International Journal of
Analytical, Pharmaceutical and Biomedical Sciences

Research Article

Volume: 1: Issue-2: April-June -2012 Copyrights@2012 ISSN: 2278-0246
Received: 10th May-2012 Revised: 14th May-2012 Accepted: 18th May-2012
Coden : IJAPBS www.ijapbs.com

GENOME WIDE ANALYSIS, MODELLING AND DNA BINDING STUDIES OF STEROL RESPONSIBLE ELEMENT BINDING PROTEIN-1(SREBP-1) OF TYPE 2 DIABETES

S.Murali Mohan[1], K.Peddanna[2], A.Ramya[3], Vinaysagar Lakka[4] and D.Jayasimha Rayalu[5*]

[1]Department of Zoology, S.S.B.N Degree and P.G College. Anantapur. Andhra Pradesh. INDIA.
[2]DST-PURSE, Sri Venkateswara University, Tirupati, Andhra Pradesh. INDIA
[3]Department of Microbiology, Sri Krishnadevaraya University, Anantapur. Andhra Pradesh. INDIA.
[4]Department of Biotechnology, Akshaya Biological Corporation. Himayat nagar, Hyderabad. INDIA.
[5]Department of Bioinformatics, DR.Rayalu's Biotech PVT. LTD. Himayat nagar, Hyderabad. INDIA

ABSTRACT: Diabetes mellitus type 2 is a metabolic disorder that is characterized by high blood glucose in the context of insulin resistance and relative insulin deficiency. A number of genes are involved in regulating lipid metabolism and insulin sensitivity, and thereby affecting the susceptibility to type 2 diabetes mellitus. Among them is the gene which is responsible for Sterol Response Element Binding Protein-1 (SREBP). SREBPs are transcription factors that bind to the sterol regulatory element DNA sequence TCACNCCAC. Mammalian SREBPs are encoded by the genes SREBF1 and SREBF2. In this work A 3D model of SREBP was generated using 2OVP (Crystal structure of Fbw7-Skp1-Cyclin E complex) as template with the help of Modeller7v7. With the aid of the molecular mechanics and molecular dynamics methods, the final model is obtained and is further assessed by Procheck and Verify 3D graph programs, which showed that the final refined model is reliable. After energy minimization study this structure was used for binding with DNA. We identified 35 genes coding for SREBP-1 and their locus regions. Multiple alignment and phylogenetic analysis showed that the total SREBP family proteins formed 6 branches. From the result we identified inhibitory region of the SREBP-1 protein while binding to DNA.

Key words: Diabetes type 2, SREBP-1, Modelling, Phylogentic analysis and DNA binding studies.

INTRODUCTION

Sterol regulatory elements binding proteins (SREBP) are transcription factors involved in Type 2 diabetes (T2DM) is a common metabolic disorder by two core defects; insulin resistance and beta cell dysfunction. Pancreatic beta cells maintain blood glucose homeostasis by their capacity to secrete insulin upon glucose stimulation and this is the most important beta cell function. SREBPs bind to the sterol regulatory element DNA sequence TCACNCCAC. Mammalian SREBPs are encoded by the genes SREBF1 and SREBF2 [1] and play an important role in adipogenesis and fatty acid homeostasis, which are basic helix loop helix Lucien zipper (bHLH) transcription factors that regulate the expression of genes in glucose metabolism, cholesterol metabolism and fatty acid metabolism in mammalian cells [2, 17].

SREBP family is comprised of two members SREBP1 and SREBP2. SREBP1 exists as two isoforms (SREBP1a and SREBP1c) derived from alternative splicing of first exon of the same gene on human chromosome 17p11.2 (SREBF-1) and SREBP-2 is encoded by a separate gene on human chromosome 22q13 (SREBF-2) [12]. SREBP-1a is the predominant isoform in the most cultured cells, intestine and spleen whereas SREBP-1c is predominates in in the liver and most other tissues, such as muscle and adipose tissue [21]. Unactivated SREBPs are attached to the nuclear envelope and endoplasmic reticulum membranes. In cells with low levels of sterols, SREBPs are cleaved to a water soluble N-terminal domain that is translocated to the nucleus.

SREBP are synthesized in endoplasmic reticulum as 130 kda precursor molecule that is transported into Golgi, where they are processed via 2-step sequential proteolytic cleavage to produce 480 amino acids active mature transcription factor that is transported to nucleus [22, 4]. These factors are activate in the transcription of target genes by binding to sterol regulating elements (SRE) or to E-box like sequence [6]. These processing event requires two separate, site-specific proteolytic cleavages are necessary for release of the transcriptionally active amino-terminal domain. These cleavages are carried out by two distinct proteases, called site-1 protease (S1P) and site-2 protease (S2P) [21] and regulatory protein designated SREBP-cleavage –activating protein (SCAP) [4].
The transcriptional activation of SREBP-1 can be up regulated by insulin [14], glucose [9], and liver X receptor [19] and suppressed by fattyacids [8,24]. SREBP-1 preferentially activates the genes involved in fatty acid synthesis, including acetyl CoA carboxylase and fatty acid synthase (FAS), whereas SREBP-2 preferentially activates genes involved in cholesterol biosynthesis such as hydroxymethylglutaryl CoA synthase, hydroxymethylglutaryl CoA reductase, faranesyl diphosphate synthase, and squalene synthase [18, 22, 10]. Glucose-stimulated hepatic GLUT2 gene expression as well as insulin effects on hepatic gene expression are mediated by SREBP-1c [14, 5]. Hepatic SREBP-1c expression is regulated by glucose, cylic adenosine monophosphate and polyunsaturated fatty acids [11]. In pancreatic β-cells, overexpression of SREBP-1c is associated with accumulation of triglycerides and impairs insulin secretion at least in part via upregulation of uncoupling protein 2.

METHODOLOGY

Sequence analysis

Reference proteins of well-established molecular function, representing each of the protein families investigated, was chosen as query sequence for searching in the human genome databases. The reference protein is SREBP (Accession number: Q12770). Searches were made using the TBLASTN tool against GenBank database non-redundant (NR), with search specifications. The other databases used were Human Genome Research Program (HGP), and Universal Protein resource Uniprot (http://www.ebi.uniprot.org/uniprot-srv/protein/uniProtView). The BLAST server used was that of the National Center for Biotechnology Information (http:// www.ncbi.nlm.nih.gov/BLAST/). As selection criteria of BLAST hits for genomic sequences, a cut off e-value of e- 10 was previously set. The genomic sequences found were used to predict putative genes contained within them. Whenever possible, genes were predicted on the basis of sequences generated by the IRGSP, since these sequences present a higher degree of accuracy. To that end, a mixed procedure was adopted combining ab initio gene prediction algorithms of genomic sequence alignments with similar sequences from expressed genes (ESTs and cDNAs). The prediction algorithms were GenScan (Burge and Karlin, 1997; http:// genes.mit.edu/ GENSCAN.html), GeneMark.hmm (Borodovsky and Lukashin, unpublished; http://opal.biology.gatech.edu/GeneMark/eukhmm.cgi). Such expressed sequences were found by BLAST searches against EST and NR databases of GenBank, using the genomic sequence as query. The algorithm of choice for the multiple alignments of protein sequences was ClustalX1.8. The multiple alignments were edited with the help of GENEDOC. All the proteins with greater than 30% identity, with at least one of the reference proteins used in the searches, were regarded as functionally similar (homologous) to the reference proteins, receiving the same name. Those sequences that did not conform to this criterion were discarded. Prediction of homology and signature sequences for the putative transporter proteins were carried out with PROSITE (http://www.ebi.ac.uk/InterProScan/) and Pfam databases. Sequences were included into families based on homology and presence of signature sequences. For topology prediction, HMMTOP was used. Protein alignments obtained with ClustalX 1.8 were used as starting points for phylogenetic analysis. Unrooted trees were prepared by the neighbor-joining method using either Clustal, PHYLIP, or and 1000 bootstrap replicates were performed. Bold lines on trees indicate protein sequences that were confirmed by cDNA sequencng**.**

3D model building

The query sequence from *Homo sapiens* was submitted to domain fishing server for SREBP protein prediction. The predicted domain *was* searched to find out the related protein structure to be used as a template by the BLAST (Basic Local Alignment Search Tool) program against PDB(Protein Data bank). Sequence that showed maximum identity with high score and less e-value were aligned (Figure 1) and was used as a reference structure to build a 3D model for SREBP protein.

The co-ordinates for the structurally conserved regions (SCRs) for SREBP protein were assigned from the template using multiple sequence alignment, based on the Needleman-Wunsch algorithm (Needleman, et al,1970,1994). The initial model of SREBP protein was built by using homology-modeling methods and the MODELLER software; a program for comparative protein structure modeling optimally satisfying spatial restraints derived from the alignment and expressed as probability density functions (pdfs) for the features restrained. The pdfs restrain C^{α}-C^{α} distances, main-chain N-O distances, main-chain and side-chain dihedral angles. The 3D model of a protein is obtained by optimization of the molecular pdf such that the model violates the input restraints as little as possible. The molecular pdf is derived as a combination of pdfs restraining individual spatial features of the whole molecule. The optimization procedure is a variable target function method that applies the conjugate gradients algorithm to positions of all non-hydrogen atoms. The structure having the least modeller objective function, obtained from the modeller was improved by molecular dynamics and equilibration methods using NAMD 2.5 software using CHARMM27 force field for lipids and proteins [20] along with the TIP3P model for water (Jorgensen , et al,1983) The energy of the structure was minimized with 10,000 steps. A cutoff of 12 Å (switching function starting at 10 Å) for van der Waals interactions was assumed. No periodic boundary conditions were included in this study. An integration time step of 2 ps was used, permitting a multiple time-stepping algorithm [7] to be employed in which interactions involving covalent bonds were computed every time step, short-range nonbonded interactions were computed every two time steps, and long-range electrostatic forces were computed every four time steps. The pair list of the nonbonded interaction was recalculated every ten time steps with a pair list distance of 13.5 Å. The short-range nonbonded interactions were defined as van der Waals and electrostatics interactions between particles within 12 Å. A smoothing function was employed for the van der Waals interactions at a distance of 10 Å. CHARMM27 force-field parameters were used in all simulations in this study. The equilibrated system was simulated for 1 ps with a 500 kcal/mol/Å2 restraint on the protein backbone under 1 atm constant pressure and 310 K constant temperature (NPT) and the Langevin damping coefficient was set to 5 ps unless otherwise stated. Finally, the structure having the least energy with low RMSD (Root Mean Square Deviation) was used for further studies. In this step, the quality of the initial model was improved.The final structure obtained was analyzed by Ramachandran's map using PROCHECK (Programs to check the Stereo chemical Quality of Protein Structures) [3] and environment profile using ERRAT graph (Structure Evaluation server) [15]. This model was used for the identification of active site and for docking of the substrate with the enzyme.

Active site Identification

Active site of SREBP-1 was identified using CASTp server. A new program, CASTp, for automatically locating and measuring protein pockets and cavities, is based on precise computational geometry methods, including alpha shape and discrete flow theory. CASTp identifies and measures pockets and pocket mouth openings, as well as cavities. The program specifies the atoms lining pockets, pocket openings, and buried cavities; the volume and area of pockets and cavities; and the area and circumference of mouth openings.

Docking method

In order to elucidate possible interactions of the protein and DNA, docking studies were performed using FRED. The relevant stereo isomers of the compounds were minimized with the MMFF force field in the Openeye package. Conformation and minimization of the compounds was performed using Omega. Fred requires a set of input conformers for each ligand. The conformers were generated by Omega and stored in a single binary file. After this the output file is used for docking.

Docking calculations were performed with FRED version 1.1 for efficient handling of large compound databases. The first stage in docking is a shape fitting process. The crude docking solutions are further tested against a pharmacophore. Various options are available for optimization with respect to the built-in scoring functions: optimization of hydroxyl group rotamers, rigid body optimization, torsion optimization, and reduction of the number of poses that are passed on to the next scoring function. Available scoring functions in FRED are Chemgauses, Chemscore, PLP, Screenscore and Shapegauses.

RESULTS

Homology Modelling of SREBP protein Domain

A high level of sequence identity should guarantee more accurate alignment between the target sequence and template structure. In the results of BLAST search against PDB, only the 2OVP which has a high level of sequence identity with the SREBP protein domain.

Structurally conserved regions (SCRs) for the model and the template were determined by superimposition of the two structures and multiple sequence alignment.

```
sp|Q12770|40-279      CCYPLLKLPLPGTGPVEFTTPVKDYSPPPVDSDRKQGEPTEQPEWYVGAPVAYVQQIFVK
2OVP                  ---------------------------------DDPGGSGTDD------IPV---------
                                                       *   *. *::       **

sp|Q12770|40-279      SSVFPWHKNLLAVDVFRSPLSRAFQLVEEIRNHVLRDSSGIRSLEELCLQVTDLLPGLRK
2OVP                  -----WDQEFLKVD--QGTL---FELILAAN---YLDIKGL--LDVTCKTVANMIKGK--
                           *.:::* **  :..*   *:*:   .     * .*:  *:  *  *:::: *

sp|Q12770|40-279      LRNLLPEHGCLLLSPGNFWQNDWERFHADPDIIGTIHQHEPKTLQTSATLKDLLFGVPGK
2OVP                  -------------TPEEIRK----TFNIKNDFT----EEEEAQVRKENQ-----------
                                  :* :: :     *: . *:     :.*   ::..

sp|Q12770|40-279      YSGVSLYTRKRMVSYTITLVFQHYHAKFLGSLRARLMLLHPSPNCSLRAESLVHVHFKEE
2OVP                  ------WCEEK----------------------------------------------------
                            : .::
```

Fig 1: Alignment through ClustalX

In the following study, we have chosen 2OVP as a reference structure for modeling SREBP protein domain. Coordinates from the reference protein (2OVP) to the SCRs, structurally variable regions (SVRs), N-termini and C-termini were assigned to the target sequence based on the satisfaction of spatial restraints. In the modeller we will get a 20 PDB out of which we select a least energy. The energy unit will be in kilo joule. All side chains of the model protein were set by rotamers. The final stable structure of the SREBP protein protein obtained is shown in Figure 2.

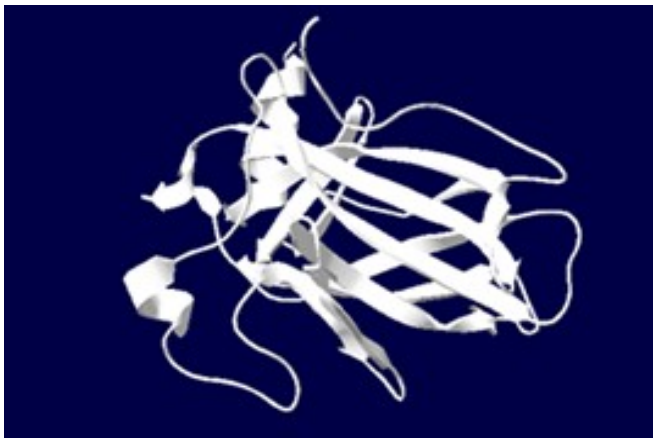

Figure 2: Modeller result

The final structure was further checked by verify3D graph and the results have been shown in Figure 3: The overall scores indicates acceptable protein environment.

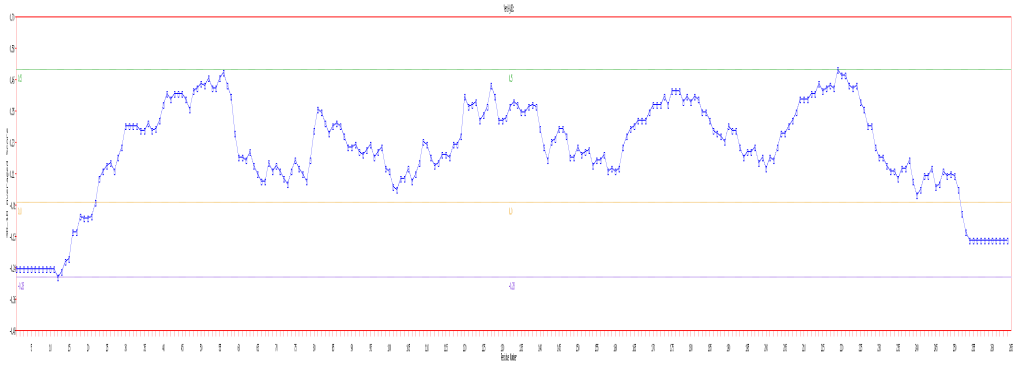

Figure 3:The 3D profiles verified results of SREBP protein model; overall quality score indicates residues are reasonably folded

Validation of SREBP protein Domain

After the refinement process, validation of the model was carried out using Ramachandran plot calculations computed with the PROCHECK program. The Ψ and θ distributions of the Ramachandran plots of non-glycine, non-proline residues are summarized in Table 1. The RMSD (Root Mean Square deviation) deviation for covalent bonds and covalent angles relative to the standard dictionary of SREBP protein was -4.27 and -0.85 Å. Altogether 100 % of the residues of SREBP protein was in favored and allowed regions. The overall PROCHECK G-factor of SREBP protein was – 2.32 and verify3D environment profile was good.

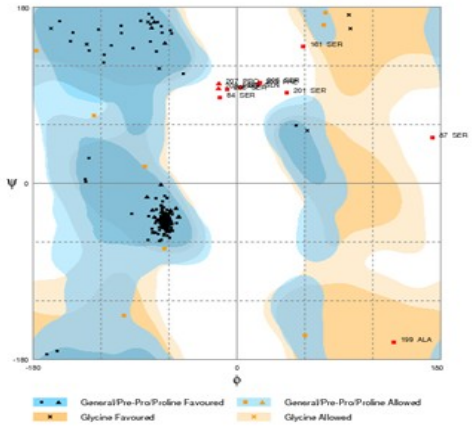

Figure-4: Ramachandran Plot

Table 1: % of residue falling in the core region of the Ramachandran's plot

% of residue in most favored regions	92.7
% of residue in the additionally allowed zones	3.1
% of residue in the generously regions	4.2
% of residue in disallowed regions	0.0
% of non-glycine and non-proline residues	100.0

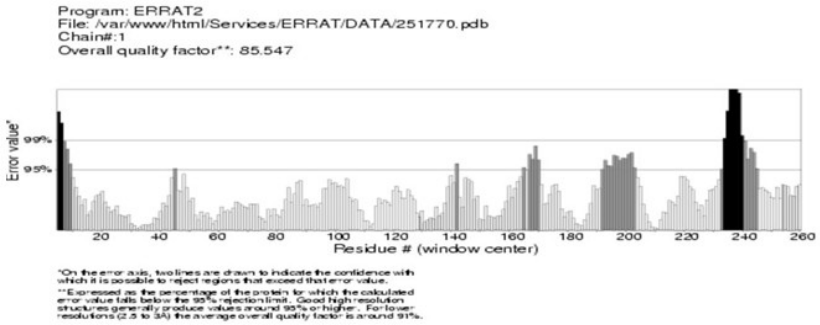

Fig 5: ERRAT result showing overall qaulity factor is 85.547

Superimposition of 2OVP with SREBP protein domain

The structural superimposition of 2OVP template and SREBP protein is shown in Figure 9. The weighted root mean square deviation of trace between the template and final refined models 0.21A^{o}. This final refined model was used for the identification of active site and for docking of the substrate with the domain SREBP protein.

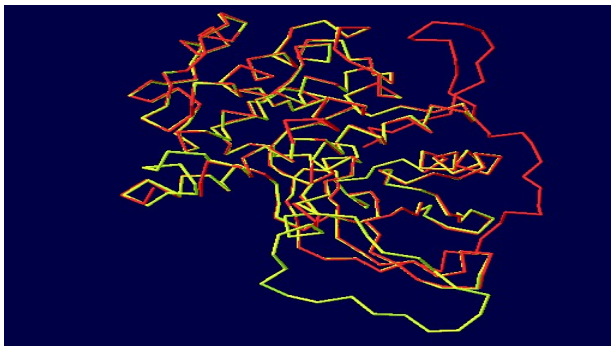

Figure 6: superimposition of SREBP protein (red colour) and 2OVP (represented in yellow color).

CAST P results

Active site Identification of SREBP protein prediction by CASTp

After selecting receptor from PDB and isolated the A-chain in SPDBV, the possible binding sites of protein was searched based on the structural comparison of template and the model build and also with CASTp server. The residues are TYR18, TYR21, LYS22, GLN25, ARG26, ARG98, GLY101, ASP102, PHE104, SER105, ARG106, TYR108, ARG109, ASP111, PHE112, ALA113, MET115, SER116, GLN118, LEU119, ARG129, THR132, VAL133, GLU136, LEU137, ARG146, VAL148, ALA149, GLU152, PHE153, GLY155, VAL156, MET157, VAL159, GLU160.

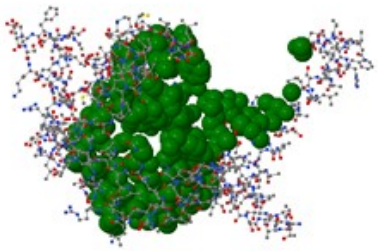

Figure 7: Representing active site Pockets of the SREBP shows highest area and volume.

Genomic overview

With the availability of the BGI and the IRGSP data in GenBank, it was possible to construct an overview of SREBP. As a starting point, protein family in SREBP, intracellular targeting was chosen for analysis. Taking specific members of this family as query sequences, searches were carried out for orthologous sequences in GenBank, HGP and Uniprot current databases using TBLASTN. After searching the databanks with TBLASTN sequences, clones having genomic sequences to the related family were taken and converted to amino acid sequences. In each family, similar sequences were removed and the sequences were subjected to PROSITE and Pfam databases to see the presence of signature sequences for the corresponding families. After subjecting the sequences to PROSITE 33 new putative genes, were predicted in Human. Together with previously reported sequences in human: 35 genes related to the SREBP protein Human (Table 2). The percent identity for all the sequences was calculated in family with the corresponding query sequence using GENEDOC. The presence of transmembrane domains was also predicted for all transporter proteins. The result was heterogeneous with majority of proteins presenting 10–12 transmembrane domains. Phylogenetic analysis of the sequences of transporters revealed that the SREBP protein were divergent, showing branches in tree view. The phylogentic analysis shows four branches indicating different transporting function to each bravnch. Some of the orthologous sequences are available as full-length cDNA clones. The expressed sequence tags were mentioned as accession numbers for the sequences.

Fig 8: Interpro Scan Results

DNA BINDING STUDIES OF SREBP

Docking of the SREBP with DNA (Homo sapiens) was performed using FRED v 2.1, which is based on Rigid Body Shape-Fitting (Open Eye Scientific Software, Santa Fe, NM). This program generates an ensemble of different rigid body orientations (poses) for each compound conformer within the binding pocket and then passes each molecule against a negative image of the binding site. Poses clashing with this 'bump map' are eliminated. Poses surviving the bump test are then scored and ranked with a Gaussian shape function. We defined the binding pocket using the ligand-free protein structure and a box enclosing the binding site.

This box was defined by extending the size of a cocrystalized ligand by 4 Å (addbox parameter of FRED). This dimension was considered here appropriate to allow, for instance, compounds larger than the cocrystallized ones to fit into the binding site. One unique pose for each of the best-scored compounds was saved for the subsequent steps. The DNA used for docking was converted in 3D with OMEGA (same protocol as above) (OpenEye Scientific Software, Santa Fe, NM). To this set, the substrate (generation of multiconformer with Omega) corresponding to the modeled protein were added

Table 2: Analysis of SREBP genes from Human

S.NO	PROTEIN	ACCESION NO	AMINO ACIDS	NO OF TRANSMEMBRANE HELICES	%IDENTITY
1	SREBP		1279		100
2	SREBP 1	BAA12111.2	1279	9	99.4135
3	SREBP 2	EAW64826.1	1023	8	99.4357
4	SREBP 3		886	4	69.3523
5	SREBP 4		352	4	74.8756
6	SREBP 5		402	1	91.1304
7	SREBP 6		1150	8	86.59
8	SREBP 7		261	0	81.3187
9	SREBP 8		364	1	21.9653
10	SREBP 9		346	1	23.3503
11	SREBP 10		394	0	80.1217
12	SREBP 11		493	1	92.6471
13	SREBP 12		272	0	72.6349
14	SREBP 13		1411	6	90.2985
15	SREBP 14		268	0	89.1057
16	SREBP 15		615	3	95.3405
17	SREBP 16		279	0	53.0102
18	SREBP 17		1350	9	90.1804
19	SREBP 18		499	1	83.3906
20	SREBP 19		873	5	99.2063
21	SREBP 20		378	1	98.6364
22	SREBP 21		440	1	31.7343
23	SREBP 22		271	1	98.8372
24	SREBP 23		258	0	98.6364
25	SREBP 24		440	1	100
26	SREBP 25		155	1	95.0777
27	SREBP 26		772	8	88.2217
28	SREBP 27		433	2	91.6667
29	SREBP 28		108	0	100
30	SREBP 29		75	0	96.1957
31	SREBP 30		184	0	99.4083
32	SREBP31		169	1	96.1538
33	SREBP 32		104	2	100
34	SREBP 33		43	0	100
35	SREBP 34		37	1	

```
SREBP21     FTLQGHSGAITTVYIDQTMVLASGGQDGAICLWDVLTGSRVSHVFAHRG---------- 322
SREBP24     FTLQGHSGAITTVYIDQTMVLASGGQDGAICLWDVLTGSRVSHVFAHRG---------- 322
SREBP20     FTLQGHSGAITTVYIDQTMVLASGGQDGAICLWDVLTGSRVSHVFAHRG---------- 260
SREBP6      FTLQGHSGAITTVYIDQTMVLASGGQDGAICLWDVLTGSRVSHMFAHRG---------- 1032
SREBP5      -QLEGHSGAITTVYIDQTMVLASGGQDGAICLWDVLTGRRVSHMFAHRG---------- 319
SREBP28     -----HSGAITTVYIDQTMVLASGGQDGAICLWDVLTGSRVSHVFAHRG---------- 55
SREBP19     FTLKGHSGAITAVYIDQTMVLASGGQDGAICLWDVLTGSRVSQTFAHRG---------- 755
SREBP13     FTLQGHSGAITSVYVDQTMVLASGGQDGAICLWDMLTGSRVSHMFAHRG---------- 1293
SREBP9      LLAARLNGALESFQFHADFDSPVEEVSHHVHSRR---------------------------- 208
SREBP22     IIASRLYGTLEMFQLQTYNRGHPVDWNFTCAYRR---------------------------- 176
SREBP10     TIVMLFLGRIDFLRLETYTQGRQIDWGFTSAYRRTHIRTGSAGSLGMFQ---------- 292
SREBP4      TCWALGSRTQGPRWGAGPWRRHAVQGGGAGPGHRHRLGAAAALPLPRAMP---------- 259
SREBP34     ------------------------------------------------------------
SREBP26     QRLIMAGTVVWIGILVYTDPAGLRNYLAAQVTEQSPLGEGALAPMPVPSG---------- 523
SREBP32     ------------------------------------------------------------
SREBP17     QRIIMAGTVIWIGILVYTDPAGIRTYLAAQVSEQSPLGDAVAGALPPHLG---------- 591
```

Fig 9: Multiple sequence alignment of SREBP Familt proteins

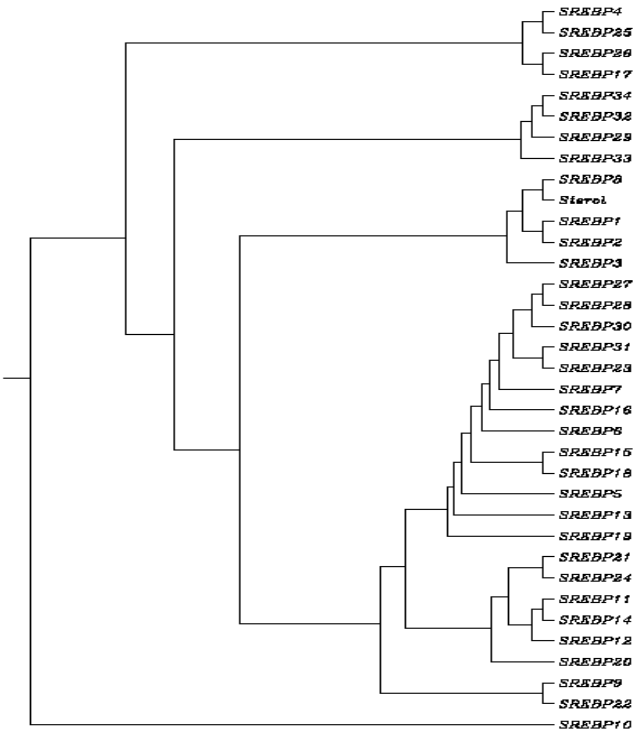

Fig 10: Phylogenetic analysis of SREBP Family proteins

Docking studies of SREBP with DNA of Homo sapiens was performed using OPEN EYE software. The docking results showed that docking energy was very good and it was -166.24 K.J/mol (Table 3).

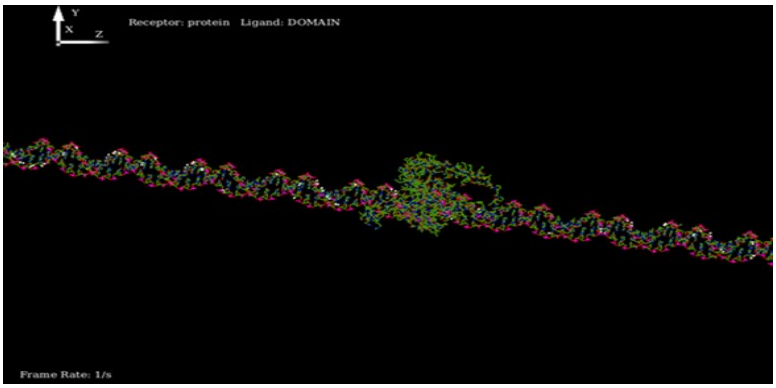

Fig 11: DNA binding studies of SREBP protein

Table 3: DNA Binding studies of SREBP-1.

S.No.	Chemscore	Screen score	Chemguass	PLP	Total
1	-7.04	-82.94	-42.76	-33.50	-166.24

CONCLUSION

Sterol Regulatory Element-Binding Proteins (SREBPs) are transcription factors that bind to the sterol regulatory element DNA sequence TCACNCCAC. In this work, we have constructed a 3D model of SREBP protein domain, from human using the MODELLER software and obtained a refined model after energy minimization. The final refined model was further assessed by ERRAT & PROCHECK program, and the results show that this model is reliable. The genes coding for SREBP protein were identified using BLAST and totally 35 genes were identified. The alignment between these gene coding proteins showed the similarity and homologous nature of proteins. Though they are similar due to mutations we can observe the different functionalities between them. This can be observed in phylogenetic analysis. In the tree view we observed 6 branches indicating different functions to each branch. SREBP channel family contains a total of 35 genes. These are numbered based on the alignment of the gene with query. The genes, which are showing more than 30% identity, are homologous sequences. In this work we have modeled 3D structure and identified related sequences for SREBP protein in Human. DNA binding studies of SREBP was performed with docking and from the result it was identified that SREBP has good inhibitory effect on the DNA of Homo sapiens.

REFERENCES

[1] Brown MS, Goldstein JL "A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood". Proc. Natl. Acad. Sci. U.S.A. 1999.96 (20): 11041–11048.

[2] Brown MS, Goldstein JL: The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell 1997.89:331–340.

[3] Brunger, A. X-PLOR, Version 3.1: A System for X-Ray Crystallography and NMR. Yale University, New Haven, CT. 1992.

[4] Espenshade PJ, Cheng D, Goldstein JL, Brown MS: Autocatalytic processing of site-1 protease removes propeptide and permits cleavage of sterol regulatory element-binding proteins. J Biol Chem 1999. 274:22795–22804.

[5] Foretz M, Guichard C, Ferre P, Foufelle F. Sterol regulatory element binding protein-1c is a major mediator of insulin action on the hepatic expression of glucokinase and lipogenesis-related genes. Proc Natl Acad Sci USA; 1999. 96: 12737–12742.

[6] Goldstein JL, Rawson RB, Brown MS.. Mutant mammalian cells as tools to delineate the sterol regulatory element-binding protein pathway for feedback regulation of lipid synthesis. Arch Biochem Biophys 2002.397(2):139-48.10.

[7] Grubmuller, H., H. Heller, A. Windemuth, and K. Schulten.. Generalized Verlet algorithm for efficient molecular dynamics simulations with long-range interactions. Mol. Sim. 1991. 6:121–142.

[8] Hannah, V. C., Ou, J., Luong, A., Goldstein, J. L., and Brown, M. S. Unsaturated Fatty Acids Down-regulate SREBP Isoforms 1a and 1c by Two Mechanisms in HEK-293 Cells J. Biol. Chem. 2001. 276, 4365–4372.

[9] Hasty, A. H., Shimano, H., Yahagi, N., Amemiya-Kudo, M., Perrey, S.,Yoshikawa, T., Osuga, J., Okazaki, H., Tamura, Y., Iizuka, Y., Shionoiri, F.,Ohashi, K., Harada, K., Gotoda, T., Nagai, R., Ishibashi, S., and Yamada, N Sterol regulatory element-binding protein-1 is regulated by glucose at the transcriptional level. J. Biol. Chem. .2000. 275, 31069–31077.

[10] Horton, J. D., Shimomura, I., Brown, M. S., Hammer, R. E., Goldstein, J. L.,and Shimano, H. Activation of cholesterol synthesis in preference to fatty acid synthesis in liver and adipose tissue of transgenic mice overproducing sterol regulatory element-binding protein-2.J. Clin. Invest. 1998. 101, 2331–2339.

[11] Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest 2002. 109: 1125–1131

[12] Hua X, Wu J, Goldstein JL, Brown MS, Hobbs HH.. Structure of the human gene encoding sterol regulatory element binding protein-1 (SREBF1) and localization of SREBF1 and SREBF2 to chromosomes 17p11.2 and 22q13. Genomics 1995. 25(3):667-73.

[13] Im SS, Kang SY, Kim SY, Kim HI, Kim JW, Kim KS et al. Glucosestimulated upregulation of GLUT2 gene is mediated by sterol response element-binding protein-1c in the hepatocytes. Diabetes ; 2005. 54: 1684–1691.

[14] Kim, J. B., Sarraf, P., Wright, M., Yao, K. M., Mueller, E., Solanes, G., Lowell,B. B., and Spiegelman, B. M. Nutritional and insulin regulation of fatty acid synthetase and leptin gene expression through ADD1/SREBP1 J. Clin. Invest. 1998. 101, 1–9.

[15] Laskoswki, R. A., MacArthur, M. W., Moss, D. S. and Thorton, J. M.. PROCHECK: a program to check the stereochemical quality of protein structures. J. Appl. Cryst. 1993. 26, 283-291.

[16] Needleman, S. B. and Wunsch, C. D. . A general method applicable to the search for similarities in the amino acid sequence of two proteins. J. Mol. Biol. 1970. 48, 443–453.

[17] Osborne TF: Sterol regulatory element-binding proteins (SREBPs): key regulators of nutritional homeostasis and insulin action. J Biol Chem 2000. 275:32379–32382.

[18] Pai, J., Guryev, O., Brown, M. S., and Goldstein, J. L. differential stimulation of cholesterol and unsaturated fatty acid bio syntesis in cells expressing individual nuclear sterol regulatory element binding protein. J. Biol. Chem. 1998. 273, 26138–26148.

[19] Repa, J. J., Liang, G., Ou, J., Bashmakov, Y., Lobaccaro, J. M., Shimomura, I., Shan, B., Brown, M. S., Goldstein, J. L., and Mangelsdorf, D. J. Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRalpha and LXRbeta.Genes Dev. 2000. 14, 2819–2830.

[20] Schlenkrich, M., J. Brickmann, A. D. MacKerell, Jr., and M. Karplus.. Empirical potential energy function for phospholipids: criteria for parameter optimization and applications. In Biological Membranes: A Molecular Perspective from Computation and Experiment. K. M. Merz and B. Roux, editors. Birkhauser, Boston, MA. 1996. 31–81.

[21] Shimomura I, Shimano H, Horton JD, Goldstein JL, Brown MS.. Differential expression of exons 1a and 1c in mRNAs for sterol regulatory element binding protein-1 in human and mouse organs and cultured cells. J Clin Invest 1997. 99(5):838-45.

[22] Shimano, H., Horton, J. D., Shimomura, I., Hammer, R. E., Brown, M. S., and Goldstein, J. L.. isoform 1c of sterol regulatory element binding protein is less active than isoform 1a in livers of transgenic mice and in cultured cells.J. Clin. Invest. 1997. 99, 846–854.

[23] Shimano, H., Shimomura, I., Hammer, R. E., Goldstein, J. L., Brown, M. S.,and Horton, J. D. Elevated levels of SREBP-2 and cholesterol synthesis in livers of mice homozygous for a targeted disruption of the SREBP-1 gene. J. Clin. Invest. 1997. 100, 2115–2124.

[24] Xu, J., Teran-Garcia, M., Park, J. H., Nakamura, M. T., and Clarke, S. D. Polyunsaturated fatty acids suppress hepatic sterol regulatory element-binding protein-1 expression by accelerating transcript decay. J. Biol. Chem. 2001. 276, 9800–9807.

[25] Yamashita T, Eto K, Okazaki Y, Yamashita S, Yamauchi T, Sekine N et al. Role of uncoupling protein-2 up-regulation and triglyceride accumulation in impaired glucose-stimulated insulin secretion in a beta-cell lipotoxicity model overexpressing sterol regulatory element-binding protein-1c. Endocrinology 2004. 145: 3566–357.