3 chamber through the equatorial pores in a mutant form of

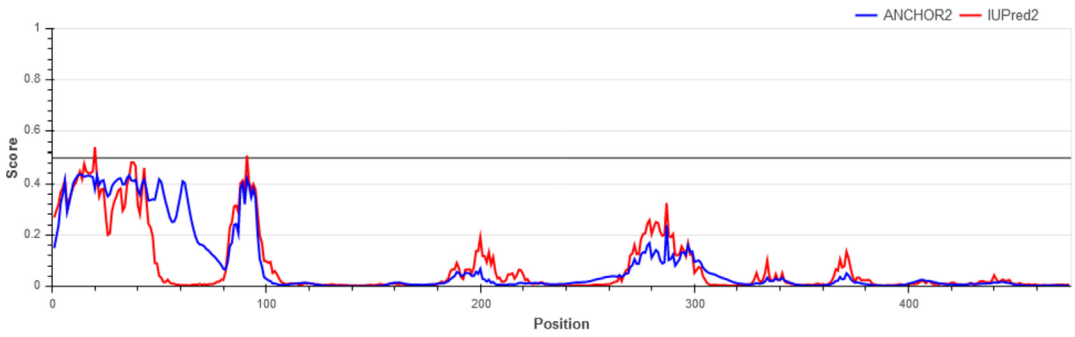

Fig. 7. The ENT3 protein contains limited disordered regions, therefore, has restricted regions as well. The figure demonstrated that both ANCHOR2 prediction (Blue Line) and IUPred prediction (Red Line) are consisting with this fact.

ENT3. Because the helix conformation can be mimicked by the destruction of the N-terminal residues or by the changes of the ENT3 N-terminal residues from the non-structured region of wild-type ENT3 upon interaction with nucleoside and nucleobase in comparison of helix form in mutant ENT3.

These results indicate that the N-terminus residues act as a regulating position to access the active sites in the binding of nucleoside and nucleobase, contributing the substrate to transport across the membrane. As it was shown in Fig. 4- access to the active sites is permitted by changes in this structure.

However, regulation of protein function appears when the binding of an effector modulates the protein's interaction with a ligand at a right site. Indeed, the mutant conformation is maintained by the placement of P.Glycine 437 Arginine and the mutation of this residue displaces constitutively activates ENT3in wild-type form, in comparison of damage N-terminus in mutant form.

It was considered the prospecting that position of the N-terminal is important in maintaining ENT3in active state. Therefore, it is necessary to identify residues that are mandatory for normal interaction. Of particular interest are the remarkable number of basic amino acid residues of the N-terminal that are not involved in the formation of the nucleoside and nucleobase binding places.

It is assumed that the unstructured ENT3 N-terminal region might affect the binding capacity of ENT3. Since the unstructured nature of the N-terminus carries significance, the data was analyzed using IUPred (Fig. 7). As mentioned in the text, there is a limited region of this protein are susceptible to mutation and disease, which are identified using the IUPred software. The ENT3protein contains 51 amino acid residues, hydrophilic N-terminus which includes a dileucine motif characteristic of lysosomal/endosomal targeting sequences [23]. Mutation of hydrophilic N-terminal region dileucine motif to alanine or its truncation caused the protein to be relocated to the cell surface both in Xenopus oocytes and in human cells, making it characterize the latter transportation activity. The unstructured N-terminal domain would be necessary for the binding process [24]. Nevertheless, the fully active protein is essential for its functional purposes and it may inactive in reactions with ligand in mutant structure. Signaling pathways in ENT3 have not yet been reported to the best of our knowledge, but it may be of interest for further studies of the mechanism of action of this protein which is demanding for some diseases (e.g H-syndrome). The starting structure was an engineered structure of *SLC29A3* sequence, as this has not yet been available as a crystal structure. On the other hand, the mutant N-terminus domain association process also suggests the possibility of changes which could be led for nucleoside and nucleobase conjugation and degradation as domain assembly proceeds. Thus, interfering with this modification could make more modified ENT3 extant to amend the efficacy of existing or developing correctors of the folding process in equilibrative nucleoside transporters.

A common mutation can display a crucial role in mitochondrial and lysosomal disorders Protein stability affects the function of a protein and it depends on its native structure [8]. In this survey, although, the overall conformation of the protein doesn't change but it can argue that stability of hENT3 using an experimental metric of the information encoded in a protein sequence and it might impair nucleoside binding. Because [7]; showed that reductions in nucleoside transport activity with all H and PHID syndrome mutants accrued with defection in subcellular localization with hENT3 mutant, impairments in protein stability with the G437R mutation. But, more studies are necessary to further identify the effects of mutant hENT3 nucleoside transport on the pathogenesis of H syndromes.

Around the time of our work [25], hypothesized a link between the mutation in Glu447Lys and the symptoms experienced, ranging from various cardiovascular anomalies to skin-dominant features including hyperpigmentation and hypertrichosis. On the other hand, [26]; considered the response of ENT3 to pH by deleting 36 amino acids from the N-terminal of ENT3. Therefore, ENT3 enable to localize on the cell surface which can cause a sharp decline in the activity of ENT3 in pH ranges above 6.5. They demonstrated that the substitution with the wrong amino acid residue in N- terminal of ENT3, cause a transition from pH-dependent transport to pH independent transport in ENT3. Thus, it is possible lysosomal homeostasis disrupt and on a larger scale propagating molecular pathogenesis of H syndrome. However, linking this computational research to their investigations on the pH sensing mechanisms of ENT3 with the aforementioned case study suggests the loss of pH sensitivity as a fourth possible consequence of N- terminal ENT3 mutations.

The 3-dimensional structure of the protein showed the domains were present in tandem repeats and it could be suggested that their function is slightly affected when they were altered in the site of mutation. Such features appear through the propagation of energetic signals from the binding site to physically distinct regions in the domain. Consequently, the mutated domain has been suggested to exhibit some allosteric features while being relatively subtle and it would be the basis of their fine regulation. These allosteric features have been described invoking both dynamic and structural changes. It can detect using some other experimental methods, including statistical coupling analysis [27], molecular dynamics simulations [28], NMR [29] and double mutant cycles used in synergy with binding kinetics [30].

In this research, we used bioinformatics tools to predict the three-dimensional structure of the wild specimen and the mutated protein sample, and then, by examining the physicochemical characteristics of both samples, to interpret the amino acid substitutions that may have the structure of protein in Iranian patients

with infections It affects the skin and causes a change in the function of protein.

A challenge in structural genomics is the prediction of the function of uncharacterized proteins. When proteins cannot be related to other proteins of known activity, identification of function based on sequence or structural homology is impossible and in such cases, it would be useful to recognize structurally conserved binding sites in connection with the protein's function.

However, the molecular mechanisms of nucleobase transport in mammals are unsolved. nucleobase transport systems have been proposed to occur in various tissues and cells. Human ENT3 (hENT3) is a 475-residue protein, which is unlike ENT1 and ENT2, the nucleoside transport function of ENT3 is maximal at pH 5.5, indicating that it may be localized to acidic intracellular compartments and it has been suggested that ENT3 is localized to lysosomes rather than allocation of the cell surface ENT1 and ENT2 distribution [31].

However, a more recent study has identified putative mitochondria targeting signal at the N-terminus of hENT3 where it regulates the transportation of native nucleosides and nucleoside drugs. The adenosine transport by mitochondria is a physiological concern which can be used in the development of new drugs, especially in cancer therapy [32].

As it is obvious the mitochondria have an essential role in regulating cellular metabolism and cell survival. It is possible that designing new drugs based on the mitochondrial ENT3 activity. This study was designed to characterize ENT3 expression by wild-type and mutant of human cells that can use to describe the effects on mitochondrial bioenergetics its role in apoptosis following ENT3 mutation [33].

ENT3 protein is an equilibrative transporter which is mediated by both efflux and influx of nucleosides through the cell membrane. This protein transports adenine, adenosine and uridine, as well as several nucleoside analog drugs, like antiviral and anticancer agents, including, cordycepin, cladribine, tubercidin, and azidothymidine [31].

hENT function in intracellular membranes, counting lysosomes. Some an anti-cancer drug like Gemcitabine is transported by hENT, which is uniquely selective for adenosine and also transports a variety of organic cation. Therefore, study the structure of ENT3 protein as an equilibrative transporter significantly increases our knowledge regarding the therapeutic role of this group of proteins in cancer therapy.

Although the mechanism of the ENT3 remains uncertain, authors suggest that the study of misfolding of ENT3 should be the first choice when considering treatment of the cutaneous manifestations of the H syndrome as a genetic disorder.

Many questions still need to be answered and future research in this field will result in exciting new discoveries that might impact other areas of biology.

The present study revealed misfolding of hENT3 protein along with 3D protein modeling. This can be valuable in developing effective therapeutic strategies against H-syndrome and thus limiting threats of some drugs in these kinds of diseases.

Finally, our systematic approaches of structure-based analysis for mutations in hENT3 protein can be widely applied to the evaluation of outcomes of mutations in different types of disease for this protein with available structural information.

4. Conclusions

In this study, by analyzing H-syndrome associated mutations in the human ENT3 protein with structural methods, it was demonstrated that mutations have a high tendency to influence the transportation of nucleosides across the membrane in H-syndrome and the improvement of the corresponding disease. Thus, this mutation is more prone to the potential clinical application as an appropriate biomarker in H-syndrome. Additionally, this research provides a noticeable model for consequence evaluation and accurate analysis and of mutations on protein-related diseases.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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