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Protein structure prediction of human connexin 30 and its mutations in hearing system

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ABSTRACT

The connexin genes family codes are the protein subunits of gap junction channels that mediate direct diffusion of ions and metabolites between the cytoplasm of adjacent cells. The role of intercellular communication, particularly between GJB2 and GJB6 (encoding CX26 and CX30) has been confirmed by evidence that certain connexin gene mutations causes sensor neural hearing loss. In this study we characterized 3D model of CX30 proteins in human. The aim of the present study was to carry out the homology modeling study of the mentioned protein using known modeling methods. The model was validated using protein structure validating tools such as RAMPAGE. In addition, mutations of CX30 in hearing system evaluated with mentioned servers. In silico modeling of proteins, Compared with template verified their possible roles and mutation effects in stability of protein structure.

Key words: CX30 protein, 3D-JIGSAWN, Verify-3D, RAMPAGE, Mupro

1. INTRODUCTION

The inner ear contains the hearing organ, the cochlea and the nerve of hearing and also a part for balance. The cochlea is Lilaceous and a bony structure that contains the sensory organ for hearing called the organ of Corti. The organ of Corti releases K+ ions when the vibrations from the stapes activate its hair cells (1). Ultra structural studies have identified two gap junction networks including that being in epithelial tissue and that of the connective tissue in cochlear duct and vestibular system (2, 3). Gap Junctions are acres of connection between two cells where ducts permits direct connection between the adjacent cells. Connexin are the protein subunits of gap junction; hexamericconnexin oligomers are ordered in the plasma membrane as connexin hemi-ducts that dock with associates in neighboring cells to produce a straight intercellular connection pathway (4). Intercellular connection via gap junctions is significant for hearing function. The critical role of intercellular connection, specially that between GJB2 and GJB6 (encoding CX26 and CX30) has been committed by proof that certain connexin gene mutations causes sensorineural hearing loss (1, 5-8). Mutations in GJB6 are associated with hearing loss and skin diseases (9, 10). Non-syndromic deafness is observed in patients carrying mutations in their GJB6coding regions (11). Grifa et al. (7) found a threonine to methionine alter at location 5 (T5M) in an Italian category influenced by bilateral middle /high-frequency audition depletion, difference amino acid conversions in GJB6 make non syndromic audition, depletion is determined in location 40 where alanine changes to valine (12). Therefore, we modeled 3D structure of CX30 and its mutations to elucidate the pathogenic role of CX30 variant in non syndromic hearing loss. The absence of an experimentally determined structure comparative or homology modeling often provides a useful 3D model for a protein that is related to at least one known protein structure. Comparative modeling predicts the 3-D structure of a given protein sequence (target) based primarily on its alignment to one or more proteins of known structure

(templates) (13-15). The GJB6 gene (AJ005585) encodes a 261-amino acid protein. The human CX30 protein shares 93% homology with mouse Cx30 and 76% identity with human CX26 which was used as our template in homology modeling (12).

2. MATERIALS AND METHODS

In this study various bio informatics tools and databases were used for homology modeling including, GenBank-NCBI, PDB (Protein Data Bank) and verify3D, ProSA, SDM and UCSF Chimera. The homology modeling method consists of four main steps: template identification, target template alignment, model building and model evaluation (16). For structure prediction Human CX30 sequence, in FASTA format was obtained from GenBank-NCBI database (NP_001103689).

2.1. Template selection and sequence alignment

BLAST was used in order to detect the related homologues of Human CX30 sequence. Here, By the BLAST searches a sequence with maximum identity and less e-value was selected as our template. Selected sequence related to Chain A of the Structure of Connexin-26 Gap Junction Channel at 3.5 Angstrom Resolution (2ZW3_A). The PDB file of 2ZW3 was downloaded from PDB and the FASTA format sequence was obtain from GenBank-NCBI.

2.2. Comparative Homology Modeling

The protein sequence of Human CX30 in one letter code was pasted in the 3D-Jigsawn (Protein Comparative Modeling Server) for creation of PDB file related to CX30. 3D-Jigsawn sends the PDB file on the e-mail. In addition, a model for CX30 mutation such as natural model was created and result obtained for models were compared with wild models.

2.3. Structure Validation and Evaluation

In order to evaluate the CX30 model, its mutations and the template Verified-3D and RAMPAGE was used and the Ramachandran plots were drawn in order to verify the quality of the modeled PDB file. In this study, the models were checked using the ProSA server in order to estimate the overall quality score (17, 18). PDB file of CX30 model, its mutations and homologous target protein were utilized in order to construct the structural model UCSF Chimera .

2.4. Mutation effect

There is a desire to command and expediently detect practically critical variants, which may be damaging or disease oriented to determine their molecular acts. For this aim, an amount of computational procedures based on amino acid arrangement, type and evolutionary information have been proposed (19, 20). PolyPhen-2 (Polymorphism Phenotyping v2) software via a Web server foretells the probable impact of amino acid substitutions on the solidity and function of human proteins using structural and comparative evolutionary considerations. It then calculates the possibility of the missense mutation being running based on a composition of complete these effects. Mupro is a set of machine learning programs to predict how single-site amino acid mutation affects protein stability. Which its results show that the prediction accuracy using sequence information alone is comparable to its tertiary structures (21). Therefore, even if you do not have protein tertiary structures available, you still can use this server in order to get a rather accurate prediction. Of course, if you provide tertiary structures, this method will take advantage of them and you might get slightly better predictions.

3. RESULTS AND DISCUSSION

In this study, the 3D structure of CX30 protein and its mutations were built by homology modeling based on a PDB file which was obtained from 3D JIGSAW by using UCSF Chimera software. The secondary structure of CX30PDB has 4 α helix and a number of turn (Figure 1). Using the BLAST search, we selected closest homologue to GJB6, which was 2ZW3 (chain A, structure of the connexin-26 gap junction channel with a 3.5 A° resolution) with the highest sequence identity of 77% and less E-value of 5e-133 with 89% similarity. Profile score above zero in the Verify 3D graph corresponds to acceptable environment of the model (22, 23). The high score of 0.59 for CX30 model and 0.56 and 0.51 respectively related to Mut.T5M and Mut.A40V indicates that environment profile of the model is acceptable (Figure 2). Score for template is a 0.56, which most parts in graph are above zero and because protein is a trans-membrane, some point is below zero.

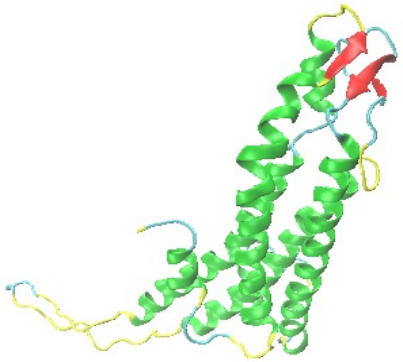

Figure 1.3d Model of connexin 30

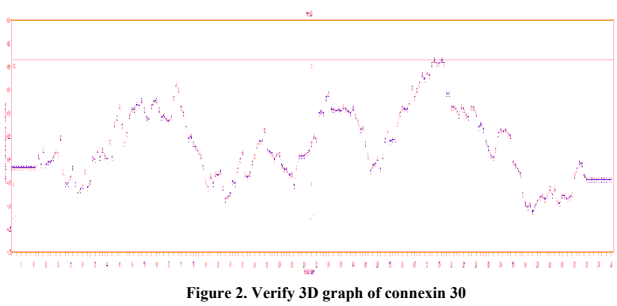

Figure 2. Verify 3D graph of connexin 30

The stereo chemical property of the model and its mutations were evaluated with Ramachandran Plot calculations using the RAMPAGE server. The Ramachandran plot of CX30.pdb protein showed that 81.9% of residues come in the most favored regions, 13.6% residues in allowed region and 4.5 % residues in outlier regions (Table 1, Figure 3a). Non-proline residues, non-glycine residue regions were 98.0% and most disallowed regions were only 2.0% in the plot (Figure 3b). A good quality Ramachandran plot has over 90% in the most favored regions but the Ramachandran plot of CX30.pdb has 81.9% of residues in the most favored regions therefore it is near to a good quality model (Table 1).

Table 1. Result summery of Ramachandran plots

Accession number	Protein	residues in favoured region%	of residues in allowed region%	residues in outlier region%
2ZW3	template	85.2	13.5	1.3
-	GJB6	81.9	13.6	4.5
-	Mut.T5M	79	15.7	5
-	Mut.A40V	81.9	13.6	4.5

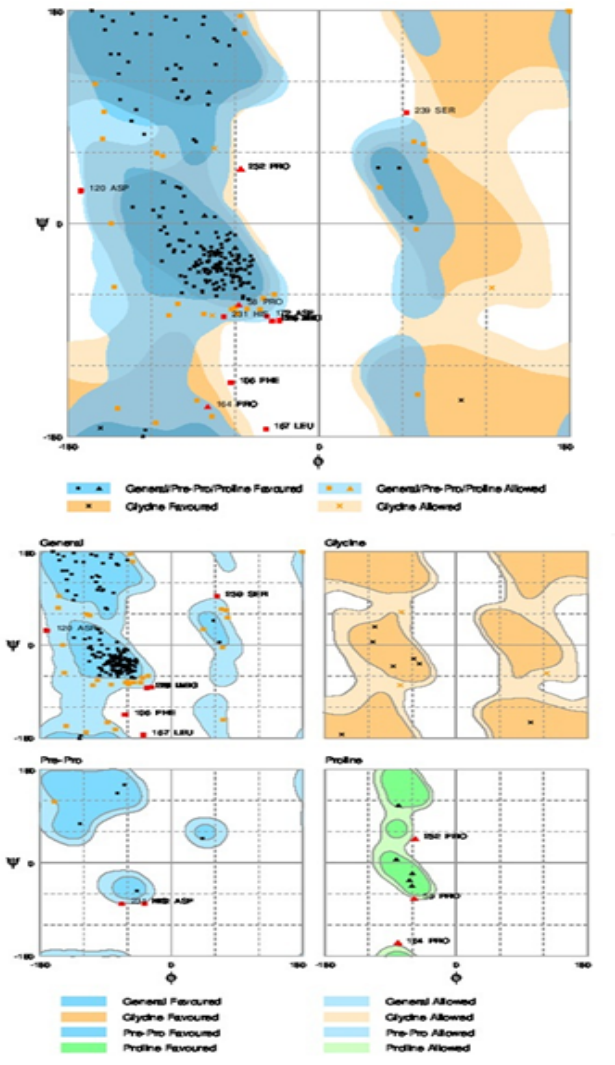

Figure 3.Ramachandran plot of 3D model of CX30 protein (a) and non proline residues and non glycine residue regions (b)

In order to evaluate the overall model quality we used ProSA Web. ProSA, which calculates an overall quality score for a definite input form. If this score is extraneous, a range particular for native proteins the structure possibly comprises of defects. Its value appears in a plot that comprises the z-scores of all analytically assessed protein chains in latest PDB. In this plot, categories of structures from different sources (X-ray, NMR) are distinguished by different colors. Related Z-score of model and template is respectively -2.5 and -3.29 which is in the desired range (Figure 4 a, b).

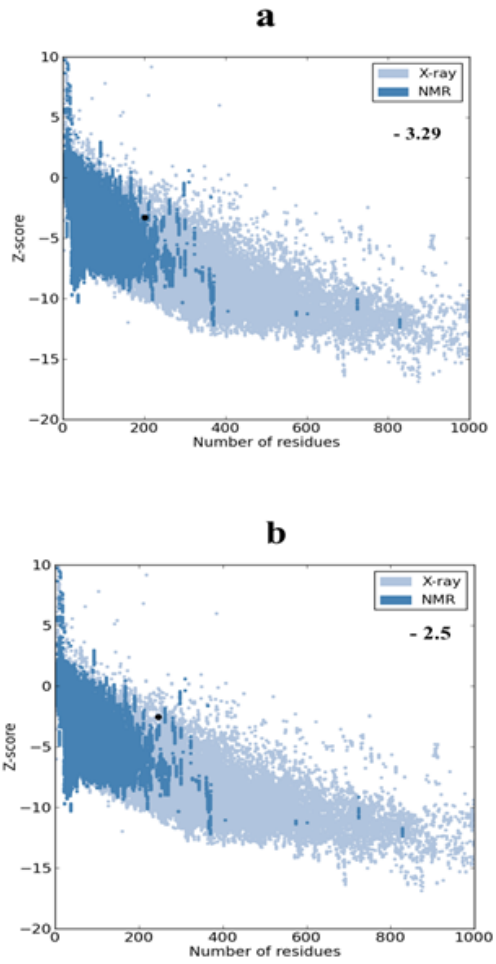

Figure 4.ProSA-web z-scores of all protein chains in PDB determined by X-ray crystallography (light blue) or NMR spectroscopy (dark blue) with respect to their length. The z-scores of template (a) and CX30 are highlighted as large dots.

In order to evaluate the mutation effect in PolyPhen-2, the sequence of protein was used. The results obtained from the analysis server predicted that mutation is probably damaging with a score of 0.999 (sensivity: 0.14, specificity 0.99) for T5M mutation and 1.000 (sensivity: 0.00, specificity 1.00) for A40V mutation. This results for MuPro server was predicted with two methods. For mutation T5M: Method 1: Support Vector Machine, used sequence information only. Effect: DECREASE the stability of protein structure (Confidence Score: -0.54992095). Method 2: Neural Network used sequence information only. Effect: DECREASE the stability of

protein structure (Confidence Score: -0.861146357356107). For mutation A40V: Method 1: Support Vector Machine, used sequence information only. Effect: INCREASE the stability of protein structure (Confidence Score: 1). Method 2: Neural Network, used sequence information only. Effect: INCREASE the stability of protein structure (Confidence Score: 0.6191749859379138). According to the results of this server it was found that in T5M mutation the hydrophilic amino acid changed to hydrophobic and reduced protein stability. However, in A40V mutation because a non-polar amino acid is created without any effect on the protein stability was seen (Figure 5).

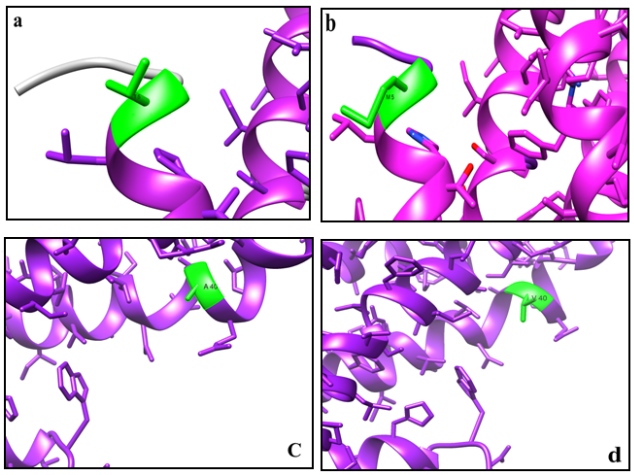

Figure 5. Structure of CX30 and mutation T5M (a, b) and mutation A40V (c, d)

4. CONCLUSION

In conclusion, we characterized 3D model of CX30 proteins in human to carry out the homology modeling study of the mentioned protein using known modeling methods.

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AUTHORS CONTRIBUTION

This work was carried out in collaboration between all authors.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

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