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***Review Article***

# COMPARATIVE *IN SILICO* ANALYSIS OF ATP1A3 GENE IN MAMMALS

Chinmoy Mishra[2*], Siddhant Sekhar Sahoo[1], Sidharth Prasad Mishra[3], Stuti Tanaya Mohanty[4]
Gangadhar Nayak[5], Kumaresh Behera[6], Kamdev Sethy[7]

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**ABSTRACT: Progress in the field of bioinformatics is useful to understand the global network of genes and their protein products. Genetic variants of the ATP1A3 gene have been suggested to be involved in the salt hypertension and feed intake, all of which may evidently affect heat production, heat loss and water mineral balance. In the present study, comparative analysis of ATP1A3 (ATPase Na+/K+ Transporting Subunit Alpha 3) proteins of different livestock species were carried out using bioinformatics tools. The results of this study indicate that most of physico-chemical properties were almost same in pig and cat. The global network analysis of ATP1A3 gene by, STRING 10 tool speculated its interaction with several other proteins like - ATP1A1, ATP1A2, ATP1A4, ATP1B1, ATP1B3, FXYD2 in different livestock species having high confidence score. The strong interaction was seen between ATP1B1 and ATP1A3 with a high score in pig. Molecular docking between ATP1A3 and ATP1B1 of pig has been carried out. Conserved region was present in all the nine studied different livestock species protein sequences despite speciation due to evolution. The present study will further support to understand the role of associated proteins in various cellular pathways in cattle. This work is also useful for the study of structural and functional analysis of ATP1A3 protein.**

**Key words**: ATP1A3, Bioinformatics, Docking, Gene, STRING

## INTRODUCTION

The ATP1A3 gene encodes the alpha-3 catalytic subunit of the $Na^+/K(^+)$-ATPase transmembrane ion pump (Rosewich *et al.* 2012). The ATP1A3 catalyzes ATP-driven exchange of 3 intracellular $Na^+$ ions for 2 extracellular $K^+$ ions across the plasma membrane (Rodacker *et al.* 2006). The movement of sodium and potassium ions helps to regulate the electrical activity of these cells and plays an important role in the signaling process that controls muscle movement. The activity of $Na^+/K^+$ ATPase also helps to regulate cell size (volume). Additionally, $Na^+/K^+$ ATPase helps to regulate a process called neurotransmitter reuptake (McGrail *et al*. 1991, Bottger *et al*. 2011, Sugimoto *et al*. 2014).

The primary role of α subunit is to bind and transport $Na^+$ and $K^+$. There are four α subunits all encoded by different genes. The α 3 subunit, encoded by ATP1A3, is the predominant α subunit expressed in neurons (Dobretsov and Stimers 2005), although many neurons also express α1. The α3 subunit has a relatively low affinity for $Na^+$ and $K^+$ as compared to α1 which enables a rapid normalization of ion gradients after intense neuronal firing (Dobretsov *et al*. 2003, Crambert *et al*. 2000). Mutations in the ATP1A3 gene are associated with rare neurological disorders *viz.* Rapid-onset Dystonia-Parkinsonism (RDP) (deCarvalho Aguiar *et al.* 2004), Alternating Hemiplegia of Childhood (AHC) (Heinzen *et al*. 2014, Rosewich *et al*. 2012), Cerebellar ataxia, Areflexia, Pes cavus, Optic atrophy and Sensorineural hearing loss (CAPOS) syndrome (Demos *et al*. 2014). Studies of cortical neurons have shown that the ATP1 α3 subunit is a receptor for an endogenous ouabain-like

*[1] PhD Scholar, Animal Breeding Division, National Dairy Research Institute, Karnal, Haryana, India.*
*[2] Assistant Professor, [5] Professor and Head, Department of Animal Breeding and genetics, College of Veterinary Sciences and Animal Husbandry, Orisha University of Agriculture and technology, India.*
*[3] Ph D Scholar, Department of Animal genetics and Breeding, [4] Ph D Scholar, Department of Veterinary Gynecology and Obstetrics, West Bengal University of Animal and fishery Sciences, West Bengal, India.*
*[6] Assistant Professor, Department of Livestock Production and Management, [7] Assistant Professor, Department of Animal Nutrition, College of Veterinary Sciences and Animal Husbandry, Orisha University of Agriculture and Technology, India.*
**Corresponding author. e-mail: drchinmoymishra@gmail.com*

molecule (agrin) modulating neuronal activity *in situ*, further supporting its role in neuronal function (Hilgenberg *et al*. 2006, Brines and Robbins 1993, Cameron *et al*. 1994). In the present study the ATP1A3 gene was analysed *in silico* to delineate its structure and function.

## COLLECTION OF DATA

The UniProt is an easily accessible database of protein sequence [http://www.uniprot.org/]. Total nine protein sequences in FASTA format were retrieved from nine different livestock species for our study (Table 1).

### Physico-chemical characterization

ProtParam [http://web.expasy.org/protoparam/] is an Expasy tool which is useful for computation of physical and chemical properties of a given protein based on its amino acid sequence. The physico-chemical properties such as theoretical isoelectric point (pI), molecular weight, and total number of positive and negative residues, extinction coefficient, half-life, instability index, aliphatic index and grand average hydrophathy (GRAVY) of all ten retrieved protein sequences were calculated using ProtParam tool.

**Table 1. List of ATPase $Na^+/K^+$ transporting subunit alpha 3 (ATP1A3).**

| Sl. No. | Organism | Accession No. |
|---|---|---|
| 1 | *Canis lupus familiaris* | F1P767 |
| 2 | *Bos taurus* | F1MR06 |
| 3 | *Ovis aries* | W5PEI8 |
| 4 | *Sus scrofa* | F1RGF9 |
| 5 | *Equs caballus* | F6QQ59 |
| 6 | *Felis catus* | M3WHH0 |
| 7 | *Cavia porcellus* | H0VH73 |
| 8 | *Oryctolagus cuniculus* | G1TQW7 |
| 9 | *Gallus gallus* | P24798 |

### Sequence alignment and phylogenetic analysis

In order to study the comparison among different protein sequences, global multiple sequence alignment (MSA) program was used for analysis of ATP1A3 protein sequences from different animals. The Clustal Omega (Sievers *et al*. 2011) tool was used for MSA analysis. Understanding phylogenetic relationship among different protein sequences had delineated evolutionary relationship of these sequences by cladogram. The Prosite, ScanProsite (De Castro *et al*. 2006) tool was used to identify the number of hits for the predicted motif [http://prosite.expasy.org/scanprosite/].

### Analysis of gene ontology and protein-protein interaction network of ATP1A3

The gene ontology of ATP1A3 for biological, molecular functions was identified using Uniprot [http://www.uniprot.org/]. The STRING (Franceschini *et al*. 2013) (Search Tool for the Retrieval of Interacting Proteins) was used for studying the protein-protein interaction network of the ATP1A3 [http://string-db.org/].

### Three dimensional structure analysis and molecular docking

The homology modeling was used to build the 3D model of ATP1A3 based on homologous structure model. The structural templates that have highest sequence homology with our target template were identified by using PSI-BLAST [NCBI, http://blast.ncbi.nlm.nih.gov/Blast] against 3D structure available in PDB databank.

**Table 2. Physico-chemical properties of protein sequences.**

| Sl. No | Accession No. | Length | Molecular Weight | Theoretical pI | Total no. of Negative | Total no. of Positive | Extinction Coefficient | Instability Index | Aliphatic Index | GRAVY |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | F1P767 | 1014 | 111794.61 | 5.26 | 126 | 101 | 96270 | 36.44 | 94.9 | -0.012 |
| 2 | F1MR06 | 1033 | 113078.86 | 5.24 | 125 | 99 | 89530 | 36.73 | 94.56 | -0.005 |
| 3 | W5PEI8 | 1036 | 112951.33 | 5.24 | 123 | 97 | 95030 | 39.26 | 90.81 | -0.041 |
| 4 | F1RGF9 | 1013 | 111690.44 | 5.25 | 126 | 101 | 96270 | 36.42 | 95 | -0.017 |
| 5 | F6QQ59 | 1026 | 113027.81 | 5.26 | 127 | 102 | 97760 | 37.04 | 94.17 | -0.03 |
| 6 | M3WHH0 | 1012 | 111592.34 | 5.25 | 126 | 101 | 96270 | 36.42 | 95.09 | -0.014 |
| 7 | H0VH73 | 1011 | 111532.29 | 5.26 | 126 | 101 | 96270 | 36.76 | 95.18 | -0.016 |
| 8 | G1TQW7 | 953 | 104328.21 | 5.29 | 116 | 94 | 76790 | 36.5 | 93.82 | -0.025 |
| 9 | P24798 | 1010 | 111284.18 | 5.23 | 125 | 100 | 96395 | 37.31 | 95.48 | 0.007 |

**Table 3. No. of hits predicted using "P P R A A V P D A V G K C R S A G I K V I M V T G D H P I T A K A I A K G V G I I S E G N E T V E D I A A R L N I P V S Q V N P R D A K A C V I H G T D L K D".**

| Sl. No. | Name Of Protein | Accession No. | Species |
|---|---|---|---|
| 1 | Sodium/potassium transporting ATPase subunit alpha3 | P24798 | *Gallus gallus (Chicken)* |
| 2 | Sodium/potassium transporting ATPase subunit alpha3 | P13637 | *Homo sapiens (Human)* |
| 3 | Sodium/potassium transporting ATPase subunit alpha3 | P136372 | *Homo sapiens (Human)* |
| 4 | Sodium/potassium transporting ATPase subunit alpha3 | P136373 | *Homo sapiens (Human)* |
| 5 | Sodium/potassium transporting ATPase subunit alpha3 | Q6PIC6 | *Mus musculus (Mouse)* |
| 6 | Sodium/potassium transporting ATPase subunit alpha3 | P06687 | *Rattus norvegicus (Rat)* |

**Table 4. Sequence distance of ATP1A3 gene between different livestock species.**

| | Canis | Bos | Ovis | Sus | Equs | Felis | Cavia | Orycto | Gallus |
|---|---|---|---|---|---|---|---|---|---|
| *Canis_lupus_familiaris* | | 0.005 | 0.008 | 0.001 | 0.001 | 0.001 | 0.001 | 0.006 | 0.005 |
| *Bos_taurus* | 0.026 | | 0.007 | 0.005 | 0.005 | 0.005 | 0.005 | 0.006 | 0.008 |
| *Ovis_aries* | 0.075 | 0.053 | | 0.008 | 0.008 | 0.008 | 0.008 | 0.01 | 0.011 |
| *Sus_scrofa* | 0.002 | 0.028 | 0.076 | | 0.002 | 0.001 | 0.001 | 0.007 | 0.005 |
| *Equs_caballus* | 0.002 | 0.026 | 0.074 | 0.003 | | 0.002 | 0.001 | 0.006 | 0.006 |
| *Felis_catus* | 0.001 | 0.027 | 0.076 | 0.001 | 0.003 | | 0.001 | 0.007 | 0.006 |
| *Cavia_porcellus* | 0.001 | 0.027 | 0.075 | 0.002 | 0.001 | 0.002 | | 0.006 | 0.005 |
| *Oryctolagus_cuniculus* | 0.04 | 0.037 | 0.088 | 0.043 | 0.04 | 0.042 | 0.042 | | 0.009 |
| *Gallus_gallus* | 0.03 | 0.054 | 0.104 | 0.03 | 0.031 | 0.031 | 0.03 | 0.071 | |

**Table 5. Interaction of ATP1A3 (*Canis lupus familiaris*) with functional nodes.**

| Sl No. | Functional node | Score | Types of evidence for the association |
|---|---|---|---|
| 1 | ATP1B1 (sodium/potassium-transporting ATPase subunit beta-1) | 0.971 | Databases, Textmining, Experiments, Coexpression |
| 2 | FXYD2( sodium/potassium-transporting ` ATPase subunit gamma ) | 0.957 | Databases, Text mining, Experiments |
| 3 | ATP1B2(ATPase, Na+/K+ transporting, beta 2 polypeptide) | 0.951 | Databases, Text mining, Experiments, Coexpression |
| 4 | ATP1B3 (ATPase, Na+/K+ transporting, beta 3 polypeptide) | 0.939 | Databases,Text mining, Experiments, Coexpression |
| 5 | FXYD6 (FXYD domain-containing ion transport regulator 6 precursor) | 0.918 | Databases, Text mining, |
| 6 | FXYD1 (phospholemman precursor) | 0.908 | Databases, Experiments |
| 7 | ATP1A2 (ATPase, Na+/K+ transporting, alpha 2 polypeptide) | 0.901 | Homology, Databases, Text mining, Cooccurence |
| 8 | ATP1A4 (ATPase, Na+/K+ transporting, alpha 4 polypeptide) | 0.901 | Homology, Databases, Text mining, Cooccurence |
| 9 | ATP1A1( sodium/potassium-transporting ATPase subunit alpha-1 precursor ) | 0.901 | Homology, Databases, Text mining, Cooccurence |
| 10 | FXYD4( FXYD domain containing ion transport regulator 4 ) | 0.900 | Databases |

**Table 6. Interaction of ATP1A3 (*Sus scrofa*) with functional nodes.**

| Sl. No. | Functional node | Score | Types of evidence for the association |
|---|---|---|---|
| 1 | ATP1B1 (Sodium/potassium-transporting ATPase subunit beta-1) | 0.998 | Databases, Experiments, Text mining, Coexpression |
| 2 | FXYD2 (sodium/potassium-transporting ATPase subunit gamma) | 0.992 | Databases, Experiments, Text mining |
| 3 | ENSSSCG00000017951 (Uncharacterized protein) | 0.961 | Databases, Experiments, Text mining, Coexpression |
| 4 | ENSSSCG00000014896 (Uncharacterized protein) | 0.943 | Databases, Experiments, Text mining, Coexpression |
| 5 | ATP1B3 (ATPase, Na+/K+ transporting, beta 3 polypeptide) | 0.943 | Databases, Experiments, Text mining, Coexpression |
| 6 | ENSSSCG00000015083 (Uncharacterized protein) | 0.917 | Databases, Text mining |
| 7 | ENSSSCG00000021374 (Uncharacterized protein) | 0.908 | Databases, Experiments |
| 8 | ATP1A4 (ATPase, Na+/K+ transporting, alpha 4 polypeptide) | 0.901 | Homology, Databases, Text mining, Cooccurence |
| 9 | ATP1A3 (sodium/potassium-transporting ATPase subunit alpha-3) | 0.901 | Homology, Databases, Text mining, Cooccurence |
| 10 | ATP1A2 (Sodium/potassium-transporting ATPase subunit alpha-2) | 0.901 | Homology, Databases, Text mining, Cooccurence |

**Table 7. Interaction of ATP1A3 (*Bos taurus*) with functional nodes.**

| Sl. No. | Functional node | Score | Types of evidence for the association |
|---|---|---|---|
| 1 | ATP1B1 (sodium/potassium-transporting ATPase subunit beta-1) | 0.988 | Databases, Text mining, Experiments, Coexpression |
| 2 | ATP1B2( ATPase, Na+/K+ transporting, beta 2 polypeptide) | 0.984 | Databases, Text mining, Experiments, Coexpression |
| 3 | FXYD2 (sodium/potassium-transporting ATPase subunit gamma) | 0.964 | Databases, Text mining, Experiments |
| 4 | ATP1B3 (ATPase, Na+/K+ transporting, beta 3 polypeptide) | 0.961 | Databases, Text mining, Experiments, Coexpression |
| 5 | FXYD6 (FXYD domain-containing ion transport regulator 6 precursor) | 0.918 | Databases, Text mining |
| 6 | FXYD7 (FXYD domain-containing ion transport regulator 7 precursor) | 0.908 | Databases, Experiments |
| 7 | FXYD1 (phospholemman precursor) | 0.908 | Databases, Experiments |
| 8 | ATP1A3 (sodium/potassium-transporting AT Pase subunit alpha-3) | 0.901 | Databases, Homology, Text mining, Cooccurence |
| 9 | ATP1A2 (Sodium/potassium-transporting AT Pase subunit alpha-2) | 0.901 | Databases, Homology, Text mining, Cooccurence |
| 10 | ATP1A4 (ATPase, Na+/K+ transporting, alpha 4 polypeptide) | 0.901 | Databases, Homology, Text mining, Cooccurence |

The criteria such as percent sequence identity, e-value, chain length and query coverage were used. The model was built by SWISS-Model using target-templates alignment. The SAVES (Structural Analysis and Verification Server) is integrated server was used for verification of models [http://nihserver.mbi.ucla.edu/

SAVES/]. The molecular docking was performed between ATP1A3 and ATP1B1 using PatchDock followed by refining the structures using FireDock [http://bioinfo3d.cs.tau.ac.il/PatchDock/]. The Patchdock based on surface patch matching and more reliable docking tools with fast search for filtering and scoring. It uses advanced data structures and spatial search pattern that resulted into several structures, thus further filtered through FireDock. Docking score and atomic contact energy (ACE) of the both complexes were calculated using Patch Dock.

## RESULT ANALYSIS

For comparative analysis of the ATPase $Na^+/K^+$ transporting subunit alpha 3 (ATP1A3) from different livestock species, computational algorithms was used. Value of most of physico-chemical properties were almost same in F1RGF9 (*Sus scrofa*) and M3WHH0 (*Felis catus*) like theoretical pI, positive R group, negative R group, molecular weight and extinction coefficients etc. (Table 2). The value of instability index of all proteins was below 40 which indicate that all nine proteins were stable. The extinction coefficients (EC) value of ATP1A3 was calculated, which help in the protein-ligand and protein-protein interaction study**.** The pI value of all the nine protein sequences was less than 7 which indicate that all proteins are acidic in nature.

Multiple sequence alignment (MSA) can give insight into sequence conservation across several species and thus allow identification of those sections of the sequence most critical to protein function (Jankun-Kelly *et al*. 2009). Further, performing MSA, it was seen that **"**P P R A A V P D A V G K C R S A G I K V I M V T G D H P I T A K A I A K G V G I I S E G N E T V E D I A A R L N I P V S Q V N P R D A K A C V I H G T D L K D"is the conserved region (identical region) in all nine different protein sequences of ATP1A3 which indicates that this peptide sequence may have been maintained by evolution despite speciation (Fig. 1). Using Scan Prosite, total 6 numbers of hits for the above mentioned conserved region was perceived (Table 3).

The phylogenetic analysis of ATP1A3 amino acid sequence revealed that cattle and sheep were much closer to each other (Fig. 2). The porcine ATP1A3 amino acid sequence was aligned with the sequence of other species (dog, cat, cow, horse, sheep, chicken, guinea pig and rabbit) and sequence distance was estimated (Table 4). The model with lowest BIC score (JTT + I -Jones Taylor

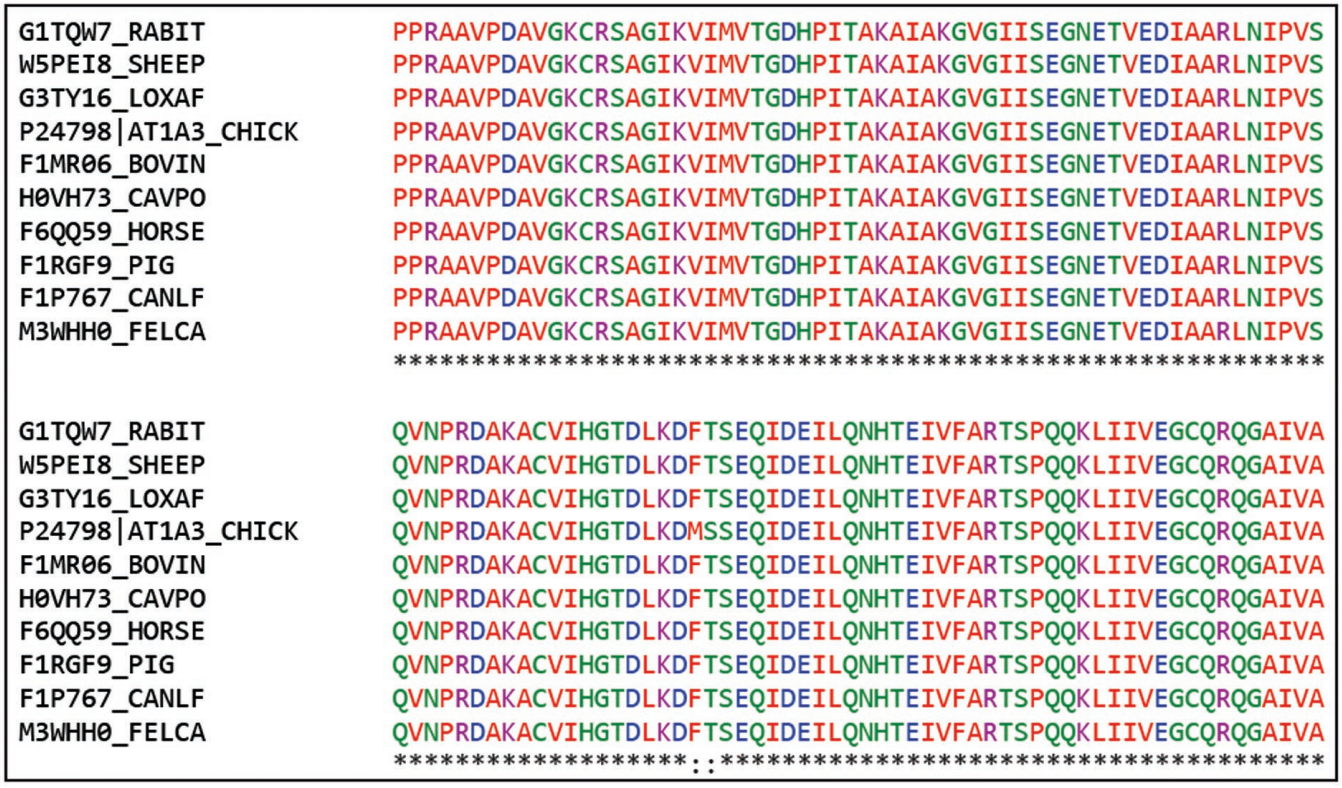


**Fig. 1. The snap sort of MSA result.**

[ "*" indicates identical in all sequences in the alignment; ":" indicates conserved substitutions; "." indicates semi-conserved. Selected conserved region is highlighted by blue box.]

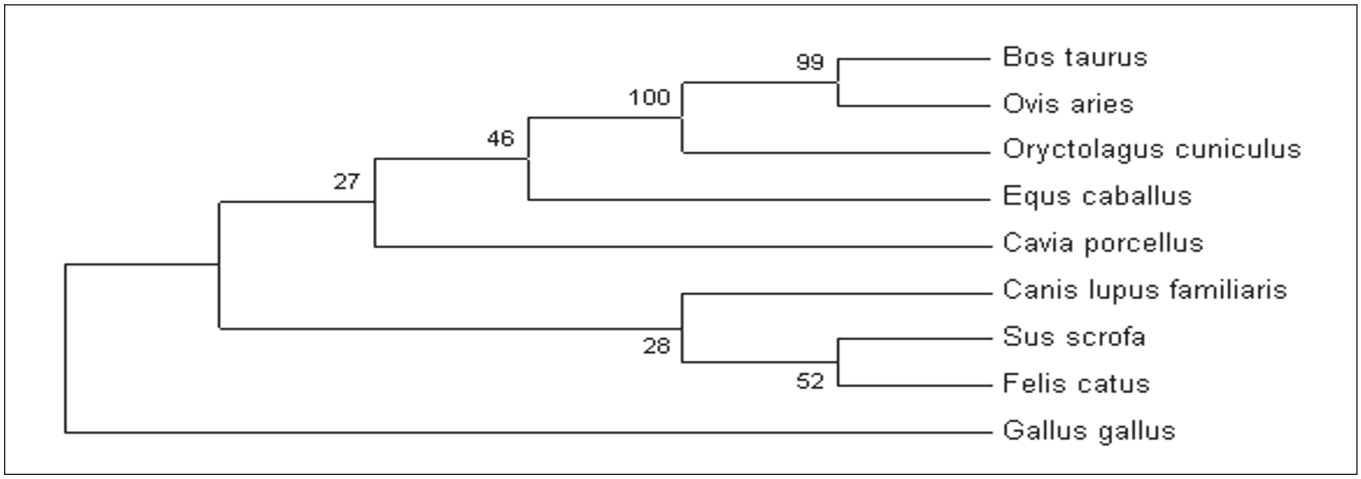


**Fig. 2. Phylogenetic tree showing the evolutionary relationships of retrieved protein sequences.**

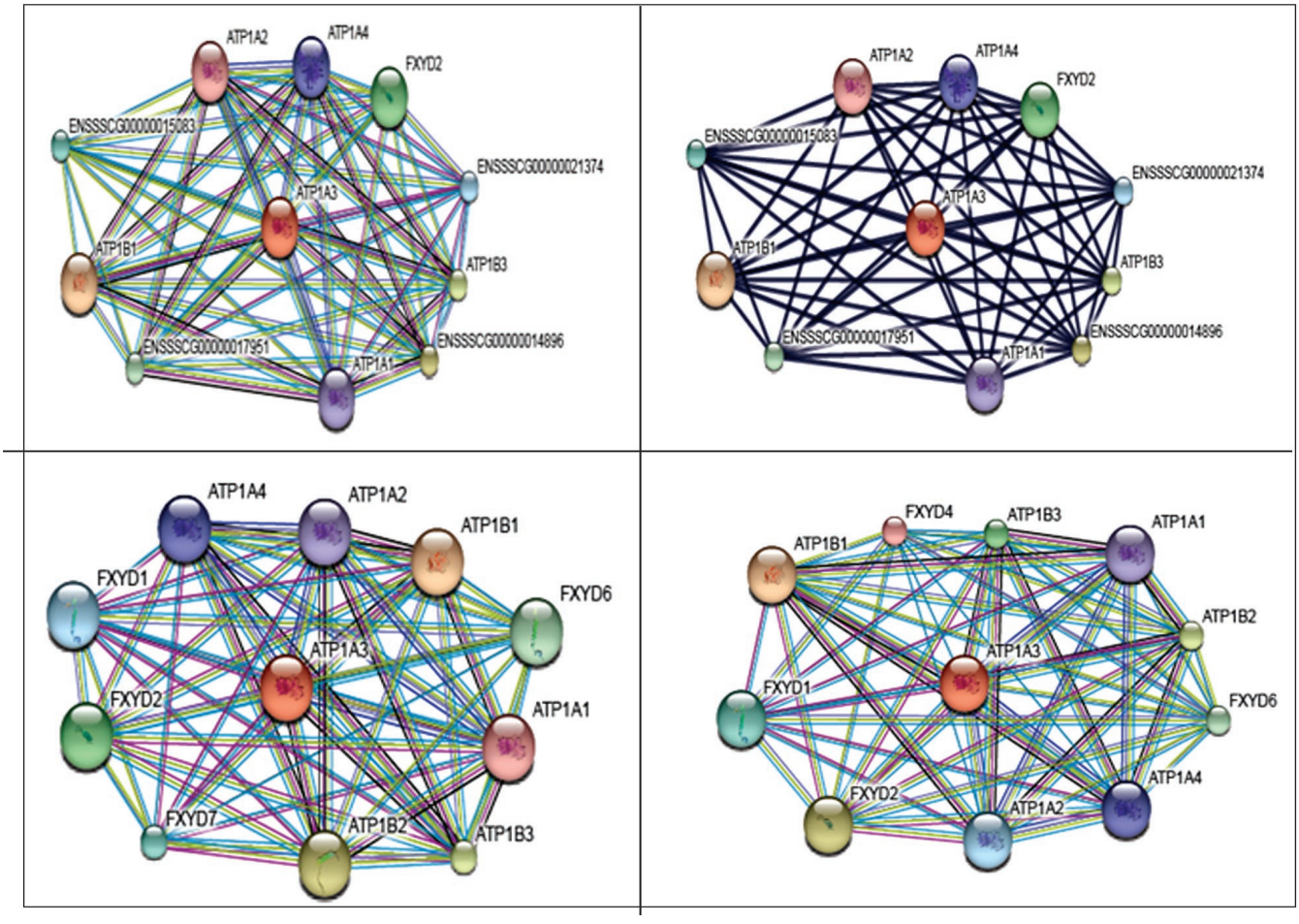


**Fig. 3. Protein interaction network of ATP1A3 and ATP1B1.**

1. (Upper-left) Evidence view of ATP1A3 protein network showing functional association with 10 proteins *(Sus scrofa*). Here, a node represents proteins; an edge represents the predicted functional associations. Different line colours represent the types of evidence for the association. Red line indicates the presence of fusion evidence; yellow line text miming evidence; Light blue line indicates database evidence; Black line indicates the co-expression evidence.
2. (Uprer-right) Confidence view of ATP1A3 network (*Sus scrofa)*. In this fig. stronger associations are represented by thicker lines.
3. (Lower-left) Evidence view of ATP1A3 protein network showing functional association with 10 proteins (*Bos taurus*).
4. (Lower-right) Evidence view of ATP1A3 network (*Canis lupus familiaris*).

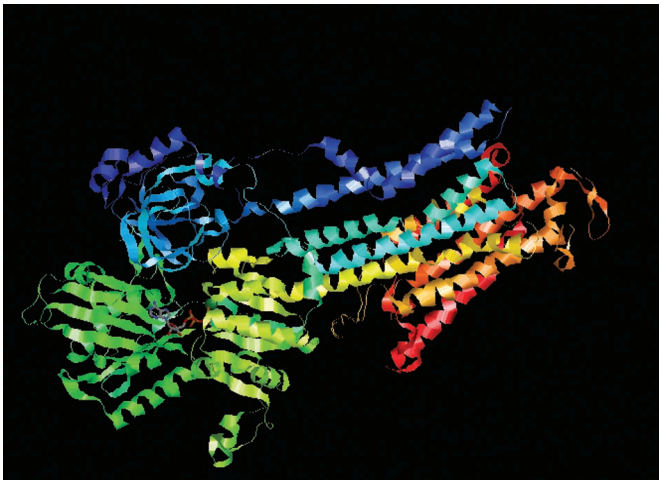

**Fig. 4. ATP1A3 protein model with conserved region in green colour obtained through homology modeling in pig.**

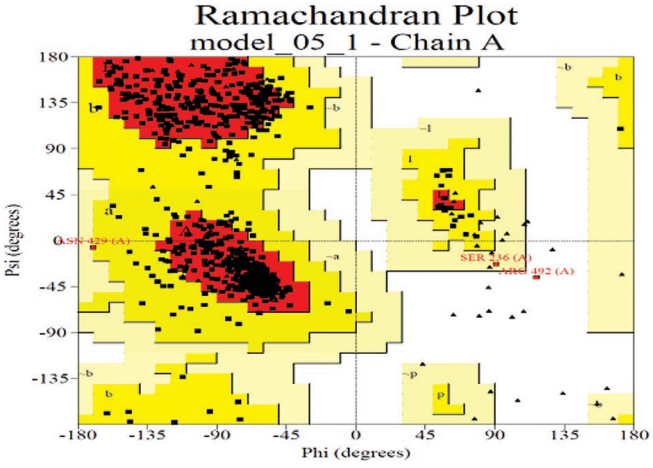


**Fig. 5. Ramachandran plot of model ATP1A3.**

[Figure showing, residues in most favoured regions 88.7%, Residues in additional allowed regions 10.9%, Residues in generously allowed regions 0.2% and Residues in disallowed regions 0.1%. Glycine residues are separately identified by triangles. The darkest areas (here shown in red) correspond to the "core" regions representing the most favourable combinations of phi-psi values.]

Plot statistics

| | | |
|---|---|---|
| Residues in most favoured regions [A,B,L] | 779 | 88.7% |
| Residues in additional allowed regions [a,b,l,p] | 96 | 10.9% |
| Residues in generously allowed regions [~a,~b,~l,~p] | 2 | 0.2% |
| Residues in disallowed regions | 1 | 0.1% |
| Number of non-glycine and non-proline residues | 878 | 100.0% |
| Number of end-residues (excl. Gly and Pro) | 2 | |
| Number of glycine residues (shown as triangles) | 73 | |
| Number of proline residues | 41 | |
| Total number of residues | 994 | |

Thornton with Invariant sites) was considered to be the best model in MEGA 7 for describing the substitution pattern. The amino acid sequence of porcine ATP1A3 showed highest identity with cat (99.99%). The

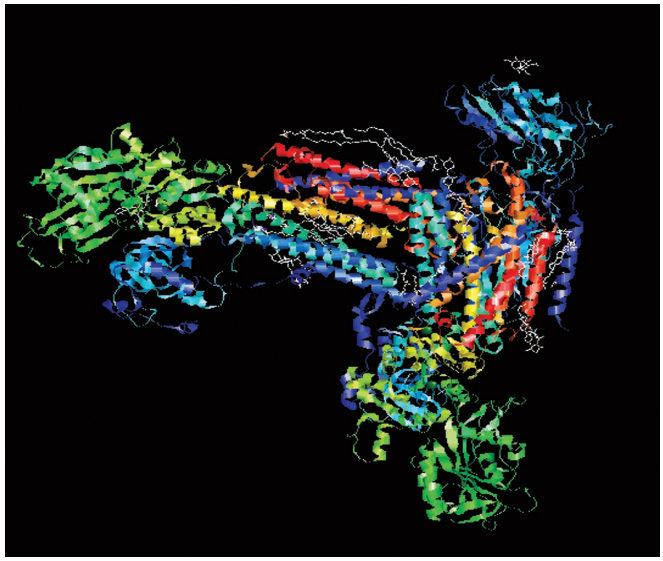

**Fig. 6. Best docking 3D model of ATP1A3 in pig from firedock.**

phylogenetic analysis of ATP1A3 amino acid sequence also revealed the close group of pig and cat (Fig. 2). All the livestock species clearly branched off as a separate cluster from poultry indicating that the ATP1A3 is functionally conserved during evolution.

Mutation in the ATP1A3 gene was associated with the disease AHC in humans. It mostly includes mutation at nucleotide positions 801$^{st}$, 815$^{th}$ and 947$^{th}$ positions which causes amino acid change Asp-Asn, Glu-Lys, Gly-Arg, respectively. Mutation at 815$^{th}$ position causes severe intellectual and motor disability followed by moderate phenotypic expression at 801$^{st}$ position. Mutation frequency was found to be least at 947$^{th}$ position (Panagiotakaki *et al*. 2015).

Protein-protein interaction investigation is a wide-ranging approach to know the organization of desire proteome (Kumari *et al*. 2015). The functional network protein study will be helpful for drug discovery to understand metabolic pathways and to predict or develop genotype-phenotype associations (Wang and Moult 2001, Wang *et al*. 2009). In order to understand network of ATP1A3 protein, analysis was done using STRING 10 which revealed that functional node - ATP1A1, ATP1A2, ATP1A4, ATP1B1, ATP1B3, FXYD2 were common in *Bos taurus, Sus scrofa and Canis lupus familiaris*. The interaction of ATP1B1 and ATP1A3 indicates the good high score in *Sus scrofa*. Protein-protein interaction networks are major part for the system-level understanding of cellular processes. All nine protein sequences were studied one by one for getting protein-protein interaction network. Here our interest to know that which functional node is common for ATP1A3 network in different animals. The analysis revealed

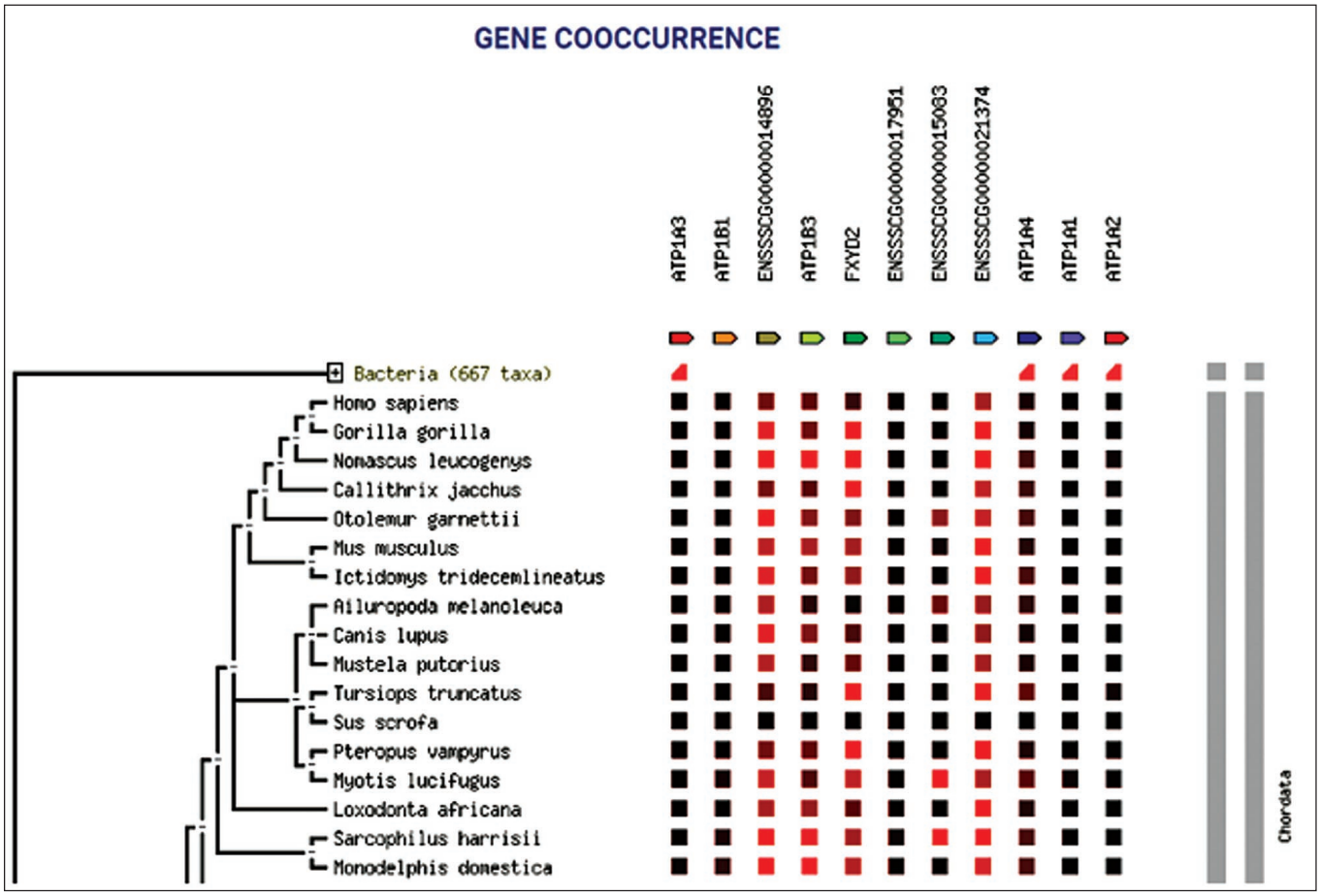


**Fig. 7. Snapshot of occurrence result conserved sequence across species.**

[Black colour indicates the 100% sequence conservation in *Sus scrofa* (inside red box).]

protein-protein interaction network only from three different animals of ATP1A3 *i.e. Bos taurus, Sus scrofa* and *Canis lupus familiaris* (Table 4, 5 and 6) and functional node-ATP1A1, ATP1A2, ATP1A4, ATP1B1, ATP1B3, FXYD2 were common in ATP1A3 protein network having high confidence score. In STRING, the functional interaction was analyzed using confidence score. Interactions with score < 0.3 were considered as low confidence, scores ranging from 0.3 to 0.7 were classified as medium confidence and scores > 0.7 yield high confidence (Franceschini *et al*. 2013). In *Sus scrofa* ATP1A3 protein network showing functional association with 10 proteins and they are ATP1A2, ATP1A1, ATP1A4, ATP1B1, ATP1B3, FXYD2, ENSSSCG00000015083, ENSSSCG00000017951, ENSSSCG00000014896, ENSSSCG00000021374 (Fig. 3). The result speculated the conserved occurrence of all 10 proteins in *Bos taurus, Sus scrofa* and *Canis lupus familiaris* with all nodes indicated 100% sequence conservation. ATP1B1 and ATP1A3 having the good high score in *Bos taurus, Sus scrofa* and *Canis lupus familiaris* which indicates the strong association. The best top ten protein-protein interaction network of ATP1A3 were demarcated.

ATP1B1 protein has shown the strong association with ATP1A3 protein in *Sus scrofa* using STRING 10 tool. So, to study the interaction at the structure level docking was done. The 3D structure of ATP1A3 of pig was obtained from Swiss-Model by performing homology modeling using retrieved homologous structures such as PDB ID; 4hqj.1.A, 3wgu.2.A, 3b8e.1.A, 4xe5.1.A with identity 87.58, 88.08, 87.65, 87.76 %, respectively (Fig. 4). The obtained 3D structure was verified with SAVES server along with Ramachandran plot which depicted 88.7% residues in most favoured regions, 10.9% residues in additional allowed regions, 0.2% residues in generously allowed regions and 0.1% residues in disallowed regions (Fig. 5). The molecular docking was performed between ATP1A3 and ATP1B1 using PatchDock followed by refining the structures using FireDock (Fig. 6). Docking score and atomic contact energy (ACE) of the both complexes were calculated using PatchDock. Both PDB structures were used for docking analysis. The docking between ATP1A3 and ATP1B1 revealed that global energy -6.83 and ACE, 2.78 were required, respectively.

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