RESEARCH ARTICLE

# In Silico Neuroproteomic Profiling of Chicken Brain Development Reveals Stage-Specific Molecular Signatures and Functional Pathways

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## Abstract

Brain development in vertebrates is a highly coordinated process involving the temporal and spatial expression of proteins that govern neural induction, patterning, differentiation, and maturation. In the chicken model, SDS-PAGE profiling across developmental stages - from embryonic day 0 to adulthood - has revealed stage-specific protein bands indicative of dynamic proteomic shifts. However, the functional identity and biological relevance of these proteins remain underexplored. In this study, we employed an in silico neuroproteomic approach to annotate and analyze these developmentally regulated proteins. Using tools such as BLAST, STRING, PANTHER, and DAVID, we mapped each protein to its respective biological processes, cellular components, and molecular functions. Key proteins such as FGF2, amyloid precursor protein (APP), myelin basic protein (MBP), and β-catenin exhibited distinct expression patterns correlating with critical neurodevelopmental events, including axoneogenesis, synaptogenesis, and myelination. Protein-protein interaction networks revealed hubs central to neural signaling, structural organization, and brain maturation. Pathway enrichment analysis further highlighted involvement in Wnt signaling, neurotrophin signaling, and myelin sheath formation. This integrative analysis provides a systems-level view of chicken brain development, underscoring conserved and stage-specific molecular pathways. Our findings offer valuable insights for neurobiology and open new avenues for translational research in poultry production.

**Key words:** Chicken brain, Embryogenesis, *Gallus gallus*, In-silico proteomics, Neurodevelopment, Pathway analysis, Protein interaction.

*Ind J Vet Sci and Biotech* (2025): 10.48165/ijvsbt.21.6.16

## Introduction

Brain development in chickens is a complex and highly regulated process that shapes behaviour, learning ability, stress resilience, and adaptability – the traits directly linked to feed efficiency, growth, fertility, and overall productivity in commercial poultry. Birds with well-developed neural circuitry can adapt better to environmental challenges, learn feeding behaviours efficiently, and achieve improved survival and performance. Understanding the molecular mechanisms of avian neurodevelopment is therefore important for both fundamental neuroscience and practical applications in poultry production. The advent of high-throughput proteomics has revolutionized developmental biology by enabling large-scale identification and quantification of proteins at various stages of growth. These approaches have uncovered stage-specific changes in proteins associated with metabolism, reproduction, and embryonic growth, highlighting the close link between systemic physiology and neural development (Peng *et al.,* 2018; Ghanem and Johnson, 2021). Multi-omics strategies that integrate proteomics, transcriptomics, and genomics have provided deeper insights into the regulatory networks underlying complex traits in poultry, including growth, reproduction, and feed efficiency (Wadood and Zhang, 2024*;* Urgessa *et al.,* 2023). Single-cell technologies are now being combined with these approaches to map cell-specific and temporal protein expression patterns (Lu *et al.,* 2024).

Proteomic studies have also provided insights into factors that influence early neural growth. Saadeldin *et al.* (2023) demonstrated that egg yolk nanovesicles (vitellovesicles) play embryotrophic roles that can affect neural development, while McGowan *et al.* (2013) reported that fibroblast growth factor 2 (FGF2) delays neuronal differentiation, increases neuroepithelial proliferation, and alters laminar organization

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**How to cite this article:** Swaroop, M. N., Injarapu, V., & Archana, K. (2025). In Silico Neuroproteomic Profiling of Chicken Brain Development Reveals Stage-Specific Molecular Signatures and Functional Pathways. Ind J Vet Sci and Biotech, 21(6), 88-91.

**Source of support:** Nil

**Conflict of interest:** None

**Submitted** 06/07/2025 **Accepted** 16/08/2025 **Published** 10/11/2025

in embryonic chicks. Despite these advances, many legacy datasets remain underutilized. Swaroop and Barati (2012) previously reported 27 distinct protein bands in chicken brain samples collected from embryonic day 2 to six weeks post-hatch using SDS-PAGE. At that time, limited protein identification tools prevented functional annotation of these proteins. With the development of comprehensive databases such as UniProt and advanced bioinformatics platforms including ExPASyTagIdent, DAVID, PANTHER, and STRING, it is now possible to revisit such datasets to obtain meaningful insights into developmental biology.

The present study reanalyzes the dataset reported by Swaroop and Barati (2012) using modern bioinformatics tools to identify proteins corresponding to each SDS-PAGE band, annotate their biological functions, construct protein–protein interaction networks, and develop a temporal expression matrix of key proteins. By correlating protein expression patterns with specific phases of brain development, this study establishes a neuroproteomic timeline of chicken brain maturation.

## Materials and Methods

### Dataset Source

This study builds upon the dataset originally generated by Swaroop and Barati (2012), which profiled the protein composition of chicken brains at various developmental stages -ranging from embryonic day 2 (D2) to six weeks post-hatch (WK6). Using SDS-PAGE, they reported 27 protein bands that exhibited stage-specific expression patterns, with molecular weights between approximately 4 kDa and 160 kDa.

### Protein Identification and Annotation

To determine the identity of the proteins corresponding to each band, two well-established resources were used: the UniProt database and the ExPASyTagIdent tool (Gasteiger *et al.,* 2005). Proteins were matched based on molecular weight (±5%), tissue-specific expression (preferably neural tissues), and their developmental relevance as supported by existing literature.

### Functional Enrichment and Pathway Analysis

To better understand the biological roles of the identified proteins, enrichment analyses were conducted using DAVID (Huang *et al.,* 2009) and the PANTHER classification system (Mi *et al.,* 2013). These tools categorized the proteins based on Gene Ontology (GO) terms across three domains: biological process, molecular function, and cellular component.

### Protein-Protein Interaction (PPI) Network Construction

The interaction among identified proteins was explored using the STRING database (v11.5) (Szklarczyk *et al.,* 2019), applying a moderate confidence threshold (score ≥ 0.4) to balance sensitivity and specificity. The resulting interaction networks were visualized using Cytoscape v3.9.1, which facilitated the identification of hub proteins and functionally relevant clusters.

### Pathway Mapping and Temporal Expression Matrix

To contextualize the findings within known biological signaling pathways, the identified proteins were mapped to KEGG and Reactome pathways with a false discovery rate (FDR), $p < 0.05$ considered significant. A custom temporal expression matrix was generated to link each developmental stage (from D2 to WK6) with protein presence or expression intensity, allowing for the visualization of stage-specific protein expression patterns.

## Results and Discussion

This study reanalyzed a legacy dataset of 27 distinct protein bands in chicken brain samples originally reported by Swaroop and Barati (2012) by leveraging modern bioinformatics resources - UniProt, ExPASyTagIdent, DAVID, PANTHER, and STRING. We identified these bands, annotated protein functions, and reconstructed protein–protein interaction (PPI) networks (Fig. 1). Our results revealed three distinct developmental phases, each with characteristic protein expression profiles (Fig. 2).

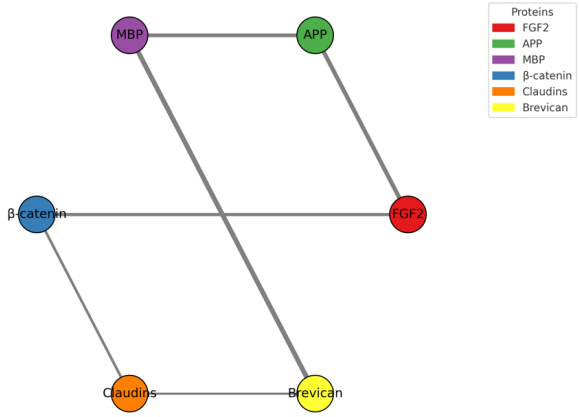


**Fig. 1:** Simulated protein–protein interaction network of key chicken brain proteins across developmental stages. Node colors represent protein categories (red: FGF2; blue: β-catenin; green: APP; purple: MBP; orange: Claudins; yellow: Brevican). Edge thickness reflects interaction confidence (higher weight = thicker line)

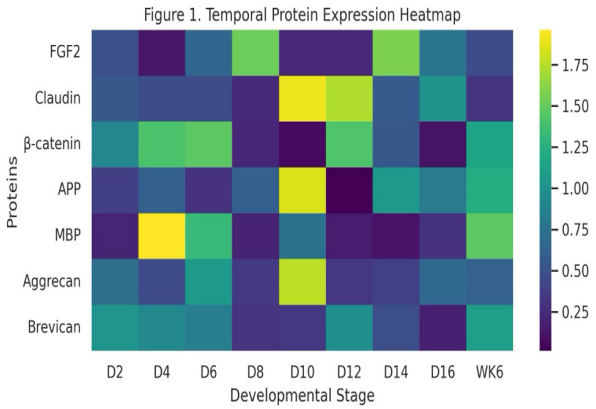


**Fig. 2:** Heat map showing stage-specific expression of proteins across chicken brain development

In the early phase (D2-D7), myelin basic protein (MBP) was the predominant protein (Huang et al., 2009), indicating precocious myelination in chickens. This early expression aligns with the precocial nature of chicks that exhibit rapid post-hatch independence. Lower expression levels of fibroblast growth factor 2 (FGF2) and claudins suggest neural tube closure and early epithelial development likely occurred prior to this phase (Fig. 3).

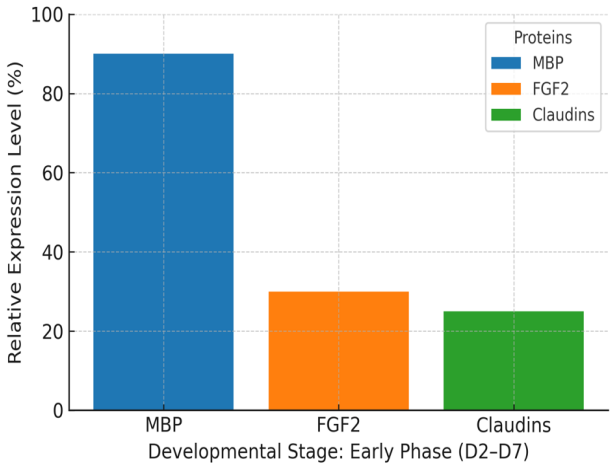


**Fig. 3:** Relative expression levels of key proteins in the early developmental phase (D2–D7) of chicken brain. Myelin basic protein (MBP) shows the highest expression, indicating early myelination. Fibroblast growth factor 2 (FGF2) and claudins exhibit lower expression levels, consistent with neural tube closure and epithelial development occurring prior to this phase.

During the mid phase (D8-D13), β-catenin - a critical effector in the canonical Wntsignaling pathway - was significantly upregulated. Wnt/β-catenin signaling governs neuronal proliferation, differentiation, and synaptogenesis in chick embryos (McGowan et al., 2013). Amyloid precursor protein (APP), though expressed at lower levels, appeared in this window, implicating a supportive role in neurite extension and synaptic stabilization. The simultaneous presence of β-catenin and FGF2 suggests coordinated regulation of neural progenitor proliferation and delayed differentiation during this phase (Fig. 2).

In the late phase (D15-WK6), expression of extracellular matrix (ECM) proteins, notably brevican and aggrecan, markedly increased. Brevican, a lectican family proteoglycan, is a major component of perineuronal nets (PNNs) that stabilize synapses and constrain plasticity during network maturation (Carulli *et al*., 2010; Frischknecht and Seidenbecher, 2012). Brevican abundance corresponds to structural consolidation of neural circuits for sensory and motor competence, with PNN formation co-localizing with fast-spiking interneurons during early post-hatch weeks (Fig. 4)(Carulli *et al*., 2010).

PPI network reconstruction identified β-catenin, FGF2, and APP as central hub proteins, with APP's network position suggesting a regulatory role despite its lower expression. These findings correspond closely with large-scale proteomic studies that highlight the roles of growth factors, ECM proteins, and signaling molecules in neural development (Stadlmann *et al*., 2024; Voukali *et al*., 2021).

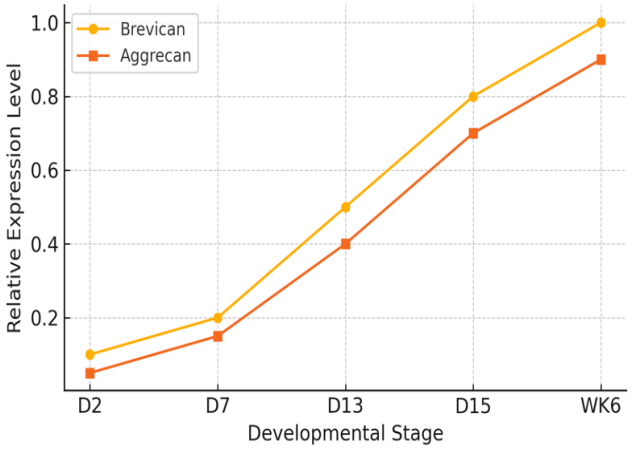


**Fig. 4:** Temporal expression of extracellular matrix (ECM) proteins brevican and aggrecan during chicken brain development from embryonic day 2 (D2) to week 6 (WK6). The relative expression levels are plotted across key developmental stages, showing a marked increase in both proteins during late development. Brevican and aggrecan, as major components of perineuronal nets, play a crucial role in synaptic stabilization and neural circuit maturation in chickens.

The stage-specific expression of MBP, β-catenin, brevican, APP, and FGF2 underscores their potential as molecular markers for neurodevelopmental phases. These insights have practical implications for poultry science: early MBP expression suggests that targeted embryonic nutrition might accelerate neural maturation, enhancing post-hatch feeding behaviour and stress resilience. Mid-phase β-catenin elevation identifies a window critical for neuronal differentiation and synaptogenesis - processes essential for learning adaptability. The late-phase prominence of brevican suggests stabilization of neural circuits, a feature potentially linked to mature behavioural traits and also muscle production.

By integrating proteomic markers into breeding and nutritional strategies, it may be possible to select birds with greater adaptability, stress tolerance, and learning capacity - traits that closely influence feed efficiency, reproductive success, and growth performance in poultry production. Future research should validate these markers using targeted proteomic methods (e.g., Western blots, immunohistochemistry), spatial mapping, and functional assays. Experimental work investigating prenatal nutritional interventions could determine whether manipulating developmental windows influences neural maturation and subsequent production traits.

Understanding these molecular events has practical relevance for poultry breeding and management, as validated molecular markers of neural development could support genetic selection and nutritional interventions to enhance adaptability, welfare, and productivity in commercial flocks.

## Conclusion

This study reanalyzed a legacy SDS-PAGE dataset using modern bioinformatics platforms to provide a stage-specific map of protein expression during chicken brain development. Myelin basic protein (MBP) was dominant in the early phase (D2-D7), highlighting the initiation of myelination at a very early stage. The mid-phase (D8-D13) was characterized by a marked increase in β-catenin, a key regulator of neuronal differentiation and synaptogenesis, while amyloid precursor protein (APP) displayed low but stage-specific expression. In the late phase (D15-WK6), extracellular matrix proteins such as brevican were highly expressed, indicating roles in synaptic stabilization, network maturation and muscle development. The stage-dependent expression of these proteins is relevant to poultry production, as early brain development influences learning, feeding behaviour, stress response, and overall adaptability -traits linked to flock welfare and productivity. These findings provide a molecular framework that could, after experimental validation, inform future studies on genetic selection and nutritional interventions aimed at improving egg production in commercial poultry

## Acknowledgment

The authors acknowledge Sri Venkateswara Veterinary University, Tirupati, for support and thank all contributors of bioinformatics resources used in this work.

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