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Structural and Functional Exploration of *UTS2B* Gene for a Possible Role in Poultry Ascites Syndrome Using *in Silico* Analysis

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Abstract

Ascites syndrome is a multifactorial disease in poultry, associated with high mortality and significant economic losses. Genomic studies have identified *UTS2B* gene as a candidate gene associated with ascites syndrome in poultry. Therefore, this study employed an *in silico* approach to explore the structural and functional characteristics of the *UTS2B* protein and its potential link to ascites. The amino-acid sequence of *UTS2B* protein was retrieved from the NCBI database and subjected to analysis through ProtParam, ProtScale, NetPhos, NetNGlyc, LocTree3, and SignalP tools to determine its sequence features and physicochemical properties. Secondary and tertiary structures were predicted using I-TASSER, AlphaFold, and trRosetta, with structural quality validated by PROCHECK (Ramachandran plot analysis) and ERRAT scores. Promoter analysis was performed using MEME Suite, TOMTOM, and GOMo to identify regulatory motifs. The *UTS2B* protein exhibited a theoretical isoelectric point of 5.67, an instability index of 42.33, and an average hydrophilicity coefficient of -0.199, indicating a hydrophilic and moderately unstable nature. It contains 12 predicted phosphorylation sites and 4 N-glycosylation sites, suggesting post-translational modifications that impact extracellular activity in peptide signalling pathways. Secondary structure analysis revealed a predominance of alpha helices and random loops. Among the predicted 3D models, the trRosetta structure achieved the highest quality, with 92.4% of residues in favorable regions of the Ramachandran plot and an ERRAT score of 85.6. Protein network analysis indicated that *UTS2B* may interact with proteins involved in pathways associated with calcium ion binding and cellular stress responses, both of which play a critical role in the pathophysiology of ascites. Promoter analysis identified regulatory motifs potentially linked to ion transport and inflammation, providing further insights into gene regulation. The findings suggest that *UTS2B* may play a role in calcium ion signaling and peptide-mediated extracellular pathways, contributing to ascites syndrome. This study presented critical bioinformatics insights into the *UTS2B* protein, providing foundational knowledge for future research aimed at understanding its role in ascites syndrome.

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Introduction

Ascites syndrome, also known as pulmonary hypertension syndrome (PHS) or "water belly" is a metabolic disorder characterized by generalized edema (Kaur *et al*., 2020), hypoxemia (Luger *et al*., 2003), right ventricular hypertrophy and heart failure (Janwari *et al*., 2018). The condition is primarily caused by metabolic stress, with high-altitude farming identified as the initial major risk factor (Hall *et al*., 1968). At higher altitudes, reduced atmospheric pressure decreases oxygen availability, resulting in pulmonary vasoconstriction and increased pulmonary arterial pressure (Ruiz-Feria *et al*., 2001).

High mortality of broiler chickens due to ascites, especially at later ages, causes significant economic losses to the poultry industry. Ascites is a multifactorial disorder mediated by several factors, such as nutritional, genetic, and environmental. The effects of different food ingredients and diets on ascites have been investigated in various studies (Jahanpour *et al*., 2020; Rajani *et al*., 2011; Tehrani *et al*., 2024). In this syndrome, the genetic background is associated with altered metabolic requirements for rapid growth in broilers, because selection of lines for high growth rate and low feed conversion increased the susceptibility of broilers to ascites (Decuypere *et al*., 2000; Hassanzadeh *et al*., 2010). In addition, selective breeding strategies, including the removal of susceptible chickens from breeding lines, have effectively improved genetic resistance to pulmonary hypertension in commercial poultry (Wideman, 2001). Moderate to high heritability has been reported for some traits associated with ascites syndrome in several studies (Lubritz *et al.*, 1995; Pakdel *et al*., 2005; Karami *et al*., 2020). It means that genetic factors play a significant role in the resistance and susceptibility of broiler chickens to ascites. Therefore, identification of genetic markers affecting ascites through marker-assisted selection (MAS) could reduce economic losses in the poultry industry with minimal impact on growth rate and feed conversion efficiency.

Advances in molecular biology, such as genomics, transcriptomics and proteomics, have revealed multiple genes (Shi *et al*., 2014), lncRNA (Ahmadian *et al*., 2024), microRNAs (Liu *et al*., 2016) and signaling molecules like endothelin-1 associated with ascites syndrome (Ruiz-Castañeda *et al.*, 2016). A total of 1421 lncRNA transcripts were identified corresponding to 921 loci and 154 target genes in kidney tissues of healthy and ascites broiler chickens (Ahmadian *et al*., 2024). Investigation of pulmonary artery tissues in broiler chickens with and without ascites revealed 29 miRNAs with significant different expressions, of which 18 and 11 showed up- and downregulated, respectively. Among 29 identified miRNAs, 20 seem to be involved in physiological processes such as hypoxia sensing response, endothelial permeability dysfunction and inflammation (Liu *et al*., 2016). Transcriptomic analysis found 390 differentially expressed genes in the liver tissue of broiler chickens in the control group compared to ascites, among which 212 and 178 genes were up- and downregulated, respectively (Shi *et al*., 2014). The kidney RNA-seq analysis identified 240 differentially expressed genes in male and female broiler chickens with ascites (Malekshahdehi *et al*., 2021). Furthermore, Davoudi and Ehsani (2019) highlighted 23 key genes involved, including *UTS2B*, a gene that encodes the urotensin 2B protein. This protein, along with those encoded by UTS2R and UTS2, plays a critical role in the G alpha signaling pathway (Davoudi and Ehsani, 2019; Cui *et al*., 2021). Despite these advances, the precise molecular mechanisms underlying ascites syndrome remain poorly understood. Structural and functional studies of *UTS2B* and its related proteins hold the potential to illuminate disease pathways. Furthermore, by unravelling the properties of *UTS2B*, researchers may better comprehend its role in the development and progression of ascites, paving the way for innovative treatment strategies. Therefore, the present study focuses on exploring the structural and functional characteristics of *UTS2B* to enhance our understanding of its contribution to ascites syndrome in poultry.

Materials and Methods

The chicken *UTS2B* gene information was sourced from the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov), specifically utilizing gene ID 404534 and protein ID NP_001383073.1. The amino acid sequence of *UTS2B* was submitted in FASTA format to the ProtParam module of the ExPASy online tool (https://web.expasy.org/protparam). This analysis provided insights into various physical and chemical properties, including amino acid composition, molecular weight, isoelectric point, instability index, and amino acid half-life coefficient. In proteins, carboxyl and amino groups are ionizable, and their charge depends on pH. Therefore, pH determines the charge of the protein molecule. In a protein molecule, the isoelectric point is defined as the pH at which the protein has no net charge (Xia, 2007). The instability index less than 40 is noted as a stable protein, as values above 40 indicate instability (Guruprasad *et al*., 1990). The half-life index of a protein is defined as the required time to degrade half of the protein present in a cell following its synthesis. It is significantly influenced by the identity of the N-terminal residue. This relationship is a key aspect of the N-end rule, which posits that specific amino acids at the N-terminus can dictate the metabolic stability

of proteins, thereby affecting their degradation rates (Bachmair *et al*., 1986).

To evaluate the hydrophilic and hydrophobic characteristics of the *UTS2B* sequence, the amino acid sequence was analyzed using the ProtScale module of the ExPASy server (https://web.expasy.org/protscale) with the Kyte-Doolittle algorithm. This assessment aids in predicting the protein's secondary structure and functional domains (Kyte and Doolittle, 1982).

The *UTS2B* amino acid sequence was analyzed for potential phosphorylation and glycosylation sites using the NetPhos (Blom *et al*., 2004) (https://services.healthtech.dtu.dk/services/NetPhos-3.1) and NetNGlyc (Gupta and Brunak, 2002) (https://services.healthtech.dtu.dk/services/NetNGlyc-1.0/) online tools.

Determining the subcellular localization of proteins provides critical insights into their biological roles, as the function of a protein is often closely associated with its cellular compartment (Wang *et al*., 2005). The subcellular location was determined by the LocTree3 (Goldberg *et al*., 2014) module of the Rostlab web resource (https://www.rostlab.org). Additionally, the presence and cleavage potential of a signal peptide within the amino acid sequence were predicted using SignalP (Teufel *et al*., 2022) software (https://services.healthtech.dtu.dk/services/SignalP-6.0).

The NCBI Protein Data Bank (PDB) was searched to identify suitable templates for *UTS2B* using BLAST, PSI-BLAST, and DELTA-BLAST algorithms. The secondary and tertiary structures of *UTS2B* were predicted using I-TASSER (Zhang, 2008) (https://zhanggroup.org/I-TASSER), AlphaFold (Jumper *et al*., 2021)(https://alphafold.ebi.ac.uk) and trRosetta (Du *et al*., 2021) (https://yanglab.nankai.edu.cn/trRosetta), with comparative analysis of the results.

The stereochemical quality of the predicted *UTS2B* models was assessed using PROCHECK (Mead *et al*., 2019) (https://www.ebi.ac.uk/thornton-srv/software/PROCHECK) and ERRAT (Pontius *et al*., 1996) (https://saves.mbi.ucla.edu) tools. This evaluation was crucial for selecting the optimal model for identifying potential ligand binding sites. Protein-protein interactions were predicted using the STRING 11.0 (Szklarczyk *et al*., 2019) database (https://string-db.org), which integrates data from various sources for protein network analysis, signaling pathway identification, and metabolic pathway analysis (Szklarczyk *et al*., 2021).

The 5’ upstream untranslated regions (5’-UTRs) of the *UTS2B* gene, approximately 1 kb in length, were retrieved from the NCBI Gene database (https://www.ncbi.nlm.nih.gov/gene/404534). To identify conserved sequence patterns, the MEME Suite (Bailey *et al*., 2015) was employed, with parameters set to P-values and E-values of less than 0.01 and 0.0001, respectively. The TOMTOM (Gupta *et al*., 2007) tool (version 5.4.1) (http://meme-suite.org/tools/tomtom) was utilized to eliminate repetitive motifs and identify known cis-regulatory elements (CREs) based on the JASPAR (non-redundant) DNA database (JASPAR CORE vertebrate). Additionally, the GOMo (Buske *et al*., 2010) tool (http://meme-suite.org/tools/gomo) was applied to explore potential ontologies associated with these motifs.

Results

Physical and Chemical Properties

The *UTS2B* protein comprises 126 amino acids with a molecular weight of 14363.60 Daltons and a molecular formula of C653H1021N167O187S5. Its theoretical isoelectric point is 5.67. Among its amino acid residues, 17 exhibit a negative charge (Asp + Glu); while 15 possess a positive charge (Arg + Lys), constituting 13.4% and 11.9% of the total amino acids, respectively. The instability index is 42.33, categorizing *UTS2B* as an unstable protein, as values above 40 indicate instability. For the *UTS2B* protein, the half-life has been empirically determined to be 30 hours *in vitro* within red blood cells.

Hydrophobicity/Hydrophilicity

The hydrophobic properties of the *UTS2B* protein were analyzed using the Kyte and Doolittle algorithm, which yielded an average hydrophobicity score of -0.199. This result, coupled with an aliphatic index of 92.86, suggests that *UTS2B* is predominantly hydrophilic. As illustrated in Figure 1, the analysis highlights specific regions of the protein with distinct hydrophobicity profiles. Notably, the amino acid residue Val20 exhibits the highest hydrophobicity, with a hydrophobicity index of 2.722, whereas Tyr52 represents the most hydrophilic residue with a value of -3.267.

These values indicate that Val20 contributes maximally to the hydrophobic character of the protein, while Tyr52 significantly enhances its hydrophilic nature. The distribution of hydrophilic residues throughout the peptide chain underscores the overall hydrophilic behavior of the *UTS2B* protein. This finding aligns with the average hydrophobicity index of -0.199, as determined using the ProtParam tool, further reinforcing the classification of *UTS2B* as a hydrophilic protein.

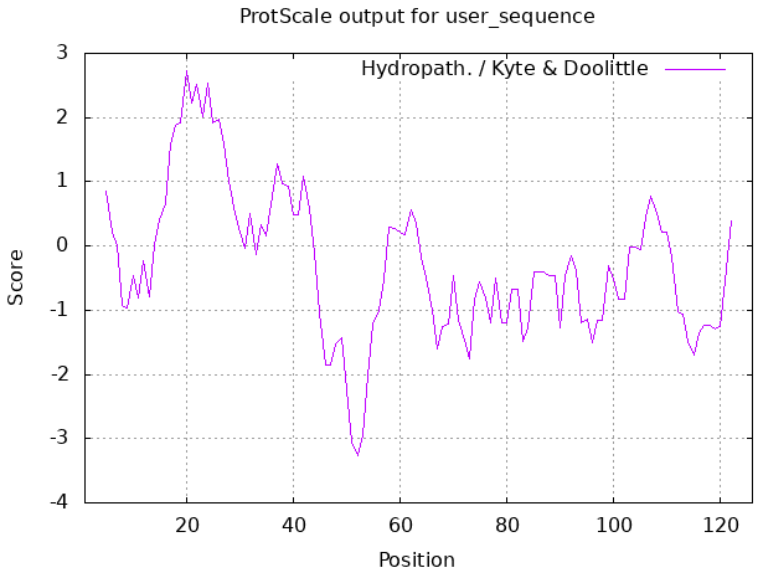


Figure 1. *UTS2B* protein hydrophilicity prediction: a positive score indicates hydrophobicity and a negative score indicates hydrophilicity; the higher the average absolute value of this index, the higher the degree of hydrophilicity.

Post-transcriptional modification

Phosphorylation plays a pivotal role in regulating gene expression by modulating protein function and activity. For the *UTS2B* protein, a comprehensive analysis identified 12 phosphorylation sites, categorized into six serine (S13, S17, S96, S101, S109, and S113), four threonine (T27, T33, T52, and T70), and two tyrosine phosphorylation sites (Y7 and Y29). Notably, serine and threonine residues constituted the majority of the phosphorylation sites. Among these, the highest predictive scores for phosphorylation were observed for S96 (0.997), T52 (0.929), and Y29 (0.738), highlighting their potential significance in functional regulation (Figure 2).

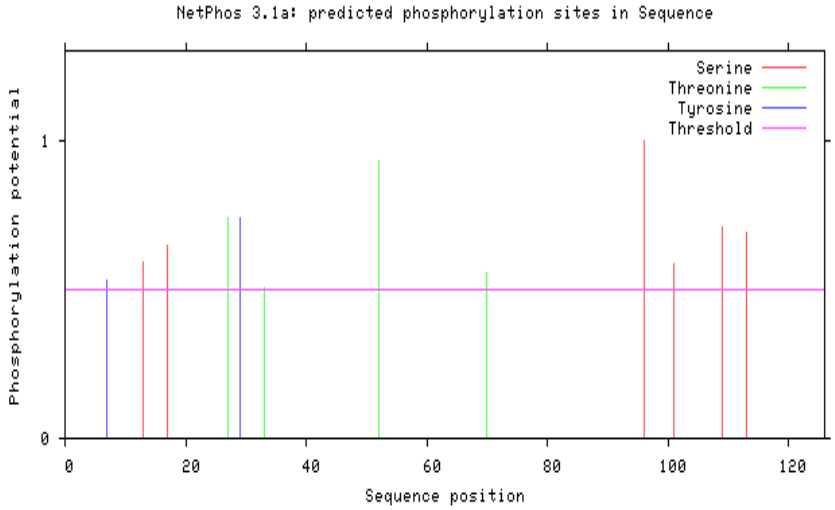


Figure 2. *UTS2B* protein phosphorylation sites: red lines indicate serine phosphorylation sites, green lines indicate threonine phosphorylation sites, blue lines indicate tyrosine phosphorylation sites, and purple lines indicate thresholds. Only phosphorylation sites with a score above the 0.5 threshold were identified.

Additionally, four N-glycosylation modification sites were identified at positions N43, N53, N63, and N73, with predictive intensities of 0.8072, 0.6131, 0.5880, and 0.5394, respectively (Figure 3).

Subcellular Location and Signaling Peptide

Our analysis predicts that the *UTS2B* protein is predominantly localized in the extracellular region, with a confidence score of 17 and an accuracy of 82%. Using a neural network algorithm, the protein

was also identified as highly likely to contain a signal peptide, with a probability of 0.9998 compared to a probability of 0.0002 for the absence of such a peptide (Figure 4). This strongly supports the hypothesis that *UTS2B* is a secretory protein. The predicted cleavage site of the signal peptide was located between amino acid residues 35 and 36, with a high confidence probability of 0.966634. These findings emphasize the functional importance of *UTS2B* in extracellular processes and its potential role as a secreted protein, which could have implications for its involvement in intercellular signaling pathways or as a therapeutic target.

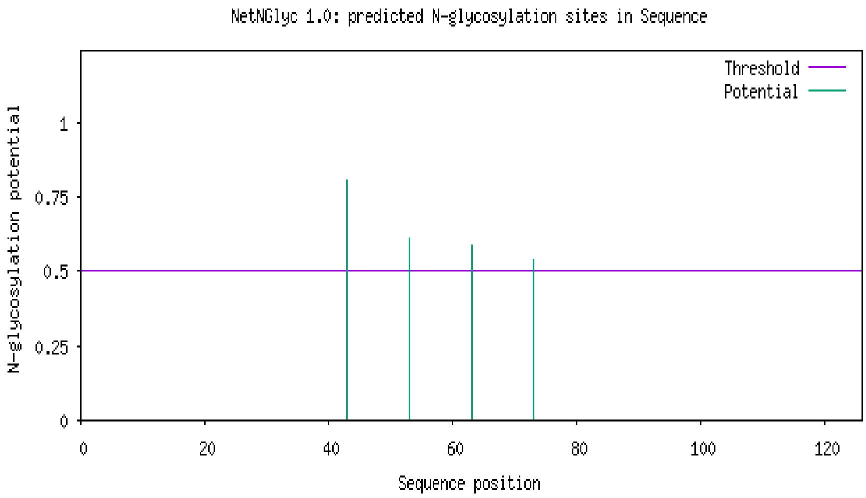


Figure 3. Glycosylation site prediction: green lines indicate potential glycosylation sites and the purple line indicates the threshold.

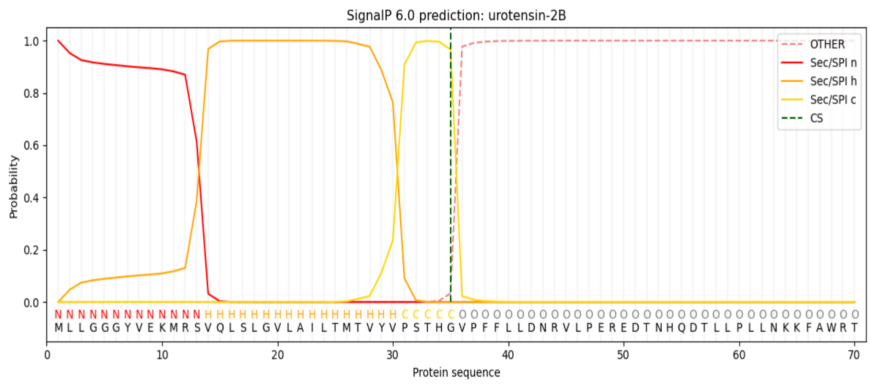


Figure 4. Signal peptide prediction: Continuous lines indicate the probability of peptide signal at the N-terminus (red), C-terminus (yellow), or center (h) (orange), the dashed green line indicates the cleavage site and the dashed pink line indicates the absence of signal peptide. At the beginning of the sequence, the red line is elevated and then decreases. The orange and yellow lines also increase and then decrease, indicating the presence of the peptide signal in these areas. At the point, the yellow line falls (end of c), the cleavage site of the signal peptide is indicated. After this site, the pink dashed line increases, which indicates the absence of signal peptide in this area.

Primary, Secondary and Tertiary Structure Prediction

The protein structure prediction relies heavily on available homologous 3D structures. For the *UTS2B* protein, a search in the NCBI Protein Data Bank revealed no homologous 3D structures, rendering traditional homology modelling methods unsuitable for its tertiary structure prediction. In such cases, *Ab initio* structure prediction provides an alternative, predicting the protein's structure based on its amino acid sequence alone, guided by physical and chemical principles (Chivian *et al*., 2003). Additionally, hybrid approaches combine the principles of homology modelling and *Ab initio* methods to find the protein 3D structure (Dorn *et al*., 2008). Tools such as Rosetta, I-TASSER, and AlphaFold have demonstrated remarkable efficacy in predicting protein structures, combining computational

efficiency with high accuracy. These methodologies expand the possibilities for investigating the structural and functional dynamics of proteins like *UTS2B*, despite the absence of homologous templates.

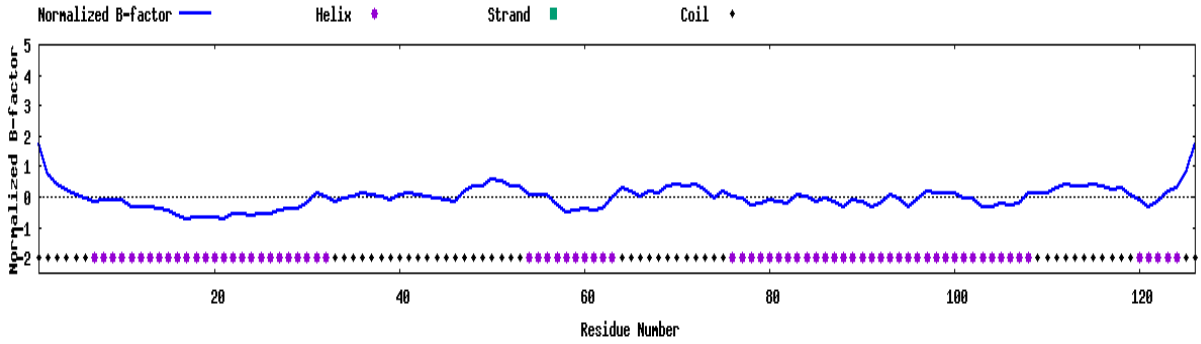


Figure 5. Secondary structure prediction: purple and black dots represent alpha helix and random coil, respectively. The blue line shows the normalized B coefficient. Negative values indicate that the residue is relatively stable in the structure.

The I-TASSER algorithm was employed to predict the secondary and tertiary structures of the *UTS2B* protein, revealing the presence of four alpha-helical regions comprising 55 amino acids and random loop structures in other regions, as shown in Figure 5. To achieve a detailed three-dimensional structure, computational modelling was conducted using I-TASSER, AlphaFold, and trRosetta. The resulting models were evaluated based on scoring metrics: I-TASSER: C-score = -3.79; TM-score = 0.10 ± 0.30.; trRosetta: TM-score = 0.317; LDDT score = 70.3; and AlphaFold: TM-score = 0.249; LDDT score = 59.1. The trRosetta model emerged as the most reliable, demonstrating a higher TM-score and LDDT score, indicative of better structural accuracy (Figure 6; Table 1). The I-TASSER model, with its lower scores, reflected less confidence in its predictions, while AlphaFold, although notable, fell short compared to trRosetta's performance.

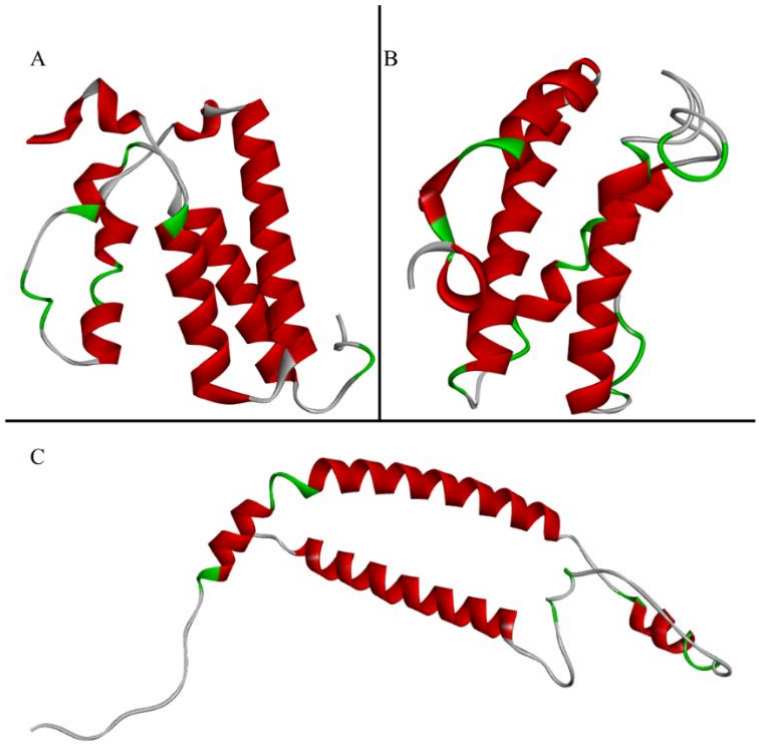


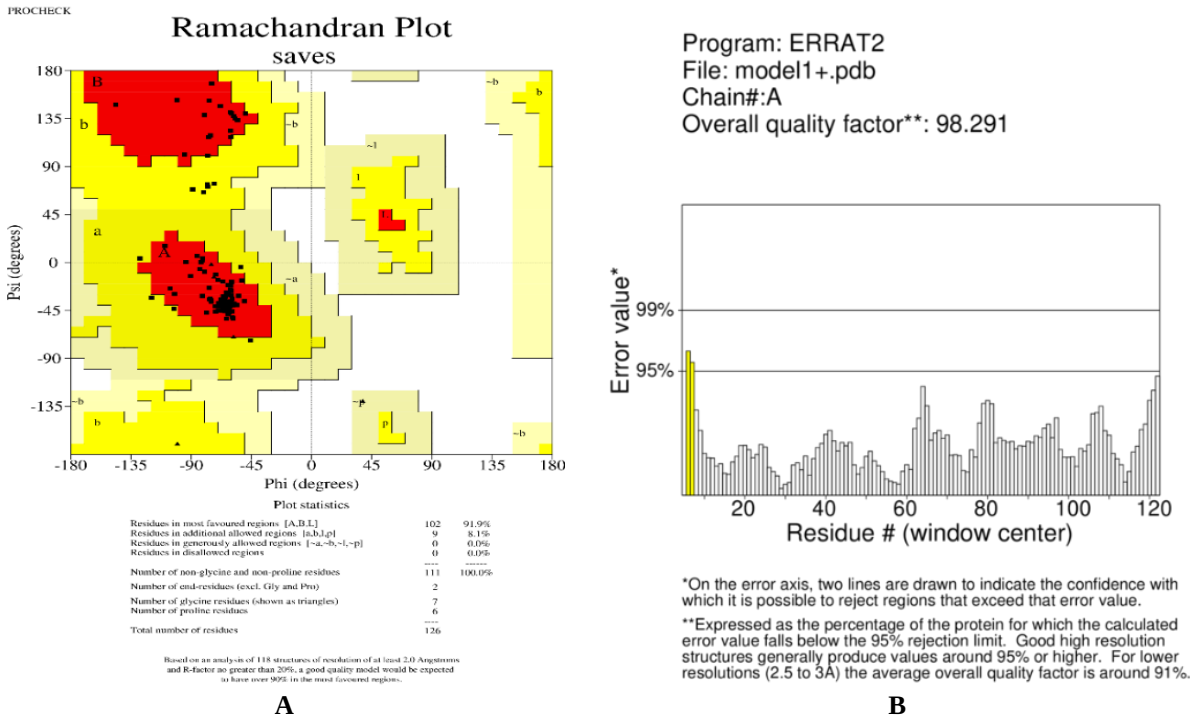
Figure 6. Tertiary structure predicted by: a) trRosetta, b) I-TASSER and c) AlphaFold. (alpha helices in red, beta sheets in green and coils in gray)

Table 1: Modelling results using trRosetta, I-TASSER and AlphaFold and validation of models with PROCHECK and ERRAT

Overall Quality Factor (%) (ERRAT)	Residues in most favored regions (%) (PROCHECK)	LDDT[1]	C-score[2]	TM-Score[3]	Software tool - model
98.29	91.9	70.3	-	0.317	trRosetta–model 1
98.18	88.3	-	-	-	trRosetta–model 2
93.10	88.3	-	-	-	trRosetta–model 3
88.79	88.3	-	-	-	trRosetta–model 4
90.35	87.4	-	-	-	trRosetta–model 5
75.42	72.1	-	-3.79	0.300	I-TASSER–model 1
93.2	65.8	-	-3.82	-	I-TASSER–model 2
83.05	63.1	-	-4.75	-	I-TASSER–model 3
88.13	53.2	-	-5	-	I-TASSER–model 4
55.08	26.1	-	-5	-	I-TASSER–model 5
77.88	77.5	60.7	-	0.255	AlphaFold–model 1
48.11	73.9	59.6	-	0.263	AlphaFold–model 2
71.58	71.2	58	-	0.255	AlphaFold–model 3
36.27	70.3	55.4	-	0.250	AlphaFold–model 4
48.81	69.4	54.3	-	0.237	AlphaFold–model 5

Note:[1]Local distance difference test, [2] Confidence score, [3]Pattern modelling score

The quality of the models was evaluated by PROCHECK, and trRosetta model showed the best and most reliable structure in terms of stereochemical properties and overall geometry compared to other structures (Table 1). Analysis of the Ramachandran plot showed that 91.9%, 8.1% and 0.0% of the residues were located in the favorable, allowed, and distant regions, respectively (Figure 7A), indicating the high quality of the predicted structure. Using ERRAT, the overall quality factor of the selected model was 98.291% (Figure 7B).



A **B**

Figure 7. Validation of the three-dimensional structure model. A) Ramachandran chart and B) ERRAT chart.

Protein-Protein Interaction Network Analysis

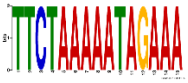
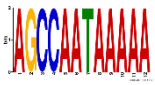
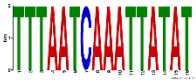
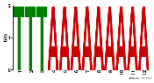
The STRING database was utilized to construct a protein-protein interaction network for *UTS2B*, as illustrated in Figure 8. This network highlights direct and indirect interactions of *UTS2B* with several chicken proteins, including CCDC50, UTS2R, UTS2, UTS2R2, LOC427799, LOC771154, RAB11FIP4, RCAN3, SNX21, TIFA, EDN2, EDN1, and PROK2. These interactions imply that *UTS2B* is integral to multiple biological pathways, particularly those regulating vascular tone and vessel diameter. Other associated pathways include neuropeptide signaling, G protein-coupled receptor signaling, hematology, blood pressure regulation, and broader physiological processes within organ systems.

Promoter motif analysis

Promoter motif analysis was performed to identify conserved motifs and cis-regulatory elements (CREs) associated with the *UTS2B* gene using the TOMTOM tool within the MEME Suite web server. The TOMTOM compares query motifs, often derived from transcription factors (TFs), to a database such as JASPAR, ranking them based on statistical significance. This tool also generates alignments for each significant match, offering insights into motif conservation. Subsequent analysis with GOMo assessed the putative activities of identified TF motifs, linking them to Gene Ontology (GO) terms related to biological processes (BP), molecular functions (MF), and cellular components (CC).

The analysis of promoter motifs identified seven significant motifs, including *Foxq1*, *MEF2A*, *Arid3a*, *Arid3b*, *Hoxa13*, *PHOX2B*, and *ZNF384*—transcription factors known to play roles in diverse biological pathways (Table 2). The biological functions associated with these TF motifs were elucidated through GOMo, revealing involvement in metal ion binding, integral membrane functions, transforming growth factor β- receptor signaling, and various transporter activities, among others (Table 2).

Table 2: The conserved motifs, related transcription factors in the promoter of the *UTS2B* gene and their GO predictions found by TOMTOM and GOMo.

	Motif	Transcription Factors	Logo	Top Specific Predictions
1	MA0040.1	Foxq1		MF metal ion binding
2	MA0052.4	MEF2A		CC integral to membrane BP transforming growth factor beta receptor signalling pathway MF serine-type endopeptidase inhibitor activity MF ion transmembrane transporter activity MF sugar transmembrane transporter activity
3	MA0151.1	Arid3a		MF calcium ion binding MF cytoskeletal protein binding
4	MA0601.1	Arid3b		MF calcium ion binding
5	MA0650.3	Hoxa13		BP hemophilic cell adhesion MF calcium ion binding
6	MA0681.2	PHOX2B		MF calcium ion binding
7	MA1125.1	ZNF384		BP biological regulation

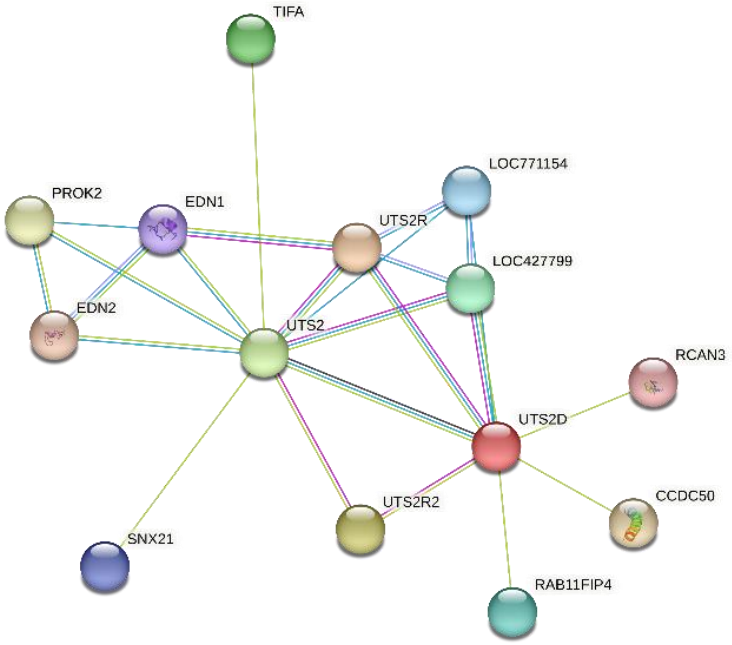


Figure 8. Protein-protein interactions of *UTS2B* with other functionally and physically correlated proteins

Discussion

The spatial structure of proteins is paramount for their biological function, and alterations in their conformation can profoundly affect their properties and activity (Todd *et al*., 2001). Through bioinformatics, we predicted the structure and function of the chicken *UTS2B* protein, providing valuable information about its mechanism of action. This understanding may enable the design of interventions to mitigate its pathogenic processes and the development of targeted therapeutic strategies.

Our analysis indicates that *UTS2B* carries a generally negative charge and is classified as an unstable protein based on the instability index (42.33). This instability is contingent upon the nature of the N-terminal residue; as certain residues are recognized by cellular degradation machinery; leading to either enhanced stability or targeted degradation. The presence of destabilizing residues can significantly shorten the half-life, with variations observed across different proteins and cellular contexts. Thus, understanding the implications of N-terminal residues is crucial for elucidating protein dynamics and regulatory mechanisms within cellular environments (Bachmair *et al*., 1986; Timms *et al*., 2019).

The results of current experiment showed that the *UTS2B* nature is predominantly hydrophilic. The dispersed arrangement of hydrophilic amino acids across the entire *UTS2B* peptide chain likely plays a critical role in the protein's solubility and interaction with aqueous environments, which may have functional implications for its biological activity. Generally, the hydrophobicity distribution of amino acids is a key factor in protein folding, influencing its functional properties (Lins and Brasseur, 1995; Nassar *et al*., 2021). For example, in a mouse model, a linear relationship was observed between immune activity and hydrophobicity of protein both *in vitro* and *in vivo* (Moyano *et al*., 2012).

Additionally, post-translational modifications such as phosphorylation and glycosylation are essential for regulating protein function and stability (Das *et al*., 2020). Disruption in post-translational modifications can lead to disease, making them potential targets for therapeutic interventions (Yang *et al*., 2024; Guo *et al*., 2024). In our study, a comprehensive analysis of *UTS2B* protein identified 12 and 4 post-translational for phosphorylation and glycosylation, respectively. It has been reported that phosphorylation and glycosylation modifications play an important role in signal pathways, immune response, and regulation of protein translation (Guo *et al*., 2022; Jiang *et al*., 2024; Li *et al*., 2023).

Determining the subcellular localization of proteins provides critical insights into their biological roles, as the function of a protein is often closely associated with its cellular compartment (Wang *et al*., 2005). The identification of a signal peptide in *UTS2B* suggests that it is a secreted protein, likely functioning in the extracellular space. This means that *UTS2B* may play a role in extracellular signaling or interaction with the cellular environment. This prediction was supported by the absence of a homologous 3D structure in protein databases, leading us to generate *de novo* 3D models of the protein. Generally, secretion refers to the process in which the synthesis and delivery of soluble proteins

to the extracellular space occurs. Protein secretion is essential for cellular function, and it plays important roles in growth, communication, survival, and homeostasis of cells (Barlowe and Miller, 2013; Farhan and Rabouille, 2011).

The homology modelling approaches are commonly used to generate the 3D protein structures with high quality. These methods have revolutionized docking and virtual screening methods in the drug discovery process (Muhammed and Aki-Yalcin, 2019). In several studies, the homology approaches have been used to design drugs for various diseases. For example, *FAM222A*, as a genetic marker for Alzheimer's disease, was investigated using diverse homology modelling approaches as target structure and drugs currently under research in clinical trials (Alabdulraheem and Durdagi, 2023). Recently, 3D structures of cancer targets have been used to discover effective chemotherapeutic agents using homology modelling methods (Masoomy *et al*., 2021; Yalcin-Ozkat, 2021). Therefore, the homology modelling approaches can serve as a reliable structural template for further studies, including experimental validation and drug design targeting *UTS2B* to mitigate the effects of ascites in poultry.

The protein-protein interaction network analysis suggests that *UTS2B* may interact with other proteins implicated in pathways related to ascites syndrome, particularly endothelin, a protein known to cause vasoconstriction and elevate blood pressure (Aharinejad *et al*., 1995; Zhou *et al*., 2008). Generally, endothelin-1, like other chemical mediators of vasoconstriction (5-hydroxytryptamine and endotoxin), can lead to excessive resistance to blood flow by causing constriction in the vessels. The expression of those mediators has been increased in the heart, lungs and serum of broiler chickens suffering from ascites (Hamal *et al*., 2010; Hassanpour *et al*., 2009; Li *et al*., 2013). Such findings underscore the functional significance of *UTS2B* in orchestrating complex biological responses, suggesting its pivotal role in chicken physiology and potential pathological states. This analysis reinforces the importance of studying *UTS2B* in relation to vascular and systemic regulatory mechanisms. A noteworthy finding of promoter analysis was the prominence of calcium ion binding, which suggests its potential role in the pathophysiology of ascites syndrome. This highlights the critical role of these transcription factors in the regulation of *UTS2B* expression and function in complex physiological and pathological contexts. The calcium signals play an important role in gene expression regulation in immune cells and other cell types (Hogan *et al*., 2003; Puri, 2020).

This study provides critical insights into the *UTS2B* protein, offering a valuable foundation for future research aimed at addressing ascites syndrome in poultry and exploring new therapeutic strategies, but acknowledges several limitations. The reliance on bioinformatics tools for predicting protein structure and function may introduce inaccuracies without experimental validation. The absence of experimental data renders the findings speculative, necessitating empirical validation. Additionally, the multifactorial nature of ascites syndrome suggests that focusing solely on *UTS2B* may not fully address the disease's complexity. Larger-scale omics studies could identify additional biomarkers or molecular pathways intersecting with *UTS2B*, providing new insights into the disease and broader applicability in veterinary medicine.

Conclusion

The intricate bioinformatics exploration of the *UTS2B* protein represents a significant advancement in elucidating its multifaceted nature and potential roles, particularly within the complex landscape of ascites syndrome. In this study, the physical, chemical, and structural properties of *UTS2B*, as well as post-translational modifications and interaction networks, were thoroughly investigated. This exploration positions *UTS2B* as a key player in cellular processes and primes the scientific community for targeted investigations into its involvement in ascites syndrome. The potential impact of *UTS2B* in blood pressure regulation opens avenues for therapeutic interventions in the multifactorial landscape of ascites disease in broilers.

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