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Original Research Article

Bio-computational analysis and characterization of Uncoupling protein 3 (UCP3) in different animals

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Abstract

Uncoupling Proteins are the unique anionic transporters present on the inner membrane of Mitochondria. These proteins are known for its association with obesity and type 2 diabetes, skeletal muscles and heart diseases in this new era, it play important role in thermogenesis by uncoupling, with production of ROS (Reactive oxygen species). Mutations causing these proteins to become nonfunctional, and thereby causing dysfunctions of the normal body activity. Characterization of these UCPs in different animals shows the protein activity and its functional approach by through computational tools. Here the amino acid distribution and aliphatic index computed by expasy protparam infers most of these proteins are membrane proteins and stable as a function in thermogenesis at wide range of temperatures 77 to 97(Aliphatic index by CLC) and Amino acid in different animals were tabulated, and the circular cladogram of UCPs were constructed. Sequence alignment was done based on consence region of UCP, proteins revealed highly conserved regions, differ maximum at 140-160 region. Protein homology prediction using (Phyre 2) reveals probability and scoring for a particular template and confidence level and percentage of Identity. The secondary structure analysis showed more number of coils and percentile turns (range: 27-34%). The positions of amino acids residues are ideal. The allowed and disallowed regions in Rampage are within the accepted limits for the model 3D structure of transport protein and mitochondrial carrier proteins was plotted 100% confidence, and percentage of identity 21% and 23% respectively. SOPMA reveals domains, motifs, and e-Values which are 1.23e-65.As these are the membrane proteins 3D structure analysis provide the importance of these proteins. SVM prot analysis for UCP3 proteins reveals functional probability; Ramachandran plot reveals phi and psi angles for the evaluation of residues with Pdb id 2LCK. By this, Uncoupling proteins are the functional markers that may consider for identification of obesity and type 2 diabetes and related disorders as competent drug targets for the disease and treatment by through bio computation and characterization.

Keywords: UCP3 protein, ROS (Reactive oxygen species), CLC work bench, Expasy Protparam, Phyre2, SOPMA, SVM prot, Ramachandran plot.

1. Introduction

UCP2 and UCP3 have been localized within 150 kb of each other on chromosome 11q13 [10]Both UCP2 and UCP3 genes consist of six coding exons. [33]With each exon encoding a putative trans membrane spanning region and at least one upstream non-coding exon.UCP3 encodes two forms of transcripts: the full-length message designated UCP3 L and UCP3 S, which truncates before the sixth coding exon via use of an alternative polyadenylation site. [13]

UCP3 are unknown for possible functions that include 1) control of adaptive thermogenesis in response to cold exposure and diet, 2) control of reactive oxygen species production by mitochondria, 3) regulation of ATP synthesis, and 4) regulation of fatty acid oxidation. [11]

In 1997 two paralegals of Ucp1 were discovered and named Ucp2 and Ucp3[7-9].These two novel uncoupling proteins share the same genomic locus being directly neighboring genes. They are about 75% identical to Each other on the amino acid level, while both share about 55% identity with Ucp1. It was soon established that these novel Ucps serve a different purpose than thermogenesis[10].The Ucp3 gene is predominantly expressed in skeletal muscle and [7]. One of the first observations doubting a role in thermogenesis - an energy wasting mechanism - was the transcriptional up regulation of the *Ucp3* gene upon starvation [11]. Additional physiological situations of increased *Ucp3* expression, among them acute exercise [12]streptozotocin induced diabetes[13] and cold exposure [14-15]. Weigle and coworkers noticed that a common physiological parameter of these situations is an elevation of circulating fatty acid levels and proposed this to be the responsiblecue [16]. It was fortified by lipid infusion experiments that did indeed lead to *Ucp3* up regulation and the function of Ucp3 to export fatty acid anions from the mitochondrial matrix [17].

Uncoupling protein 3 (UCP3) has significant amino acid homology to UCP2 (71%), and expression of UCP3 in humans is much greater in skeletal muscle than in any other tissue [18-19]. Several studies have shown that these uncoupling proteins are regulated by dietary alterations[18,20,21] thyroid hormones [21] and agonists of the β-3 adrenergic receptor, supporting the hypothesis that UCP2 and UCP3 could play an important role in energy metabolism and body weight regulation.

The biochemical properties of the protein as measured in mitochondrial proton leak assays by parallel recording of membrane potential and oxygen consumption infer a role for Ucp3 in the defense against radical oxygen species (ROS), mitigating their generation by mild uncoupling[22]. This possible function is corroborated by the finding that a product of ROS induced lipid peroxidation, 4- hydroxy-2-nonenal, specifically induces uncoupling by Ucp3 and that even a small reduction of membrane potential markedly decreases ROS production[22]. A controversial hypothesis which takes into account both physiological and biochemical data emphasizes that the export of fatty acids or hydroperoxy fatty acids and subsequent protonated re-influx of a certain fraction into the matrix would result in a net proton import detectable as mild uncoupling [24,25].

2. Materials and methods

Protein sequence retrieval: The Protein Sequences of Uncoupling Protein 3 of different Animals (12 sequences) were retrieved in FASTA format from NCBI database (Table1) used to get different parameters for analysis.

Amino acid Composition: The amino acid composition of selected proteins were computed using the tool CLC free workbench (www.clc.bio.com/..../clc-main-workbench), tabulated in (Table-2).[**Figure**:1]

Primary structure analysis - Percentages of hydrophobic and hydrophilic residues were calculated from the primary structure analysis by SOPMA (Table-3).

Physio-chemical parameters: ProtParam (http://www.expasy.org/tools/protparam.html)[26] computes various physicochemical properties that can be deduced from a protein sequence. No additional information is required about the protein under consideration [27].

The physicochemical parameters, theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction coefficient,instability index [28], aliphatic index [29] and grand average hydropathy (GRAVY)[31] were computed using the Expasy'sProtParam server [30], and tabulated in (Table 4).

SVM prot analysis (http://jing.cz3.nus.edu.sg/cgi-bin/svmprot.cgi.) which is a protein function prediction tool, and classification of distantly related proteins can be Analyzed.(Table-5).

Secondary structure prediction: The secondary structure was predicted by self-optimized prediction method with alignment by SOPMA server [32] [Table-3]

Sequence Homology Analysis: Method employed in alignment of divergent protein sequences, it is used to align divergent sequences in order, locally reduced gap penalties to encourage the opening up of new gaps at these positions.(**Figure**2) By CLC work bench sequence alignment and circular cladogram was done.(Figure3).

Tertiary structure Prediction: Tertiary structure prediction [33,34] (Figure 5) of Uncoupling proteins was performed using bioinformatics tools (www.sbg.bio.ic.ac.uk/phyre2/index.cgi). The modeled 3D structure were evaluated and validated with RAMPAGE (mordred.bioc.cam.ac.uk/…/rampage.php).(**Figure**4,6) and Ramchandran Plot Analysis for Tertiary structure (Figure7)

Table 1: Protein sequences retrieved from different animals

Aliphatic index	Soelectric point	Weight	Modification date	Description	Name	Organism	Length	Sequence type	Information
84.513	9.61	33.772 kDa	Not Available	Mitochondrial uncoupling protein 3 (Susscrofa)	gi|47522914|ref|NP_999214.1|	Not Available	308aa	Protein	gi|47522914|ref|NP_999214.1|
97.818	9.58	33.13 kDa	Not Available	Mitochondrial uncoupling protein 3 (gallusgallus)	gi|45383892|ref|NP_989438.1|	Not Available	307aa	Protein	gi|45383892|ref|NP_989438.1|
84.188	9.72	33.91 kDa	Not Available	Mitochondrial uncoupling protein 3 (*Musmusculus)*	gi|6678495|ref|NP_033490.1|	Not Available	308aa	Protein	gi|6678495|ref|NP_033490.1|
81.948	9.73	34.014 kDa	Not Available	Mitochondrial uncoupling protein 3 (*Rattusnorvegicus*)	gi|7110733|ref|NP_037299.1|	Not Available	308aa	Protein	gi|7110733|ref|NP_037299.1|
94.968	9.44	34.216 kDa	Not Available	Mitochondrial uncoupling protein 3 isoform UCP3L (Homo sapiens	gi|4507807|ref|NP_003347.1|	Not Available	312aa	Protein	gi|4507807|ref|NP_003347.1|
94.727	9.25	29.782 kDa	Not Available	Mitochondrial uncoupling protein 3 isoform UCP3S (Homo sapiens	gi|13259546|ref|NP_073714.1|	Not Available	275aa	Protein	gi|13259546|ref|NP_073714.1|
96.547	9.58	33.148 kDa	Not Available	Mitochondrial uncoupling protein 3 (*Meleagrisgallopavo*)	gi|734703834|ref|NP_001290093.1|	Not Available	307aa	Protein	gi|734703834|ref|NP_001290093.1|
81.543	9.55	34.205 kDa	Not Available	Mitochondrial uncoupling protein 3 (*Bos Taurus*)	gi|28849931|ref|NP_776635.1|	Not Available	311aa	Protein	gi|28849931|ref|NP_776635.1|
82.83	9.5	34.063 kDa	Not Available	Mitochondrial uncoupling protein 3 (*Antechinusflavipes*)	gi|42742053|gb|AAS45212.1|	Not Available	311aa	Protein	gi|42742053|gb|AAS45212.1|
79.614	9.37	34..19 kDa	Not Available	Mitochondrial uncoupling protein 3 (*Ovisaries*)	gi|820867025|ref|NP_001295510.1|	Not Available	311aa	Protein	gi|820867025|ref|NP_001295510.1|
83.408	9.57	34.37 kDa	Not Available	Mitochondrial uncoupling protein 3 (*Canis lupus familliaris*)	gi|50978696|ref|NP_001003047.1|	Not Available	311aa	Protein	gi|50978696|ref|NP_001003047.1|
77.314	9.65	33.92 kDa	Not Available	Mitochondrial uncoupling protein 3 (*Sparusaurata*)	gi|189031437|gb|ACD74889.1|	Not Available	309aa	Protein	gi|189031437|gb|ACD74889.1|

Table 2: frequency of Amino Acid for different Uncoupling Protein 3

Amino Acid	gi|47522914|ref|NP_999214.1|	gi|45383892|ref|NP_989438.1|	gi|6678495|ref|NP_033490.1|	gi|7110733|ref|NP_037299.1|	gi|4507807|ref|NP_003347.1|	gi|13259546|ref|NP_073714.1|	gi|73470834|ref|NP_001290093.1|	gi|28849931|ref|NP_776635.1|	gi|42742053|gb|AAS45212.1|	gi|820867025|ref|NP_001295510.1|	gi|50978696|ref|NP_001003047.1|	gi|189031437|gb|ACD74889.1|
Alanine (A)	0.088	0.101	0.091	0.081	0.087	0.095	0.101	0.087	0.100	0.077	0.087	0.091
Cysteine (C)	0.023	0.026	0.023	0.023	0.026	0.029	0.026	0.029	0.023	0.026	0.026	0.023
Aspartic Acid (D)	0.036	0.033	0.029	0.032	0.042	0.047	0.033	0.039	0.035	0.039	0.035	0.042
Glutamic Acid (E)	0.032	0.033	0.036	0.036	0.026	0.025	0.033	0.029	0.035	0.029	0.032	0.029
{Phenylalanine (F)	0.039	0.036	0.052	0.052	0.048	0.040	0.036	0.051	0.045	0.051	0.045	0.055
Glycine (G)	0.078	0.085	0.088	0.088	0.077	0.084	0.085	0.084	0.080	0.084	0.080	0.097
Histidine (H)	0.013	0.003	0.016	0.016	0.010	0.011	0.003	0.016	0.010	0.019	0.013	0.010
Isoleucine (I)	0.039	0.039	0.039	0.039	0.035	0.040	0.039	0.045	0.045	0.042	0.045	0.045
Lysine (K)	0.049	0.029	0.045	0.042	0.048	0.047	0.029	0.045	0.048	0.045	0.048	0.045
Leucine(L)	0.088	0.111	0.081	0.078	0.087	0.080	0.107	0.087	0.087	0.080	0.087	0.065
Methionine (M)	0.039	0.023	0.042	0.045	0.042	0.036	0.026	0.045	0.048	0.045	0.039	0.052
Aspargine (N)	0.036	0.029	0.023	0.023	0.029	0.029	0.029	0.026	0.035	0.026	0.029	0.032
Proline(P)	0.062	0.059	0.055	0.058	0.058	0.058	0.059	0.051	0.048	0.055	0.055	0.036
Glutamine (Q)	0.045	0.039	0.039	0.039	0.045	0.044	0.039	0.045	0.039	0.048	0.045	0.039
Arginine(R)	0.062	0.075	0.068	0.0710	0.058	0.055	0.075	0.061	0.058	0.055	0.061	0.068
Serine(S)	0.058	0.062	0.049	0.052	0.058	0.055	0.062	0.064	0.055	0.068	0.058	0.055
Threonine (T)	0.078	0.075	0.078	0.081	0.080	0.084	0.075	0.084	0.090	0.084	0.090	0.084
Valine(V)	0.091	0.101	0.097	0.097	0.099	0.098	0.101	0.074	0.074	0.084	0.080	0.087
Trtptophan (W)	0.008	0.007	0.010	0.010	0.006	0.004	0.007	0.006	0.010	0.006	0.006	0.010
Tyrosine(Y)	0.036	0.036	0.036	0.036	0.038	0.040	0.036	0.039	0.035	0.039	0.039	0.036

Figure 1: Circular cladogram (Tree topology) of Uncoupling Protein 3 of different animals

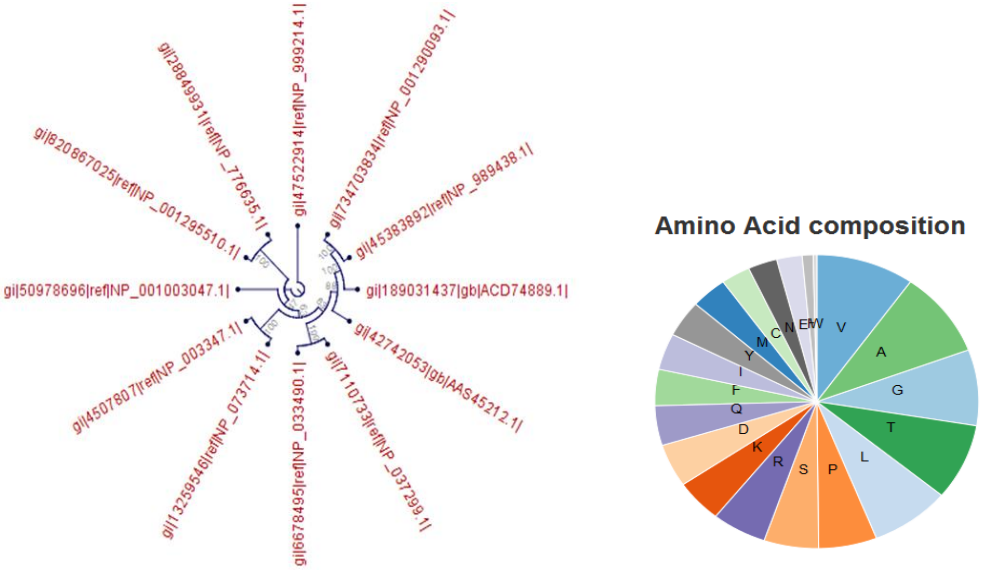


Figure 2: Sequence alignment

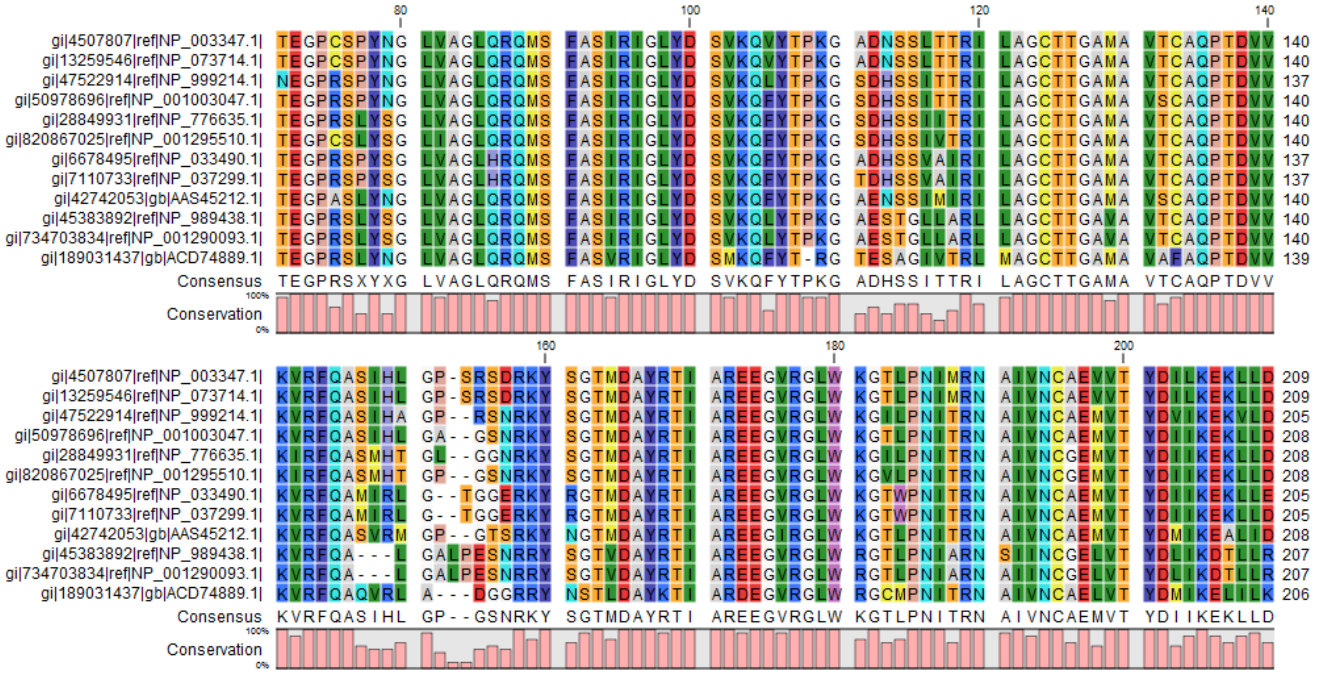
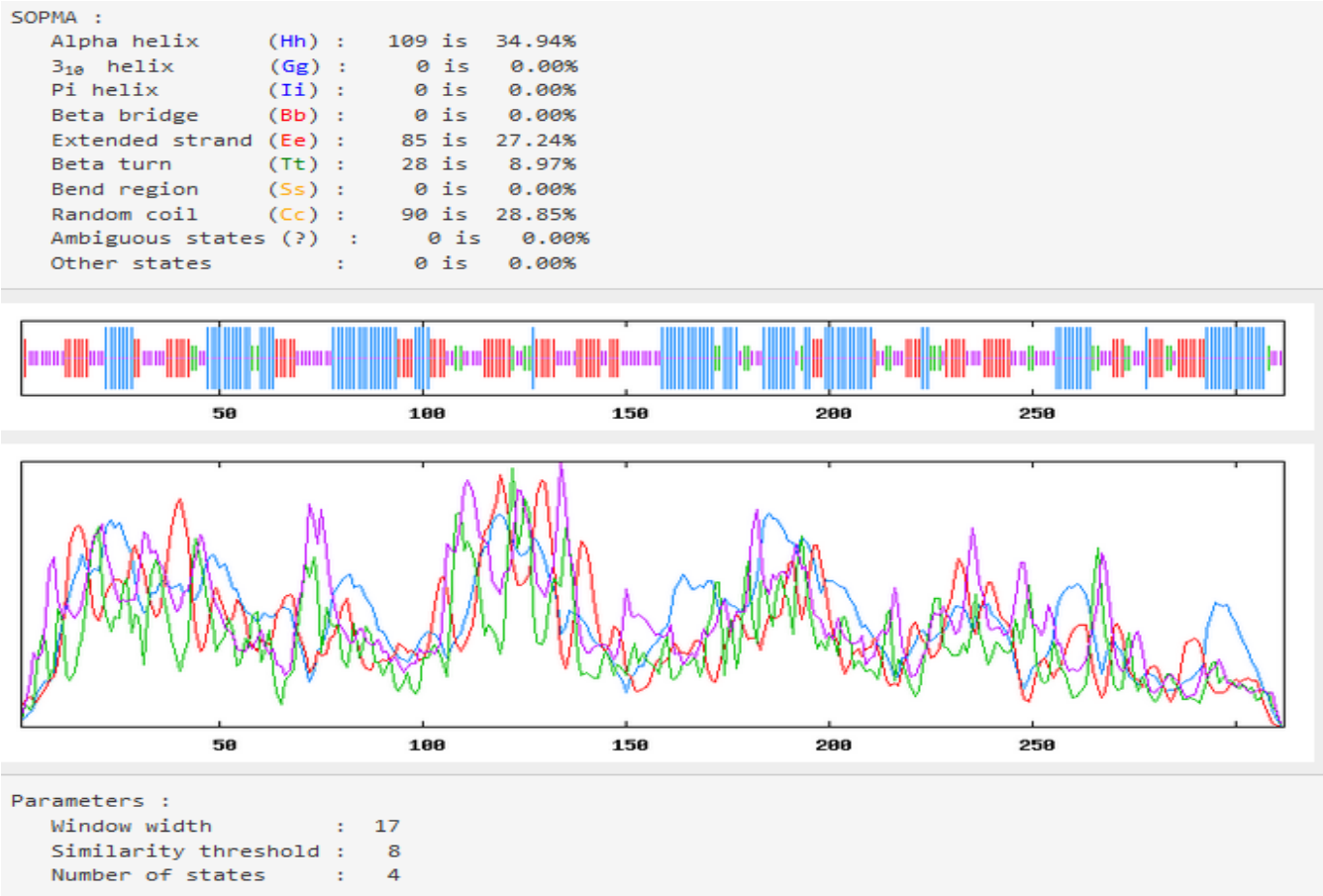


Figure 3: SOPMA prediction for Human Ucp3 L form



NPS@: Network Protein Sequence Analysis TIBS 2000 March Vol. 25, No 3 [291]:147-150

Table 3: Expasy protparam analysis for various parameters

Acccession No.	PI	Mol. Wt.	- R	+R	EC	II	AI	Gravy
097649	9.09	29782.05	20	28	0.752	37.02	84.73	0.063
P55916	9.31	34215.8	22	33	0.844	39.31	84.97	0.075
P56501	9.61	33910.6	21	35	0.97	40	84.19	0.076
077792	9.46	34205.7	21	33	0.844	45.9	81.54	0.018
P56499	9.63	34014.7	21	35	0.967	42.09	81.95	0.041
Q9N219	9.46	34137.6	21	34	0.846	42.07	83.41	0.006
Q6TGS8	10.02	33736.4	17	36	0.931	34.75	88.35	0.072
Q5XQS4	9.44	33673.1	21	33	0.858	40.06	84.51	-0.018
Q90X50	9.5	33148.6	20	32	0.826	39.73	96.55	0.194
V8N8K6	9.96	32481.6	19	43	0.921	41.26	74.93	-0.194
D5FGC8	9.54	33920.3	22	35	0.981	39.83	77.31	0.023
R4TWYP	9.24	34190.6	21	31	0.845	44.22	79.61	0.01

Table 4: SVM Prot Analysis

	Probability(%)											
Function	UCP3S [Homo sapiens]	UCP3L [Homo sapiens]	Rattus norvegicus	mus musculus	Gallus gallus	Sus scrofa	Bos taurus	Meleagris gallopavo	Canis lupus familiaris	Ophiophagus hannah	Sparus aurata	Ovis aries
	NP_ 073714.1	NP_ 003347.1	NP_ 037299.1	NP_ 033490.1	NP_ 989438.1	NP_ 999214.1	NP_ 776635.1	NP_ 001290093.1	NP_ 001003047.1	ETE 57968.1	ACD 74889.1	NP_ 001295510.1
EC3.2 Hydrolases - Glycosylases	98.6	89.3	—	—	—	68.5	71.3	—	71.3	73.8	85.4	—
TC2.A Electrochemical Potential-driven transporters - Porters (uniporters, symporters, antiporters)	73.8	86.8	99	99	—	85.4	96.1	58.6	83.9	76.2	85.4	97.5
EC1.14 Oxidoreductases - Acting on paired donors with incorporation or reduction of molecular O_2	—	65.4	89.3	86.8	—	71.3	—	—	71.3	—	83.9	—
TC3.A.3 P-type ATPase (P-ATPase) family	—	58.6	—	—	58.6	58.6	58.6	58.6	58.6	—	58.6	—
transmembrane receptor (metabotropic glutamate family)	—	58.6	58.6	—	—	58.6		58.6	58.6	—	58.6	58.6
Actin binding	—	58.6	58.6	58.6	—	58.6	58.6	58.6		—	58.6	58.6
Metal-binding	—	—	—	—	65.4	62.2		62.2	62.2	58.6	—	—
Transmembrane	89.3	99.2	99	—	98.1	99.1	99	97	99	—	99	99.1
Magnesium-binding	58.6	—	—	—	—		—	—	—	—	—	—
Zinc Binding	—	—	—	—	—	68.5	—	—	85.4	83.9	62.2	68.5
Outer membrane	—	—	—	—	—	58.6	58.6	—	—	—	58.6	58.6
Coat proteins	—	—	—	—	73.8	—	—	71.3	71.3	—	62.2	—

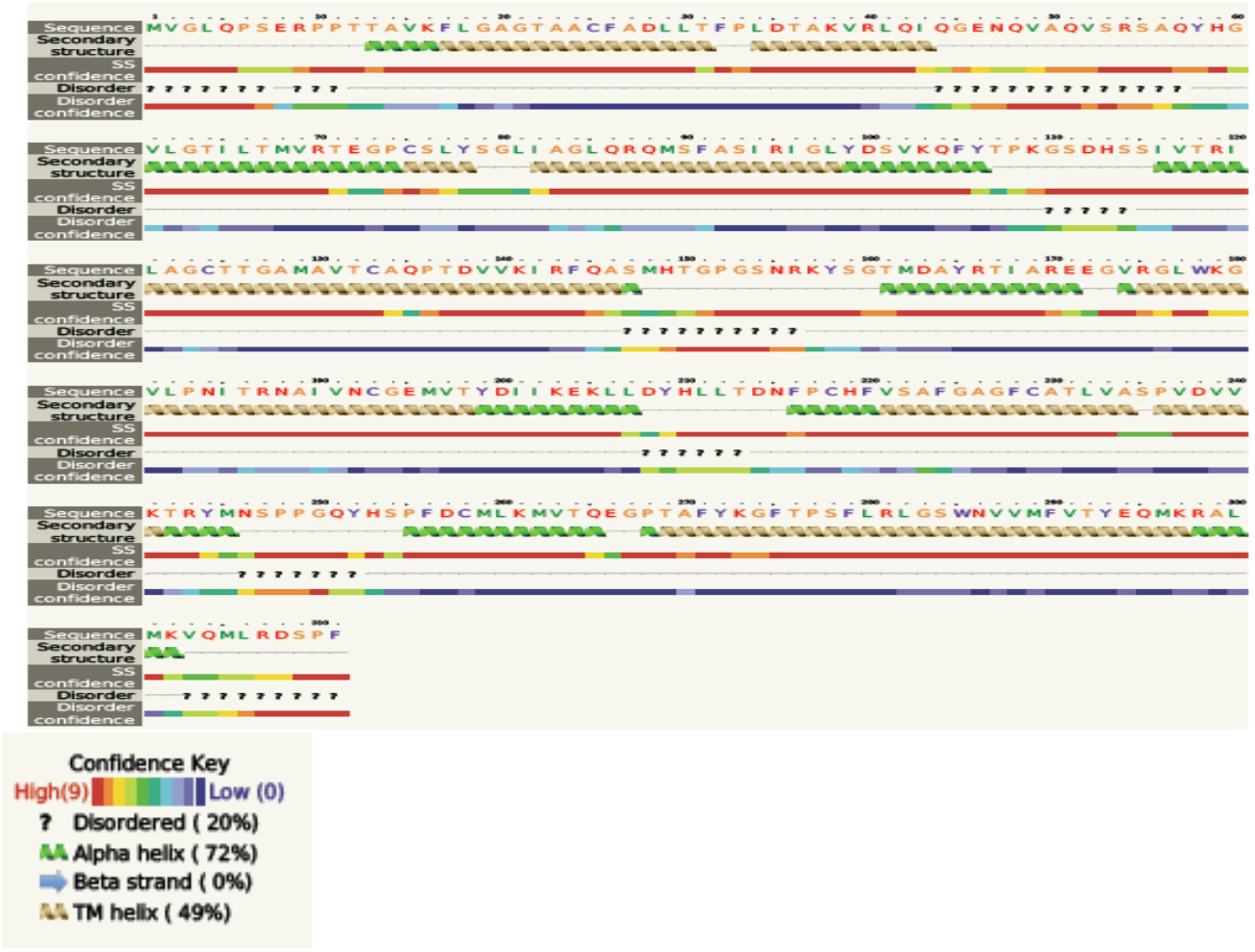


Figure 4: The structure is evaluated and validated for disorder confidence for secondary structure of UCP3

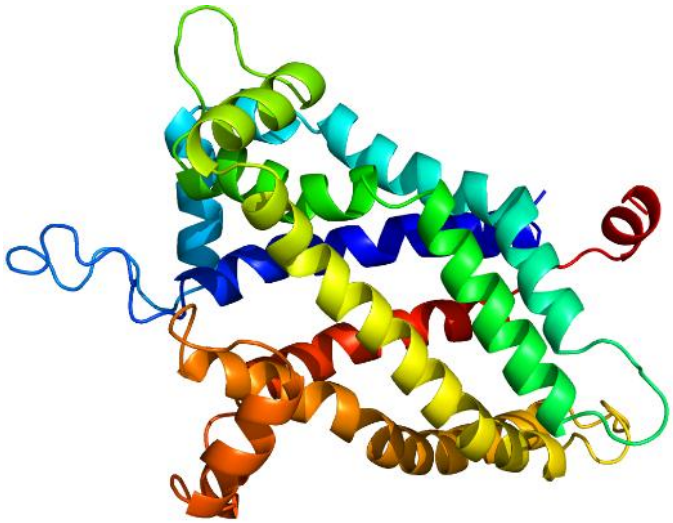


Figure 5: Modelled 3D Tertiary structure of UCP3

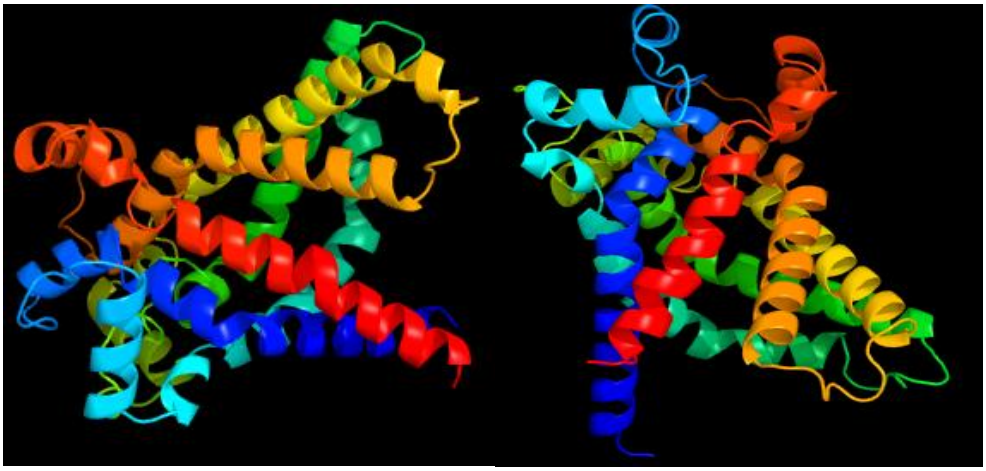


Figure 6: Mitochondrial transport and carrier protein of Human Uncoupling protein 3.

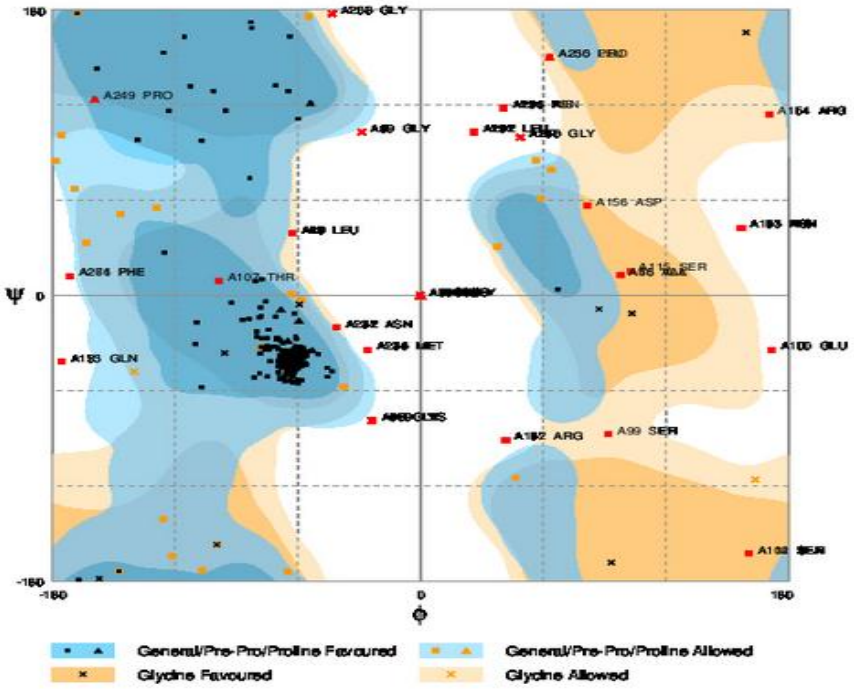
Transport protein of UCP3

Job Description	stucture_		
Confidence	100.00%	Date	Wed Aug 3 08:05:45 BST 2016
Rank	2	Aligned Residues	275
% Identity	21%	Template	c4c9qB_
PDB info	**PDB header:**transport protein	**Chain:** B: **PDB Molecule:**adp, atp carrier protein 3;	**PDBTitle:** structure of yeast mitochondrial adp/atp carrier isoform 32 inhibited by carboxyatractyloside (p21 crystal form)
Resolution	3.20 Å		
Model Dimensions (Å)	**X**:54.842 **Y**:52.888 **Z**:48.459		

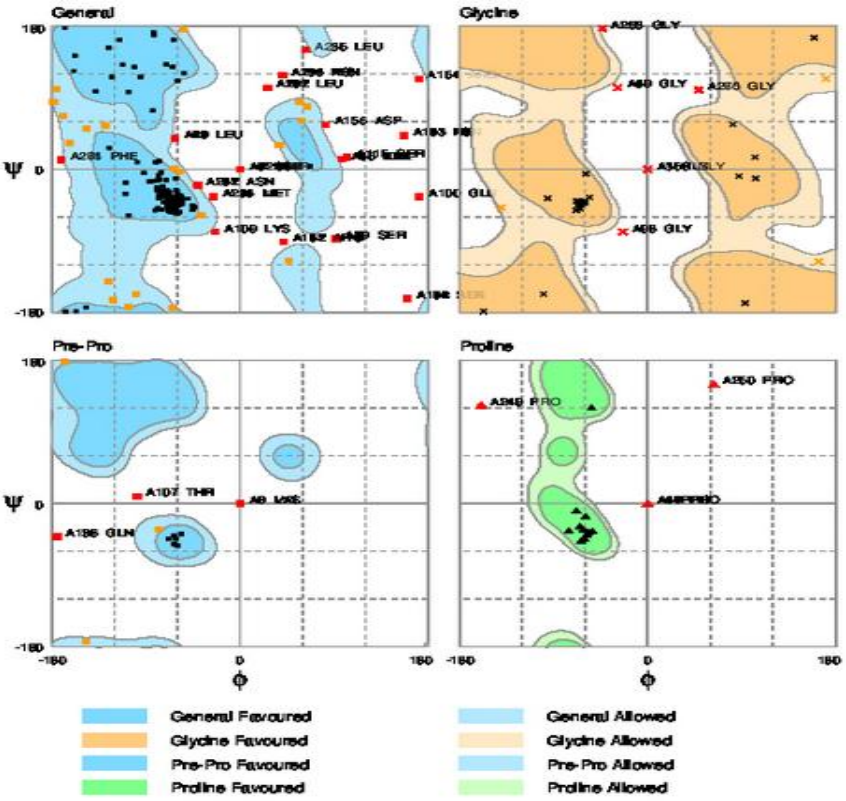
Mitochondrial carrier of UCP3

Job Description	stucture_		
Confidence	100.00%	Date	Wed Aug 3 08:05:45 BST 2016
Rank	3	Aligned Residues	284
% Identity	23%	Template	d1okca_
SCOP info	Mitochondrial carrier	Mitochondrial carrier	Mitochondrial carrier
Resolution	2.2		
Model Dimensions (Å)	**X**:50.524 **Y**:67.305 **Z**:50.678		

Figure 7: Ramchandran Plot Analysis for Tertiary structure



Ramchandran Plot for Uncoupling Protein with Pdb id : 2lck.1



Evaluation of Residues for both the Ψ and Φ angles.
Number of residues in favoured region (~98.0% expected) : 496 (83.8%)
Number of residues in allowed region (~2.0% expected) : 45 (7.6%)
Number of residues in outlier region : 51 (8.6%)

3. Results and Discussion

3.1. Amino acid composition:

The results of Primary sequence analysis of 12 Uncoupling protein 3 revealed the sequence length of all amino acids is found to be 307 amino acid in number, except UCP3S, tabulated in (Table 1), by through CLC work bench, we conclude that Tryptophan has least frequency in all UCPs. and Alanine, Glycine, valine present in large frequency in all uncoupling proteins. The aliphatic index computed the mitochondrial UCP3 protein of Galusgallus shows highest aliphatic index 97.81 with 33.13kDa.

Most abundant amino acids are serine, arginine, and threonine which are tabulated in CLC work bench.Abundant was found to be leucine in chicken, which promotes stimulation of insulin, this may act as a marker for obesity and type 2 diabetes levels in human. Serine, has a major role in formation of myelination sheath, Arginine and threonine has role in the phenomenon of neurotoxicity and nervous system function, one of the major causes for maintenance of proton gradient activity across the mitochondrial membrane that can be seen in NP_001290093.1 and NP_989438.1 (Table 2).

3.2. Primary sequence analysis:

The result of primary structure analysis suggests that most of the UCP3s are conserved and consence sequence had similarity in all the uncoupling proteins and each UCP has differed from one another in the region 140 to 160 nucleotide sequence alignment (Figure2). Presence of less content of Tryptophan that may lead to depression, Insomnia, Chronic fatigue etc.

The Alpha helix, extended strand, beta turn and random coil was predicted by through SOPMA, where 109 is 34.94%, 85 is 27.24%, 28 is 8.97%, 90 is 28.85% respectively.

3.3. Physico-chemical parameters:

The average molecular weights of uncoupling Protein3 were found in between 32481.6 – 34215.8 KDa, except O97649.

Protparam of Expasy computes atomic composition of carbon, hydrogen, Nitrogen, Oxygen, Sulphur is for a range of (1478, 2384, 410, 422 and 15) wavelength, 280nm is favored because proteins absorb strongly there while other substances commonly in proteins solution, do not.

Extinction co-efficient of uncoupling proteins at 280nm is ranging from 0.75- 0.98 M^{-1} Cm^{-1}.

ProtParam server predicted that O97649,P55916,P56501,O77792,P56499,Q9N2I9,Q6TGS8 ,Q5XQS4,Q90X50,V8N8K6,D5FGC8,R4TWY5, are having Asp+Glu no. is 20-32 infers ATP-dependent RNA activity part of neurotoxic activity.

Isoelectric point is the pH at which the surface of protein is covered with charge but net charge of protein is zero. pI of UCPs found to be membrane proteins Computed isoelectric point of proteins > 10 soluble in basic buffers. Isoelectric point is predicted ranges from 9.09 – 10.02 (Table 4). Useful for developing buffer system for purification of proteins.

The Aliphatic index of a protein is defined as the relative volume occupied by aliphatic side chains:alanine, valine, isoleucine, and Leucine of ACD7488, NP_003347.1, and with highest Aliphatic index of NP_989438.1 having 77.31, 84.96, and 97.81 respectively, which infers positive factor for thermo-stability between the species. [35]

The Grand Average hydropathy (GRAVY values) showed that all UCP3 proteins are hydrophilic ranging from 0.01 to -0.194, supports the soluble nature of uncoupling proteins. Though it can play a role in substrate recognition as membrane protein. Here the protein sequences showing negative that indicates stability of the protein. In particular, hydrophobic amino acids can be involved in binding/recognition of ligands.

A protein whose instability index is smaller than 40 are predicted as stable, and a value above 40 predicts that the protein may be unstable, here the instability index of all proteins found to be less than 50. [36] (Table 4)

Support vector machines (SVM) method for the classification of proteins with diverse sequence distribution. SVMProt shows a certain degree of capability for the classification of distantly related proteins and homologous proteins of different function and thus may be used as a protein function prediction tool that complements sequence alignment methods. It has been employed in protein studies including protein–protein interaction prediction probability, fold recognition, solvent accessibility and structure prediction. The prediction accuracy ranges from 58.6 to 99.1% in this study. Thus SVM classification of protein functional probability, a potentially developed into a protein function prediction tool to complement methods based on sequence similarity and clustering.

Based on the Classification of proteins of our interest and its values we predicted (Table5)we can come into conclusion that, these proteins may show the following functional probability and its action in mitochondrial anionic trans membrane protein, Electro chemical gradient, Metal binding sites, bonding involved trans membrane action and its probability functional percentage causing obesity in Type 2 diabetes is concerned.

3.4. Secondary structure prediction:

SOPMA was employed for calculating the secondary structural features of the selected protein sequences considered in this study. This method calculates the content of α-helix, β-sheets, turns, random coils and extended strands. SOPMA is a neural network based methods; global sequence prediction may be done by this sequence method.[37]

The secondary structure of alpha helix, beta turn, extended strand, random coil ranging from 27-34% predicted. The secondary analysis showed that UCP3 contain more random coils and alpha helices (range: 28-34%) in UCP3 L of humans. Sequence secondary structure and with Disorder confidence was generated with the confidence key disordered 20% and Trans membrane helix 49%.

Being hydrophobic, Leucine prefers to be buried in protein hydrophobic cores. It also shows a preference for being within alpha helices more so than in beta strands. The very high coil structural content of UCP3 is due to the rich content of more flexible glycine and hydrophobic Proline amino acids. Proline has a special property of creating links in polypeptide chains and disrupting ordered secondary structure.

SOSUI that predicts a part of the secondary structure of proteins from a given amino acid sequence (AAS). The main objective is to determine whether the protein in question is a soluble or a trans membrane protein. Here it is at all sequences of UCP3 are soluble proteins and has average hydropathy ranges from -0.03 to 0.200.

3.5 Sequence homology Analysis:

Multiple Sequence alignment by CLC work bench tool. Homology sequences revealed significant conserved (Leucine) and semi conserved regions (Proline, Alanine). Residues conserved for 90 % and above is 59 which is 42.75 % Residues conserved for 50 % and based on conserved and consensus, each sequence is differed in the region around 150 (Figure 2) and the Circular cladogram was generated for the tree view representation of UCP3 proteins of different animals (Figure 3).

3.6 Tertiary structure Prediction: The Tertiary structure Analysis of UCP3 proteins reveals the ideal 3D structures of PDB IDs were generated through Phyre2 (Figure 2). which predicts UCP3 S of human uncoupling protein , which shows highest % i.d. of 65%, with the use of psi-BLAST found 100% confidence which shows single highest scoring template (Figure 5). [38] Mitochondrial transport protein and carrier protein with 21% and 23%

identity with 100% confidence Modeled 3D structures were developed that is represented (Figure 6).

Ramchandran plot [39] (Figure 4) is an indication of the stereo chemical quality of the model taken for the structural analysis. Ramchandran plot displays the main chain torsion angles phi, psi (, ψ); (Ramchandran angles) in a protein of known structure. Dihedral angle checks Ramchandran plot shows phi-psi distribution. Each residue is classified according to its region 'favoured', 'allowed', 'generous', or 'disallowed'. Residues in the generous and disallowed regions are high-lighted on the plot.

Validation of results determined that the distribution of amino acid residues were at the most favorable region in the Ramchandran plot (more than 83.8%). The Crystallographic structures was developed for the PDB Ids 2lck.1 that may inturn functions as that of UCP3 shown in (Figure 7). In most favored regions in R-Plot suggests, the predicted UCP3 proteins is of good Quality. Very useful in molecular medicine as obesity and diabetes marker for designing a drug or to know the disease by through this in the field of biomedicine [40].

4. Conclusion

The present analysis entitles members of Uncoupling proteins selected from uniprot database showing high conservation suggests their functional similarity. In our studies we depicted that some of the protein families which has metal binding, trans membrane binding, immunity and functional probability of protein by through training, testing, independent evaluation by SVM Analysis prediction can say high sensitivity of UCP3 family proteins in all animals, which Play role in maintaining proton gradient and as unique transporter and mutation cause failure of normal functioning of uncoupling and blockage that may be lead to accumulation of acetyl CoA in and outside the mitochondria that affects lipotoxic pathway. From the present analysis it can be concluded that selected UCP3 proteins have high degree of homology between the animals.

Prediction infers that these are membrane proteins could result in better interaction with water [41]. Uncoupling proteins are important in maintenance of gradient, proton efflux and related disease conditions like obesity and type 2 diabetes, neuroscience, and pharmacology. These UCP3 components are highly variable and functionally complex and they offer many research opportunities[42].

UCP3 in its mutational effect and its emerging importance can turn into potential markers in obesity and type 2 diabetes related diseases and some deadly disorders which open new areas for future research.

In the present study the sequence and structure analysis of Uncoupling protein was done by various tools and software's. Based on the findings it could be concluded that further characterization UCP3 proteins is novel and will be important for evaluating how the regulation of this proteins is related in the complications connected to diet and over body mass and related complications. Although UCP3 proteins were initially identified as key members which participate in regulating the Proton Uncoupling of acetyl CoA and maintenance of Reactive Oxygen Species (ROS) and their strong association with obesity/diabetes and different types of disorders. Because of the functionality of these UCP3 proteins is rather complex, further studies may help to establish the relevance of individual family members as predictive markers and therapeutic targets for unbalanced diet leading to obesity and diabetes. Moreover, bioinformatics studies will aid in the development of improved molecular tools for the study of UCP3 proteins. Identification of novel UCP3 protein functions and crucial signaling events provide additional targets and new therapeutic approaches. Although significant progress has been made towards elucidating its role in causing disease and role as a marker in detecting the disease. Additional work needed to fulfill the regulation of UCP3 protein as if obesity and diabetes is considered.

Moreover, bioinformatics studies will aid in the development of improved molecular tools for the study of proteins like UCP3. It is becoming clear that UCP3 may have many important functions, and hydropathicity might contribute to its role in signaling and immunogenic responses. Identification of novel UCP3 definitive functions and crucial signaling events provide additional targets and new therapeutic approaches. Further work will be required in order to fully understand the role of uncoupling of mitochondria as a membrane protein.

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