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INSILICO ANALYSIS AND HOMOLOGY MODELLING OF TBC1D3 PROTEIN

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Abstract: Type 2 diabetes is the most common form of diabetes. In type 2 diabetes body does not use insulin properly. TBC1D3 protein is able to maintain the insulin pathway open so that the cells still take up glucose. In the current study structure analysis of TBC1D3 protein was done by using protparam, GOR IV tools. This gives the primary & secondary structure analysis of the protein. The structure was modelled with Swiss prot sever and validate with PSVS.

Keywords: Diabetes2, Homology modelling, structure prediction, Insilico analysis

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INTRODUCTION

Our body gets energy by making glucose from foods like bread, potatoes, rice, pasta, milk and fruit. To use this glucose, our body needs insulin. Insulin is a hormone that helps our body controls the level of glucose (sugar) in our blood. Insulin is a small protein with a molecular weight of about 6000 Daltons. It is synthesized in significant Quantities only in beta cells of the Pancreas.Diabetes is a disease in which blood glucose levels are above normal. People with diabetes have problems converting food into energy [1]. Diabetes is the most common metabolic disorder associated with many complications. The condition develops due to abnormalities in carbohydrate metabolism and insulin synthesis. Diabetes is also a genetically inherited disorder. Type 2 diabetes is a disease in which our pancreas does not produce enough insulin, or our body does not properly use the insulin it makes. If we have type 2 diabetes, glucose builds up in our blood instead of being used for energy [2]. The research showed that the protein, called TBC1D3, is able to maintain the insulin pathway open so that the cells still take up glucose (The research is published online Feb 13 2013 in PLoS One). Some study also show that TBC1D3 expression substantially delayed ubiquitination and degradation of insulin receptor substrate-1 (IRS-1). This effect is achieved through suppression of serine phosphorylation at S636/639, S307 and S312 of IRS-1, which are key phosphorylation sites required for IRS-1 degradation[3]. The expression of double palmitoylation mutant TBC1D3:C318/325S resulted in protein mislocalization and enhanced growth factors-induced TBC1D3 degradation. Moreover, ubiquitination of TBC1D3 via CUL7 E3 ligase complex was increased by mutating the palmitoylation sites, suggesting that depalmitoylation of TBC1D3 makes the protein more available for ubiquitination and degradation [4].

A .Structure Prediction

Protein structure prediction is the prediction of the three-dimensional structure of a protein from its amino acid sequence. Genome sequencing projects are producing linear amino acid sequences, but full understanding of the biological role of these proteins will require knowledge of their structure and function [5]. Computational structure prediction methods will provide valuable information for the large fraction of sequences whose structures will not be determined experimentally.

B .Homology modelling

Homology modelling is a computational technique, within structural biology, to determine the 3d structure of proteins [6]. As the name suggests homology modelling predicts structure based on sequence homology with known structure. The structure of a protein is uniquely determined by its amino acid sequence [7]. In practice, homology modelling is a multistep process that can be summarized in seven steps:

1. Template recognition and initial alignment

2. Alignment correction

3. Backbone generation

4. Loop modelling

5. Side-chain modelling

6. Model optimization

7. Model validation

MATERIALS AND METHODS

A. Primary structure prediction

For physio-chemical characterization, theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction coefficient , instability index, aliphatic index and grand average hydropathy (GRAVY) were computed using the Expasy ProtParam server (http://expasy.org/cgi bin/protpraram).

B. Secondary structure prediction

Secondary structure of this protein was predicted using the FASTA sequences of 345 amino acid, and predicted using GOR IV.

C. Homology modelling

The protein sequence was subjected for comparative homology modelling via Swiss model to generate putative 3D model. (http://swissmodel.expasy.org/)

D. Validation

The validation of the modelled structure was carried out using Protein Structure Validation Suite (PSVS) tool. Structural analysis was performed and the 3-d coordinate file was visualized and analyzed in Rasmol. (http://psvs-1_4-dev.nesg.org/)

RESULTS AND DISCUSSION

A. Primary structure prediction

In this study primary structure of TBC1D3 was predicted using Expasy's ProtParam server. Results showed that TBC1D3 had 291 amino acid residues and the estimated molecular weight 33400.2. The calculated isoelectric point (pI) is useful for at pI the solubility is least and the mobility in an electric field is zero. Isoelectric point (pI) is the pH at which the surface of protein is covered with charge but net charge of protein is zero. The calculated isoelectric point (pI) was computed to be 9.46. The computed value is more than 7 indicate that the protein is basic The maximum number of amino acid present in the sequence was found to be leucine(Leu) (9.3%) and the least was that of tripsine(Trp)(2.1%). The total number of negatively charged residues (Asp + Glu):35 and the total number of positively charged residues (Arg + Lys):44.The Aliphatic index: 76.74. Aliphatic index is defined as the relative volume of a protein occupied by aliphatic side chains and high index refer as positive factor for the increase of thermostability of the protein.[8] While the instability index (50.43) provides the estimate of the stability of protein in a test tube. The Grand Average Hydropathicity (GRAVY) value is low -0.654, indicates better interaction of the protein with water.

B .Secondary structure prediction

The secondary structure is composed of alpha helix and beta sheets and the secondary structure is predicted using GOR IV Table 1 from which it is clear that random coil is predominantly present i.e. 45.02%, followed by Alpha helix

and Extended strand the percentage is 36.77% and 18.21% respectively. Extended strand was found to be least frequent.

This is graphically represented in Figure 1

C. Tertiary structure prediction

The tertiary structure was modelled by Swiss model workspace and visualize in Rasmol visualizer (Figure2). The modelled structure was validated by PSVS (Fig 3) Ramachandran plot was plotted.

Result-

Sequence of TBC1D3 protein in fasta format and its accession number is as follow

GenBank: AAH78140.1

GenPept Graphics

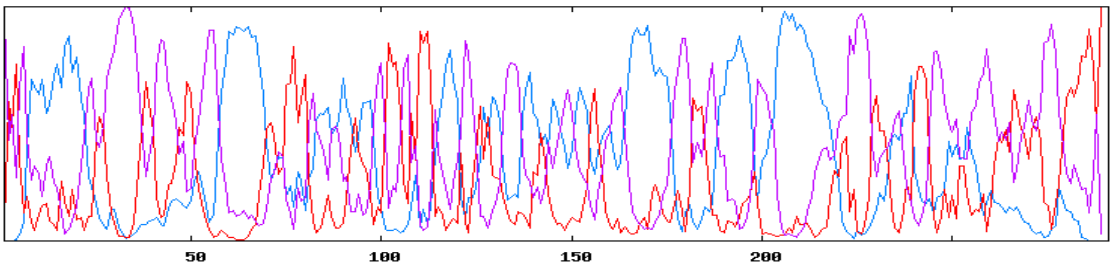
>gi|50416992|gb|AAH78140.1|TBC1D3 protein [Homo sapiens]

MDVVEVAGSWWAQEREDIIMKYEKGHRAGLPEDKGPKPFRSYNNNVDHLGIVHETELPPLTAREAKQIRREISRKSKW
VDMLGDWEKYKSSRKLIDRAYKGMPMNIRGPMWSVLLNTEEMKMKNPGRYQIMKEKGKRSSEHIQRIDRDVSGTLR
KHIFFRDRYGTKQRELLHILLAYEEYNPEVGYCRDLSHIAALFLLYLPEEDAFWALVQLLASERHSLQGFHSPNGGTVQGL
QDQQEHVVATSQPKTMGHQSASRRRPGVARGHVFATGSLIPGPGMRTLCSSILGPL

Figure 1:- Secondary structure of TBC1D3 protein by using GOR IV

```
        10        20        30        40        50        60        70
         |         |         |         |         |         |         |
MDVVEVAGSWWAQEREDIIMKYEKGHRAGLPEDKGPKPFRSYNNNVDHLGIVHETELPPLTAREAKQIRR
ccccccccchhhhhhhhhhhhhhhcceeccccccccccceeecccccceeeecccccchhhhhhhhhhh
EISRKSKWVDMLGDWEKYKSSRKLIDRAYKGMPMNIRGPMWSVLLNTEEMKMKNPGRYQIMKEKGKRSSE
heeecceeeeeeccchhhhcchhhhhhhhhccceeeeecccceeeechhhhhhccccchhhhhhccccchh
HIQRIDRDVSGTLRKHIFFRDRYGTKQRELLHILLAYEEYNPEVGYCRDLSHIAALFLLYLPEEDAFWAL
hhhhhcccccccchhhhhhhhhhcccchhhhhhhhhhhhhhccccceeecccchhhhhhhhhcccccchhhhhh
VQLLASERHSLQGFHSPNGGTVQGLQDQQEHVVATSQPKTMGHQSASRRRPGVARGHVFATGSLIPGPGM
hhhhhhhhhhcccccccccceeeecccceeeeeeeccccccccccccccccccccccceeecccccccccc
RTLCSSILGPL
eeeeeeeeecc
```

```
Sequence length :   291
GOR4 :
   Alpha helix      (Hh) :   107 is  36.77%
   3₁₀ helix        (Gg) :     0 is   0.00%
   Pi helix         (Ii) :     0 is   0.00%
   Beta bridge      (Bb) :     0 is   0.00%
   Extended strand  (Ee) :    53 is  18.21%
   Beta turn        (Tt) :     0 is   0.00%
   Bend region      (Ss) :     0 is   0.00%
   Random coil      (Cc) :   131 is  45.02%
   Ambigous states  (?)  :     0 is   0.00%
   Other states          :     0 is   0.00%
```



helix *sheet* *coil*

Figure 1:- Graphical representation of Secondary elements in TBC1D3 protein

Figure 2:- Three dimensional structure of TBC1D3 protein

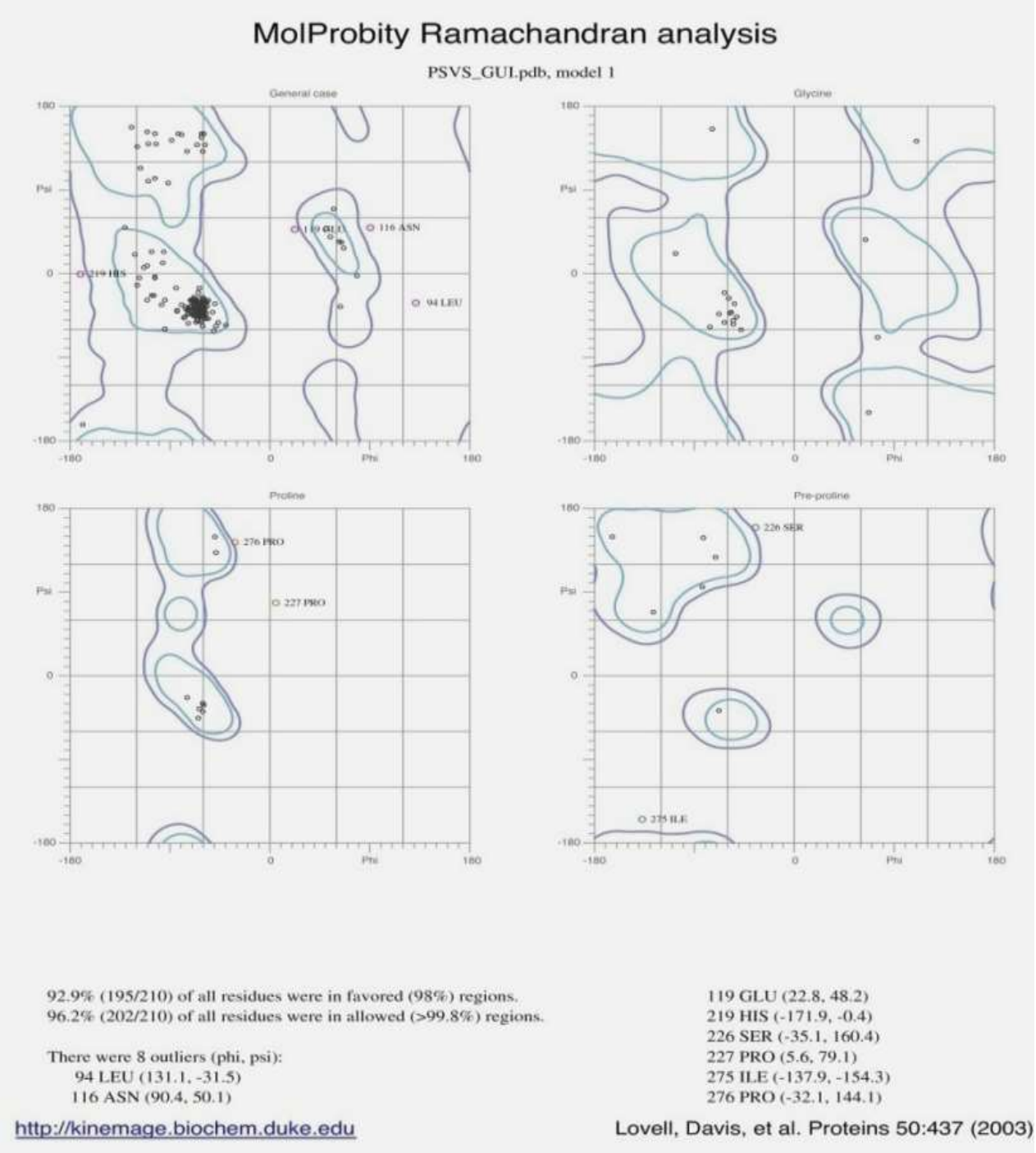


Figure 3:- Validation of modelled protein with PSVS

CONCLUSION

Diabetes is a disease in which blood glucose level are above normal. Insulin helps our body to control glucose in blood. For diabetic patients insulin is used for treatment. Insulin user show higher risks of overall and mortality. In the present study the structure analysis of TBC1D3 protein was done by various tools. On this basis it can conclude that further characterization of TBC1D3 protein will be important for the treatment against diabetes type2.

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