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HOMOLOGY MODELING, SECONDARY STRUCTURE PREDICTION AND EVALUATION OF *NKCC2* PROTEIN RESPONSIBLE FOR BARTTER'S SYNDROME

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Abstract

Bartter's syndrome is a rare autosomal recessive disorder due to the mutation of the sodium potassium chloride cotransporter 2 gene (NKCC2) in the 15th chromosomes of *Homo sapiens* (15q15-15q21.1). This syndrome results in potassium wasting due to defect in reabsorption mechanism in thick ascending limb of loop of Henle (TALH), which leads to ionic imbalance. The structure of the NKCC2 protein coded by the NKCC2 gene is not available in the Protein Data Bank (PDB). The structure of the protein is modeled using the homology modeling tool SWISS MODEL. The secondary structure of the protein is predicted by using TMP red and PHD. The NKCC2 protein model is evaluated by various evaluation tools such as What Check, Prot param, Anolea, Prosa and Errat. The model is refined using energy minimization software's by considering the evaluation results. Hence the structure evaluation of NKCC2 protein may lead to the identification of new targets in developing drugs for Bartter's syndrome.

Keywords: Bartter's syndrome, NKCC2, Homology modeling, Secondary Structure Prediction

1. Introduction

Bartter's syndrome is a renal tubular salt-wasting disorder in which the kidneys cannot reabsorb chloride in the Thick Ascending Limb of loop of Henle (TALH) and the Distal Convoluted Tubule (DCT). The condition is thought to be caused by a defect in the kidney's ability to reabsorb potassium. As a result, an excessive amount of potassium is excreted from the body. This is also known as potassium wasting [1]. Bartter's syndrome is hereditary, which is caused by a recessive gene; thus, a person with the disorder has inherited two recessive genes for the disorder, one from each parent. The abnormal gene causes the kidney to excrete excessive amounts of sodium, chloride and potassium, resulting

in electrolyte abnormalities. The loss of sodium and chloride leads to mild dehydration, which causes the body to produce more renin and aldosterone (hyperaldosteronism). The increase in aldosterone increases potassium and acid secretion in the kidneys, leading to Hypokalemia and metabolic alkalosis. Bartter's syndrome can also be divided into different subtypes based on the genes involved. Bartter's syndrome type-I is caused by mutations of sodium-potassium-chloride co transporter 2 (NKCC2) genes [2] located in the chromosome 15 of human (15q15-15q21.1) encoding NKCC2 proteins that transport ions across renal cells. Characterization of Bartter's syndrome is given [3].

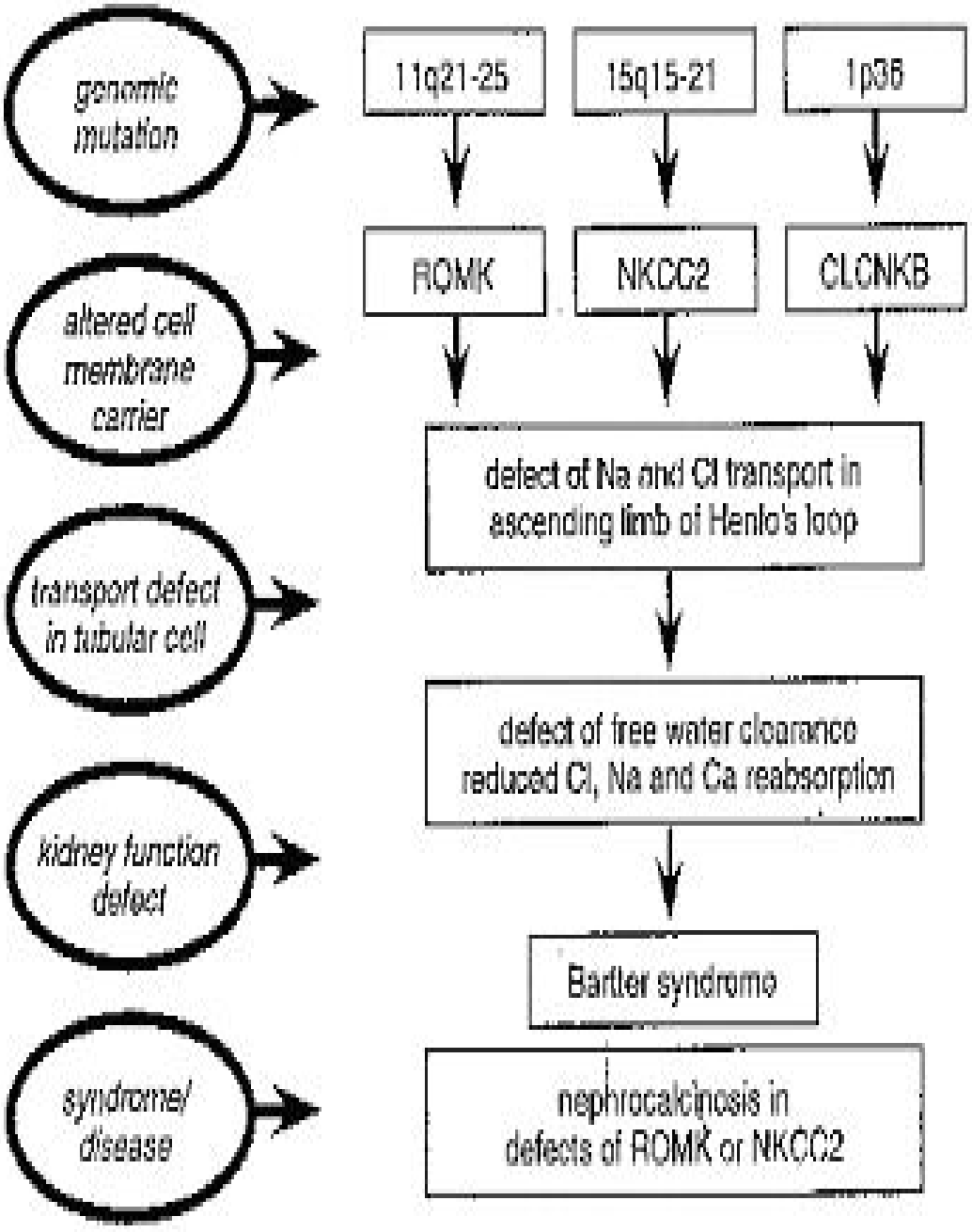


Figure 1-Characterization of Bartter's syndrome.

The Na-K-Cl co transporters are a family of integral membrane proteins that mediate the coupled transport of Na+, K+, and Cl- across the plasma membrane. All NKCCs described to date are characterized by a strict dependence of transport on the three ions, Na, K, and Cl (Forbush *et al.,* 1983). In most tissues, the binding stoichiometry is 1Na: 1K: 2Cl per transporter and, therefore, the transport process is not associated with net movement of charge (Haas, 1989). Mutation of the NKCC2, located on chromosome 15, leads to reduced Na^+ and Cl^- reabsorption in the TAL, with subsequent salt wasting and hypokalemia. Protein structure prediction is the prediction of the three-dimensional structure of a protein from its amino acid sequence that is, the prediction of a protein's tertiary structure from its primary structure. It is one of the most important goals pursued by bioinformatics and theoretical chemistry. Despite community-wide efforts in structural genomics, the output of experimentally determined protein structures typically by time-consuming and relatively expensive X-ray crystallography or NMR spectroscopy is lagging far behind the output of protein sequences. Due to advancement in computational approaches much progress is being made to overcome these problems. The structure evaluation of the protein lead to a better understanding of the function of the protein and act as an excellent target for designing specific drugs.

2. Materials and Methods

Tools and softwares used

2.1 ProtParam

ProtParam is a tool, which allows the computation of various physical, and chemical parameters for a given protein stored in Swiss-Prot or TrEMBL or for a user entered sequence.

2.2 Blast Similarity Search

BLAST stands for Basic Local Alignment Search Tool. It is used to compare a novel sequence with those contained in nucleotide and protein databases by aligning the novel sequence with previously characterized genes. The emphasis of this tool is to find regions of sequence similarity.

2.3 Clustalw Multiple Sequence Alignment

ClustalW is a fully automatic, general purpose global multiple sequence alignment program for DNA or proteins. It produces biologically meaningful multiple sequence alignments of divergent sequences. It calculates the best match for the selected sequences, and lines them up so that the identities, similarities and differences can be seen.

2.4 RCSB PDB

The Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) provides a variety of tools and resources for studying the structures of biological macromolecules and their relationships to sequence, function, and disease. The RCSB is a member of the world wide PDB (wwPDB) whose mission is to ensure that the PDB archive remains an international resource with uniform data. This site offers tools for browsing, searching, and reporting that utilize the data resulting from ongoing efforts to create a more consistent and comprehensive archive.

2.5 SWISS-MODEL

SWISS-MODEL is a fully automated protein structure homology-modeling server, accessible via the Expasy web server, or from the program Deep View (Swiss Pdb-Viewer). The purpose of this server is to make Protein Modeling accessible to all biochemists and molecular biologists World Wide.

2.6 DEEPVIEW - SWISS-PDB VIEWER

Deep View - Swiss-PdbViewer is an application that provides a user-friendly interface allowing analyzing several proteins at the same time. Working with these programs greatly reduces the amount of work necessary to generate models, as it is possible to thread a protein primary sequence onto a 3D template and get an immediate feedback of how well the threaded protein will be accepted by the reference structure before submitting a request to build missing loops and refine side chain packing. Swiss-PdbViewer can also read electron density maps, and provides various tools to build into the density. In addition, various modeling tools are integrated and command files for popular energy minimization packages can be generated. In a polypeptide chain, which consists of N-C and C –C bonds are relatively free to rotate. These rotations are represented by the torsion angles Phi and Psi respectively. The Phi/Psi plot (Ramachandran plot) was the first serious verification tool available for protein structures. There are three regions in the Ramachandran plot namely the allowed region, the disallowed region and the core region. The occurrence of the amino acids in the core region is mostly accepted, while in the allowed region is also accepted but in the disallowed region is not accepted.It has proven to be very difficult to improve the appearance of a Ramachandran plot while refining a structure. Therefore it is even more than 30 years after its design still useful as a posteriory quality check for a protein.

2.7 WHAT IF

WHAT IF is a versatile molecular modeling package that is specialized on working with proteins and the molecules in their environment like water, ligands, nucleic acids, etc.

2.8 ERRAT

Errat is a protein structure verification algorithm that is especially well-suited for evaluating the progress of crystallographic model building and refinement. The program works by analyzing the statistics of non-bonded interactions between different atom types. A single output plot is produced that gives the value of the error function vs. position of a 9-residue sliding window. By comparision with statistics from highly refined structures, the error values have been calibrated to give confidence limits. This is extremely useful in making decisions about reliability.

2.9 Pro SA

ProSA-web provides an easy-to-use interface to the program ProSA which is frequently employed in protein structure validation. ProSA calculates an overall quality score for a specific input structure. If this score is outside a range characteristic for native proteins the structure probably contains errors. A plot of local quality scores points to problematic parts of the model which are also highlighted in a 3D molecule viewer to facilitate their detection. The z-score indicates overall model quality. Its value is displayed in a plot that contains the z-scores of all experimentally determined protein chains in current PDB In this plot, groups of structures from different sources (X-ray, NMR) are distinguished by different colors. It can be used to check whether the z-score of the input structure is within the range of scores typically found for native proteins of similar size.

2.10 TM pred

The TMpred program makes a prediction of membrane-spanning regions and their orientation. The algorithm is based on the statistical analysis of TMbase, a database of naturally occuring transmembrane proteins. The prediction is made using a combination of several weight-matrices for scoring.

2.11 ANOLEA

ANOLEA (Atomic Non-Local Environment Assessment) is a server at the SWISS-MODEL server, provided by the Biozentrum , University of Basel, and the Swiss Institute of Bioinformatics that performs energy calculations on a

protein chain, evaluating the "Non- Local Environment" (NLE) of each heavy atom in the molecule. The energy of each pairwise interaction in this non-local environment is taken from a distance-dependent knowledge based mean force potential that has been derived from a database of 147 non-redundant protein chains with a sequence identity below 25% and solved by X-Ray crystallography with a resolution lower than 3 Å .

3. Results and Discussion

The NKCC2 protein present in the apical membrane of thick ascending limb of loop of Henle (TALH) is responsible for Bartter's syndrome. The structure of the NKCC2 protein is not available in the Protein Data Bank. The sequence of NKCC2 protein is obtained from NCBI Protein database.

Results of Protparam

Number of amino acids: 1099

Molecular weight: 121450.2

Theoretical pI: 7.18

Amino acid composition: CSV format

Ala	(A)	93	8.5%
Arg	(R)	43	3.9%
Asn	(N)	62	5.6%
Asp	(D)	45	4.1%
Cys	(C)	22	2.0%
Gln	(Q)	30	2.7%
Glu	(E)	63	5.7%
Gly	(G)	83	7.6%
His	(H)	16	1.5%
Ile	(I)	89	8.1%
Leu	(L)	100	9.1%
Lys	(K)	65	5.9%
Met	(M)	26	2.4%
Phe	(F)	60	5.5%
Pro	(P)	38	3.5%
Ser	(S)	76	6.9%
Thr	(T)	57	5.2%
Trp	(W)	17	1.5%
Tyr	(Y)	34	3.1%
Val	(V)	80	7.3%
Pyl	(O)	0	0.0%
Sec	(U)	0	0.0%

Total number of negatively charged residues (Asp + Glu): 108
Total number of positively charged residues (Arg + Lys): 108
Atomic composition:

Carbon	C	5483
Hydrogen	H	8595
Nitrogen	N	1435
Oxygen	O	1574
Sulfur	S	48

Formula: $C_{5483}H_{8595}N_{1435}O_{1574}S_{48}$

Total number of atoms: 17135
Extinction coefficients:
Extinction coefficients are in units of M^{-1} cm^{-1}, at 280 nm measured in water.
Ext. coefficient 145535 Abs 0.1% (=1 g/l) 1.199, assuming ALL Cys residues appear as half cysteine
Ext. coefficient 144160 Abs 0.1% (=1 g/l) 1.188, assuming NO Cys residues appear as half cysteine
Instability index:
The instability index (II) is computed to be 32.58
This classifies the protein as stable.
Aliphatic index: 96.38
Grand average of hydropathicity (GRAVY): 0.091

CLUSTALW Result

The retrieved sequence is submitted in BLASTP for template identification. Several similar sequences were found and those sequences with the query sequence are submitted in ClustalW Multiple Sequence Alignment. The D181a Variant Of Catalase Hpii of E.coli is having highest alignment score (alignment score value-42, BLAST score-28.5, E-value-9.2) with that of the query sequence and it is taken as the template sequence (PDB ID – 1P7Y/A).

CLUSTAL W (1.83) Multiple Sequence Alignments

Sequence type explicitly set to Protein
Sequence format is Pearson
Sequence 1: query| 1099 aa
Sequence 2: template|pdb|1P7Y| 753 aa
Start of Pairwise alignments

```
CLUSTAL 2.1 multiple sequence alignment


gi|134254459|ref|NP_000329.2|      MSLNNSSNVFLDSVPSNTNRFQVSVINENHESSAAADDNTDPPHYEETSF 50
gi|34809674|pdb|1P7Y|D             MSQHNEK-------------------NPHQHQSPLHDSSEAKPGMDSLAP 31
                                   ** :*..                  * ::..*.  *..   *  :. :

gi|134254459|ref|NP_000329.2|      GDEAQKRLRISFRPGNQECYDNFLQSGETAKTDASFHAYDSHTNTYYLQT 100
gi|34809674|pdb|1P7Y|D             EDGSHRPAAEPTPPGAQPTAPGSLKAPDTRNEKLNSLEDVRKGSENYALT 81
                                    * :::    .  ** *    . *:: :* : . .      : .  *  *

gi|134254459|ref|NP_000329.2|      FGHNTMDAVPKIEYYRNTGSISGPKVNRPSLLEIHEQLAKNVAVTPSSAD 150
gi|34809674|pdb|1P7Y|D             TNQGVRIADDQNSLRAGS---RGPTLLEDFILREKITHFDHERIP----E 124
                                    .:..  *  : .   .:    **.: .  :*. :    .:  :.    :

gi|134254459|ref|NP_000329.2|      RVANGDGIPGDEQAENKEDDQAGVVKFGWVKGVLVRCMLNIWGVMLFIRL 200
gi|34809674|pdb|1P7Y|D             RIVHARGSAAHGYFQPYKS-LSDITKADFLSDPNK-------ITPVFVRF 166
                                   *:.:. * ...   :  :.  :.:.* .::..           . :*:*:

gi|134254459|ref|NP_000329.2|      SWIVGEAGIGLGVLIILLSTMVTSITGLSTSAIATNGFVRGGGAYYLISR 250
gi|34809674|pdb|1P7Y|D             STVQGGAGS---------ADTVRAIRGFATKFYTEEGIF----------- 196
                                   * : * **          :  * :* *::*.  : :*:.

gi|134254459|ref|NP_000329.2|      SLGPEFGGSIGLIFAFANAVAVAMYVVGFAETVVDLLKESDSMMVDPTND 300
gi|34809674|pdb|1P7Y|D             ----DLVGNNTPIFFIQDAHKFPDFVHAVKPEPHWAIPQGQSAHDTFWDY 242
                                       :: *.   ** : :*  .. :* ..       : :.:*      :

gi|134254459|ref|NP_000329.2|      IRIIGSITVVILLGISVAGMEWEAKAQVILLVILLIAIANFFIGTVIPSN 350
gi|34809674|pdb|1P7Y|D             VSLQPETLHNVMWAMSDRGIPRSYRTMEGFGIHTFRLIN----------- 281
                                   : :  .    :: .:*  *:  . ::   : :  :  *

gi|134254459|ref|NP_000329.2|      NEKKSRGFFNYQASIFAENFGPRFTKGEGFFSVFAIFFPAATGILAGANI 400
gi|34809674|pdb|1P7Y|D             ----------------------AEGKATFVRFHWKPLAGKASLVWDEA 307
                                                        ::*:. *  *     *... *.  :

gi|134254459|ref|NP_000329.2|      SGDLEDPQDAIPRGTMLAIFITTVAYLGVAICVGACVVRDATGNMNDTII 450
gi|34809674|pdb|1P7Y|D             QKLTGRDPDFHRR-----------------------------------ELW 323
                                   .       *   *                                     :
```

```
gi|134254459|ref|NP_000329.2|     SGMNCNGSAACGLGYDFSRCRHEPCQYGLMNNFQVMSMVSGFGPLITAGI 500
gi|34809674|pdb|1P7Y|D            EAIEAGDFPEYELGFQLIPEEDE-----FKFDFDLLDPTK-----LIPEE 363
                                  ..::... .   **:::   ..*     :  :*:::. ..     : .

gi|134254459|ref|NP_000329.2|     FSATLSSALASLVSAPKVFQALCKDNIYKALQFFAKGYGKNNEPLRGYIL 550
gi|34809674|pdb|1P7Y|D            LVPVQRVGKMVLNRNPDNFFAENEQAAFHPGHIVPGLDFTNDPLLQGRLF 413
                                  : ..   .   *   *. * *  ::  ::. ::..    .*:  *:* ::

gi|134254459|ref|NP_000329.2|     TFLIAMAFILIAELNTIAPIISNFFLASYALINFSCFHASYAKSPGWRPA 600
gi|34809674|pdb|1P7Y|D            SYTDTQISRLGGPN----------------------FHEIPINRP----- 436
                                  ::  :    * .                         **    : *

gi|134254459|ref|NP_000329.2|     YGIYNMWVSLFGAVLCCAVMFVINWWAAVITYVIEFFLYVYVTCKKPDVN 650
gi|34809674|pdb|1P7Y|D            --------------------------------------------------

gi|134254459|ref|NP_000329.2|     WGSSTQALSYVSALDNALELTTVEDHVKNFRPQCIVLTGGPMTRPALLDI 700
gi|34809674|pdb|1P7Y|D            ------TCPYHNFQRDGMHRMGIDTNPANYEPNSINDNWPRETPP----- 475
                                        : .* .   :.:.   :: :  *:.*:.*  .    * *

gi|134254459|ref|NP_000329.2|     THAFTKNSGLCICCEVFVGPRKLCVKEMNSGMAKKQAWLIKNKIKAFYAA 750
gi|34809674|pdb|1P7Y|D            ------------------GPK------------------RGGFESYQER 488
                                                    **:                   :. ::::

gi|134254459|ref|NP_000329.2|     VAADCFRDGVRSLLQASGLGRMKPNTLVIGYKKNWRKAPLTEIENYVGII 800
gi|34809674|pdb|1P7Y|D            VEGNKVRE------RSPSFG---------EYYSHPRLFWLSQTPFEQRHI 523
                                  * .: .*:      ::..:*          * .: *   *::       *

gi|134254459|ref|NP_000329.2|     HDAFDFEIGVVIVRISQGFDISQVLQVQEELERLEQERLALEATIKDNEC 850
gi|34809674|pdb|1P7Y|D            VDGFSFELSKVVRPYIRERVVDQLAHIDLTLAQAVAKNLGIELT------ 567
                                   *.*.**:. *:    :   :.*: :::  * :   :.*.:* *

gi|134254459|ref|NP_000329.2|     EEESGGIRGLFKKAGKLNITKTTPKKDGSINTSQSMHVGEFNQKLVEAST 900
gi|34809674|pdb|1P7Y|D            -------------------------DDQLNITPPPDVNGLKKDPSLSLY 591
                                                          *..:* : . .*. :::.   :

gi|134254459|ref|NP_000329.2|     QFKKKQEKGTIDVWWLFDDGGLTLLIPYILTLRKKWKDCKLRIYVGGKIN 950
gi|34809674|pdb|1P7Y|D            AIPDGDVKGRVVAILLNDEVRSADLLAILKALKAKGVHAKLLYSRMGEVT 641
                                   : . : ** : .  * *:   : *:. : :*: *  ..**     *::.

gi|134254459|ref|NP_000329.2|     RIEEEKIVMASLLSKFRIKFADIHIIGDINIRPNKESWKVFEEMIEPYRL 1000
gi|34809674|pdb|1P7Y|D            ADDGTVLPIAATFAGAPSLTVDAVIVPCGNIADIADNGDANYYLMEAYKH 691
                                    :   : :*: ::     .*  *:   **    :. ..   ::*.*:

gi|134254459|ref|NP_000329.2|     HESCKDLTTAEKLKRETPWKITDAELEAVKEKSYRQVRLNELLQEHSRAA 1050
gi|34809674|pdb|1P7Y|D            LKPIALAGDARKFK--ATIKIADQGEEGIVEADS--------------- 723
                                   :.     *.*:*  :. **:*   *.: * .

gi|134254459|ref|NP_000329.2|     NLIVLSLPVARKGSISDLLYMAWLEILTKNLPPVLLVRGNHKNVLTFYS 1099
gi|34809674|pdb|1P7Y|D            ---------ADGSFMDELLTLMAAHRVWSRIPKIDKIPA---------- 753
                                          *  . :.:** :   . : ..:* :  : .
```

Sequences (1:2) Aligned. Score: 10.757
Sequences (2:2) Aligned. Score: 100
Sequence: 2 Score: 5443
Alignment Score 42

HOMOLOGY MODELING OF NKCC2 PROTEIN

The query sequence (NKCC2 protein) and the template sequence (The D181a variant of Catalase Hpii from E.coli) are submitted in the SWISS MODEL and the model obtained is viewed with the help of Swiss PDB Viewer.

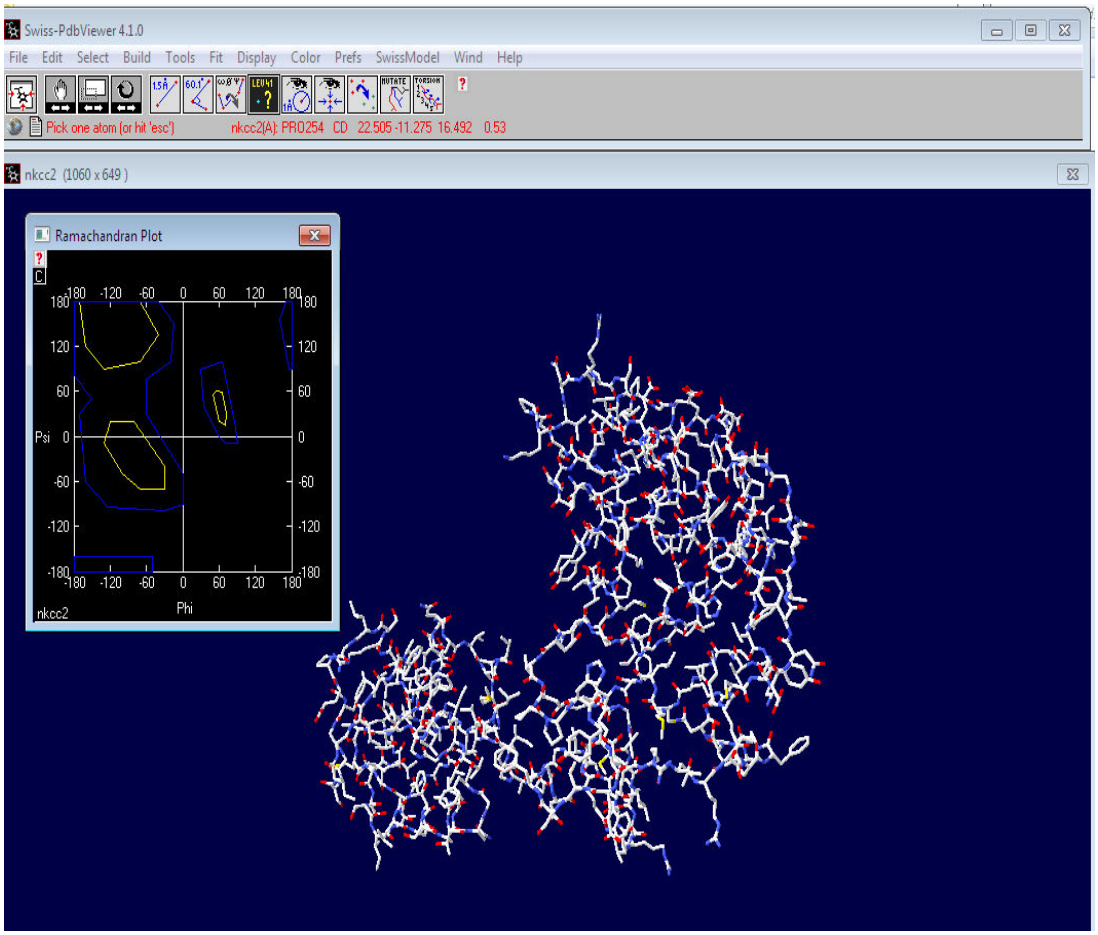


Figure 2-Modeled Structure of Nkcc2 Protein.

WHAT CHECK REPORT

Structure Z-scores, positive is better than average:

1st generation packing quality	:	-7.384
2nd generation packing quality	:	-9.515
Ramachandran plot appearance	:	-1.644
chi-1/chi-2 rotamer normality	:	1.753
Backbone conformation	:	-2.929

RMS Z-scores, should be close to 1.0:

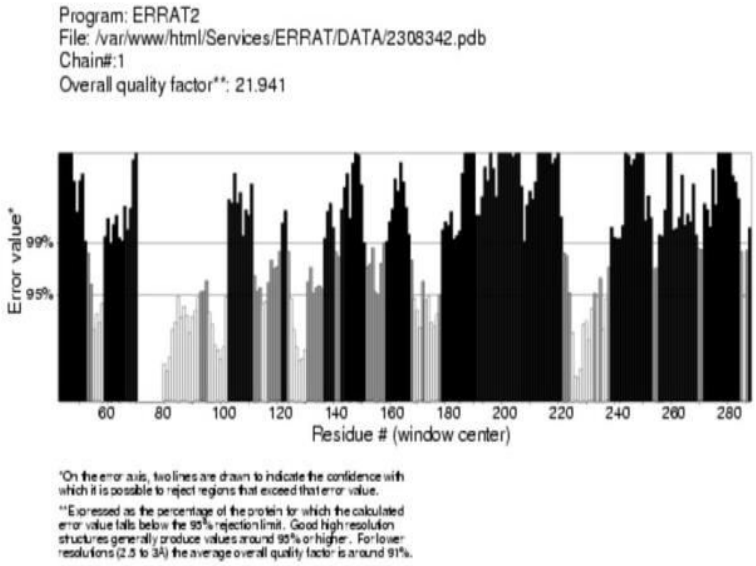
Bond lengths	:	0.691
Bond angles	:	1.070
Omega angle restraints	:	1.034

Side chain planarity : 1.078

Improper dihedral distribution : 1.388

Inside/Outside distribution : 1.255 (unusual)

ERRAT RESULTS



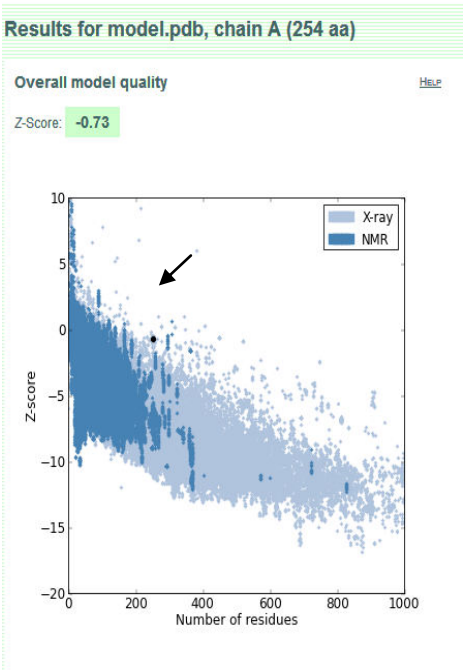

```
21.292  -35.107 -4.386  84.941  24.607  71.138
WARNING: Frame  71      Below Minimum Interaction Limit.
WARNING: Frame  72      Below Minimum Interaction Limit.
WARNING: Frame  73      Below Minimum Interaction Limit.
WARNING: Frame  74      Below Minimum Interaction Limit.
WARNING: Frame  75      Below Minimum Interaction Limit.
WARNING: Frame  76      Below Minimum Interaction Limit.
WARNING: Frame  77      Below Minimum Interaction Limit.
WARNING: Frame  78      Below Minimum Interaction Limit.
WARNING: Frame  79      Below Minimum Interaction Limit.
chain 1:    Residue range 43 to 288
```

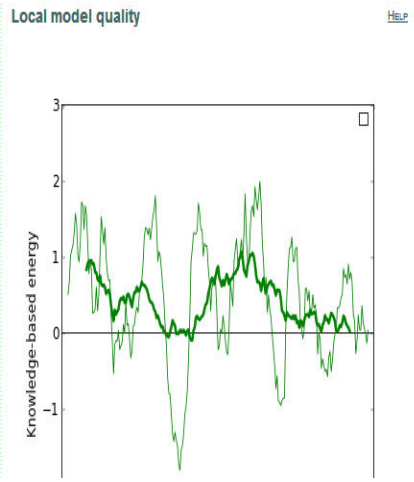
Figure 3-ERRAT showing the overall quality factor of chain 1.

ProSA Result

Figure 4

(a) **(b)**

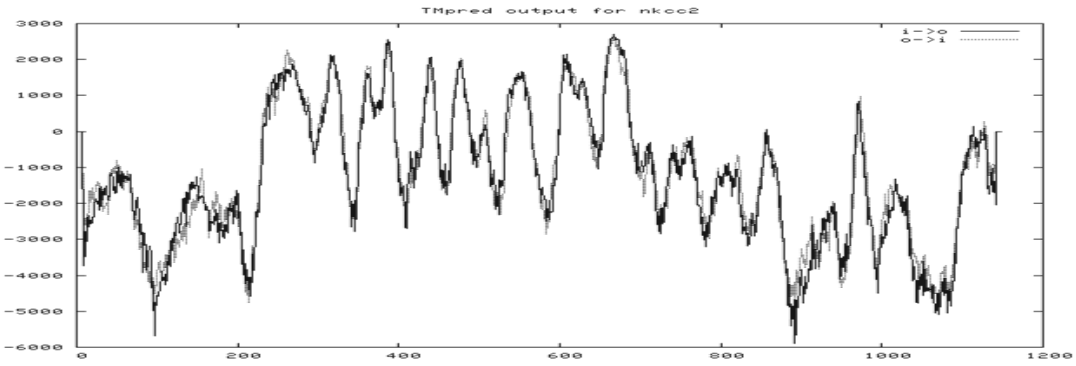




TMPred Result

Figure 5

(a)



(b)

1.) Possible transmembrane helices

The sequence positions in brackets denominate the core region.
Only scores above 500 are considered significant.

Inside to outside helices : 15 found

from	to	score	center
258 (258)	279 (277)	1875	269
307 (309)	327 (325)	2128	317
354 (356)	371 (371)	1624	362
379 (379)	395 (395)	2547	387
430 (433)	449 (449)	2077	441
467 (469)	489 (485)	1984	477
500 (500)	518 (518)	174	509
539 (546)	564 (564)	1661	554
598 (596)	618 (618)	2147	609
618 (621)	638 (638)	1467	629
657 (657)	685 (677)	2681	668
847 (849)	870 (865)	47	857
962 (964)	981 (981)	842	972
1100 (1106)	1129 (1122)	145	1114
1113 (1119)	1138 (1136)	96	1128

Outside to inside helices : 13 found

from	to	score	center
253 (253)	274 (274)	2252	263
307 (309)	326 (326)	2135	317
352 (352)	369 (369)	1825	361
379 (379)	395 (395)	2407	387
430 (430)	449 (449)	1912	441
470 (470)	489 (489)	2055	479
499 (499)	519 (519)	585	509
542 (546)	566 (564)	1637	556
598 (603)	623 (620)	1875	612
657 (659)	676 (676)	2706	667
847 (847)	870 (866)	50	857
964 (964)	983 (983)	965	974
1114 (1119)	1138 (1138)	298	1128

(c)

2.) Table of correspondences

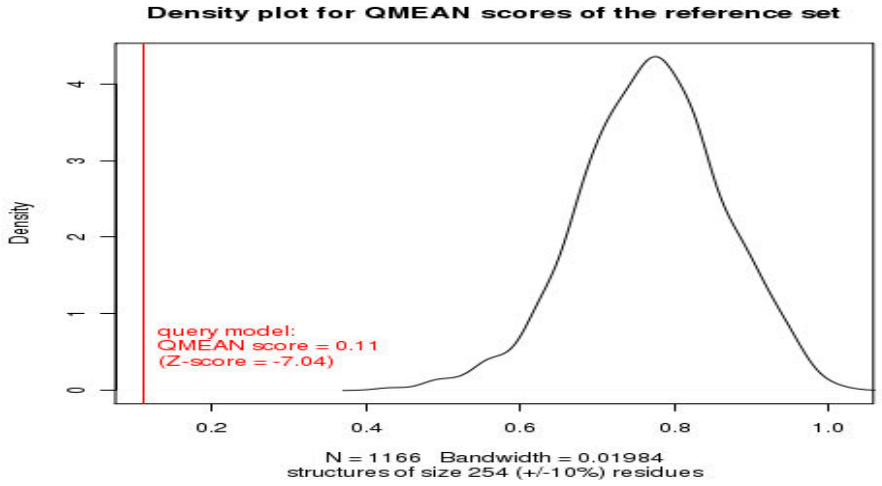
Here is shown, which of the inside->outside helices correspond to which of the outside->inside helices.

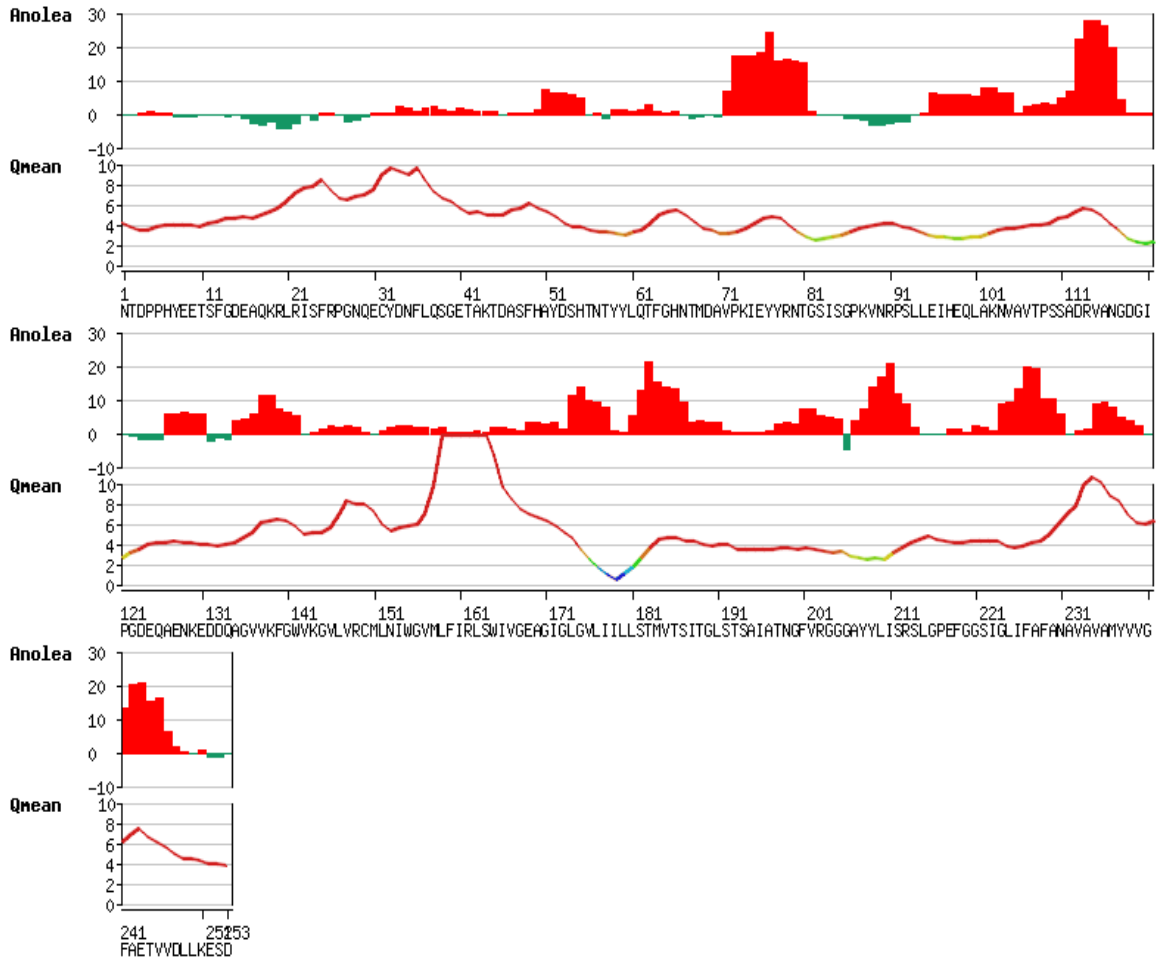
Helices shown in brackets are considered insignificant.
A "+"-symbol indicates a preference of this orientation.
A "++"-symbol indicates a strong preference of this orientation.

inside->outside		outside->inside
258- 279 (22) 1875	|	253- 274 (22) 2252 ++
307- 327 (21) 2128	|	307- 326 (20) 2135
354- 371 (18) 1624	|	352- 369 (18) 1825 ++
379- 395 (17) 2547 +	|	379- 395 (17) 2407
430- 449 (20) 2077 +	|	430- 449 (20) 1912
467- 489 (23) 1984	|	470- 489 (20) 2055
(500- 518 (19) 174)	|	499- 519 (21) 585 ++
539- 564 (26) 1661	|	542- 566 (25) 1637
598- 618 (21) 2147 ++	|	598- 623 (26) 1875
618- 638 (21) 1467 ++	|	
657- 685 (29) 2681	|	657- 676 (20) 2706

ANOLEA result

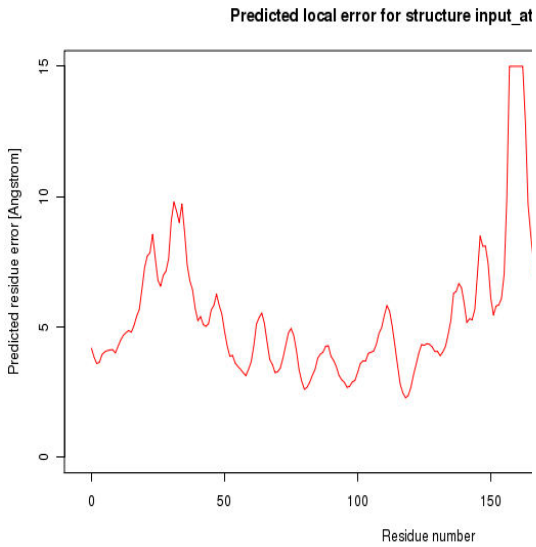
Figure 6 (a)



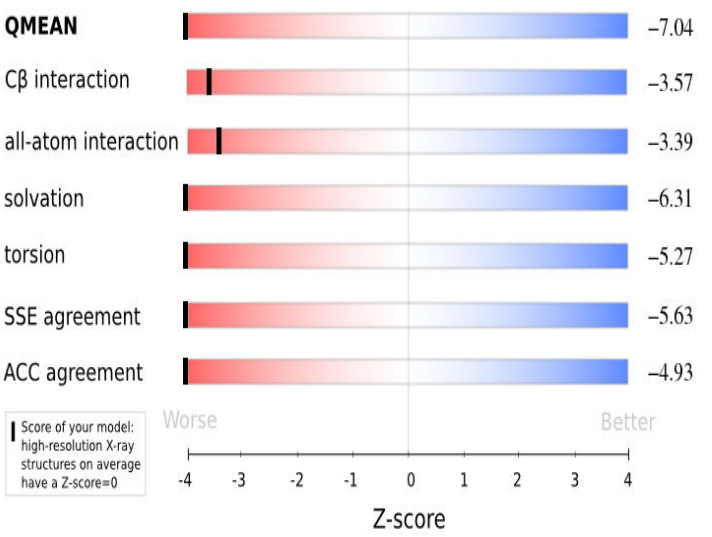

Local Scores: [+/-]
Anolea
Qmean
NTDPPHYEETSFGDEAQKRLRISFRPGNQECYDNFLQSGETAKTDASFHAYDSHTNTYYLQTFGHNTMDAVPKIEYYRNTGSISGPKVNRPSLLEIHEQLAKNWAVTPSSADRVANGDGI
PGDEQAENKEDDQAGVVKFGWVKGVLVRCMLNIWGVMLFIRLSWIVGEAGIGLGVLIILLSTMVTSITGLSTSAIATNGFVRGGGAYYLISRSLGPEFGGSIGLIFAFANAVAVAMYVVG
FAETVVDLLKESD

(b)

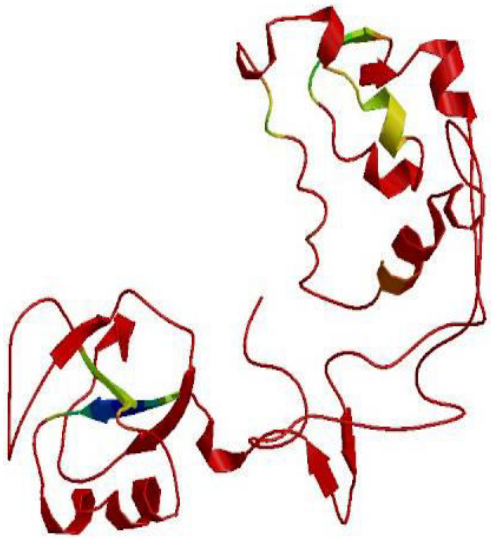
(c)


Predicted local error for structure input_at
Predicted residue error [Angstrom]
Residue number

(d)



(e)



The molecular weight of the protein and its pI value are found to be 121450.2 and 7.18 respectively. The instability index value of 32.58 implies that the model of the protein is good. The Grand Average of Hydropathicity (GRAVY) reveals that the protein is slightly hydrophobic. A reliable three dimensional model of NKCC2 protein is created using SWISS Model and visualized using SWISS PDB Viewer in which Ramachandran plot is shown (fig.2). The positive Z scores obtained from What Check report revealed that the model of NKCC2 protein is good. The NKCC2 protein structure is verified by ERRAT 2 where the overall quality factor is 21.941 (fig.3) that shows the model to be refined further. ProSA Z-score is -0.73 and dark blue dot (fig.4a) shows that the groups of the input structure is within the range of scores typically found for native proteins of similar size. ERRAT results revealed 15 inside to outside helices and 13 outside to inside helices (fig.5b) and table of correspondence (fig.5c) shows the strong preferential helices. Annolea plot shows the model plot with error determination (fig 6a) where the red portions of the plot implies error. Fig 6b shows a Qmean value of 0.11 and Z-score of -7.04.predicted local error plot is shown in fig 6c and fig 6d presents the Z-scores for all the parameters like Cβ interaction, solvation, torsion.the 3D image of the protein is shown in fig 6e.

4. Conclusion

The 3D and secondary structures of NKCC2 protein responsible for Bartter's syndrome is modeled and evaluated. The evaluated results showed that the model is good and can be used as a target for drug interaction. Since the structure of

NKCC2 protein is not available in the PDB the model can be uploaded with confirmed findings using crystallographic and diffraction studies.

5. References

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