104 (2025) 105032

Contents lists available at ScienceDirect

Poultry Science

journal homepage: www.elsevier.com/locate/psj

Cloning, phylogenetic analysis, tissue expression profiling, and functional roles of *NPC1L1* in chickens, quails, and ducks

Xin Shu [a], Ziwei Chen [a], Hui Wang [a], Bingjie Xu [a], Li Liu [a], Jilong Zhang [a], Xiaotong Zheng [a,b], Jianfei Chen [a,b,*]

[a] Jiangsu Key Laboratory of Sericultural Biology and Animal Biotechnology, School of Biotechnology, Jiangsu University of Science and Technology, Zhenjiang, China
[b] Key Laboratory of Silkworm and Mulberry Genetic Improvement, Ministry of Agriculture and Rural Affairs, Sericultural Scientific Research Center, Chinese Academy of Agricultural Sciences, Zhenjiang, China

ARTICLE INFO

Keywords:
Poultry
NPC1L1
mRNA sequence
Gene expression profile
Cholesterol

ABSTRACT

The Niemann-Pick C1-Like 1 (**NPC1L1**) protein, primarily expressed in the epithelial cells of the small intestine, is essential for cholesterol absorption from both dietary intake and biliary secretion. Despite this conserved function across mammals, the full-length coding sequence of NPC1L1 remains uncharacterized in key avian models including chicken (*Gallus gallus*), quail (*Coturnix japonica*), and duck (*Anas platyrhynchos*). In this study, we successfully cloned the full *NPC1L1* mRNA sequence in chicken, quail, and duck, including the entire 5′ and 3′ untranslated regions, utilizing rapid amplification of cDNA ends methods. Phylogenetic analysis across 12 species, comprising four avian and eight representative mammalian species, revealed that the NPC1L1 sequences in the main poultry species exhibit a high degree of similarity. Despite the phylogenetic divergence of poultry NPC1L1 sequences from their mammalian counterparts, protein sequence alignment revealed that the cholesterol-sensing peptides of NPC1L1 are conserved across all species examined in this study. These findings imply that the NPC1L1 in poultry may also play a role in cholesterol transport. Analysis of tissue gene expression profiles in chickens, quails, and ducks indicated that *NPC1L1* is predominantly expressed in the duodenum, jejunum, and liver. Additionally, experiments on medium-to-cell cholesterol transit in primary intestinal epithelial cells confirmed that chicken NPC1L1 is capable of efficiently transporting cholesterol into cells. Further experiments are required to elucidate the biological function of poultry NPC1L1. In summary, this study successfully cloned the full-length sequence of *NPC1L1* from chickens, quails, and ducks, and conducted a comprehensive analysis of their evolutionary history and expression patterns. This research establishes a foundation for future investigations into the role of poultry NPC1L1 in cholesterol transport.

Introduction

Cholesterol is a vital lipid molecule that is integral to numerous physiological processes related to growth and development (Luo et al., 2020; Schade et al., 2020). In humans and mice, Niemann-Pick C1-Like 1 (**NPC1L1**) is a polytopic transmembrane protein situated at the apical membrane of enterocytes and the canalicular membrane of hepatocytes. It serves as a sterol transporter, facilitating the absorption of intestinal cholesterol from dietary intake or bile acids and balancing hepatobiliary cholesterol excretion (Betters and Yu, 2010; Jia et al., 2011; Xiao et al., 2023). Furthermore, NPC1L1 is implicated in several pathological conditions, including cancer and non-alcoholic fatty liver disease (Zhang et al., 2022; Xu et al., 2023). Current understanding of NPC1L1 in avian species remains limited, with only two studies reported to date. The first demonstrates that lycopene supplementation in laying hens preserves egg production indices while reducing yolk cholesterol deposition and downregulating cholesterol-transport genes, including NPC1L1 (Orhan et al., 2021). The second reveals through transcriptomic profiling that Muscovy duck reovirus infection induces hepatic lipid dysregulation via differential expression of NPC1L1 and other metabolic regulators (Wang et al., 2018). Notably, the complete sequence of *NPC1L1* remains uncharacterized in major poultry species like chicken (*Gallus gallus*), quail (*Coturnix japonica*), and duck (*Anas platyrhynchos*), significantly hindering mechanistic exploration of its cholesterol transport functions.

This paper belongs to Genetics and Molecular Biology.

* Corresponding author.
E-mail address: jfchen@just.edu.cn (J. Chen).

https://doi.org/10.1016/j.psj.2025.105032
Received 14 January 2025; Accepted 13 March 2025
Available online 15 March 2025

The *NPC1L1* sequences for goose and quail are available in existing databases, theoretically suggesting the presence of *NPC1L1* in poultry species such as chickens and ducks. Research conducted on chickens has demonstrated the detection of the NPC1L1 protein in the small intestine using human NPC1L1 antibodies (Orhan et al., 2021), thus supporting the presence of NPC1L1 in poultry. The absence of complete *NPC1L1* sequences in avian genomic databases—particularly for chicken, quail, and duck—highlights a critical knowledge gap in comparative lipid metabolism. Consequently, this study aims to clone the full-length *NPC1L1* cDNA sequence of major avian species, conduct phylogenetic analysis among these species, examine its gene expression profile, and explore its potential roles in cholesterol transport.

Materials and methods

Animals and sample collection

The animal protocols were approved by the Institutional Animal Care and Use Committee (**IACUC**) of Jiangsu University of Science and Technology (GQ20230302, Zhenjiang, China). Animal care and handling practices were followed by the IACUC guidelines.

The duck samples consisted of Beijing ducks (*Anas platyrhynchos*) purchased from the market (n=6). All ducks were maintained under uniform environmental conditions and provided with unrestricted access to water. The experimental ducks were euthanized using CO_2 anesthesia. Samples from the chest skin, heart, liver, spleen, lung, kidney, proventriculus, duodenum, jejunum, ileum, cecum, and colon were rapidly frozen in liquid nitrogen and subsequently stored at −80 °C for RNA extraction. The chicken samples were derived from Arbor Acres broilers (*Gallus gallus*), which were previously utilized in a study by Zheng et al. (2022). The quail samples were obtained from 40-day-old Chinese yellow quails (*Coturnix coturnix*), as used in research by Shu et al. (2024). Duodenum tissues of chickens, quails, and ducks were used for rapid amplification of cDNA ends (**RACE**) experiments. All the tissue samples were utilized for gene expression profile analysis.

Cloning full-length cDNA of NPC1L1

Total RNA was extracted from individual tissue samples utilizing the RNAiso Plus reagent (Takara Biotech Co., Ltd, Beijing, China) in accordance with the manufacturer's instructions. The integrity of the extracted RNA was assessed via agarose gel electrophoresis, and its concentration was determined using a Nanophotometer N60 Touch (IMPLEN, Munich, Germany). Primers targeting conserved regions of *NPC1L1* were designed through multi-species homologous alignment to clone partial sequences, with detailed oligonucleotide information provided in Table S1. The RACE experiment was conducted following the protocol provided by the HiScript-TS 5′/3′ RACE Kit (Vazyme Biotech Co., Ltd, Nanjing, China). Briefly, for the 5′ and 3′ *NPC1L1* RACE, total RNA underwent reverse transcription with different adapters separately. Gene-specific primers (**GSPs**) targeting the cloned partial *NPC1L1* sequences were paired with universal adapter primers for primary PCR, followed by nested PCR using nested GSPs (**NGSPs**) and nested universal adapter primers to enhance specificity. 5′/3′ GSPs and NGSPs were designed using the Vazyme cloud online software (http://cloud.vazyme.com), with sequences listed in Table S1. The 5′/3′ RACE PCR purified amplification products were cloned using the Ultra-Universal TOPO Cloning Kit (Vazyme) and sequenced via the Sanger method (Shangya Biotechnology Co., Ltd., Hangzhou, China).

Phylogenetic analysis

To investigate the conservation of *NPC1L1* and its protein sequences across species, a phylogenetic analysis of 12 species was performed. Since the chicken, duck, and quail *NPC1L1* has been cloned in this study, and its homologs in 8 species, including goose (*Anser cygnoides*), human (*Homo sapiens*), pig (*Sus scrofa*), cattle (*Bos taurus*,), green monkey (*Chlorocebus sabaeus*), mouse (*Mus musculus*), dog (*Canis lupus familiaris*), sheep (*Ovis aries*), and rabbit (*Oryctolagus cuniculus*) were downloaded from the NCBI website. The *NPC1L1* mRNA evolutionary history was inferred using the Maximum Likelihood method and General Time Reversible model. The evolutionary history of NPC1L1 protein was inferred by using the Maximum Likelihood method and JTT matrix-based model (Jones et al., 1992). All positions with less than 90 % site coverage were eliminated, i.e., fewer than 10 % alignment gaps, missing data, and ambiguous bases were allowed at any position (partial deletion option). Evolutionary analyses were conducted in MEGA 11 software (Tamura et al., 2021).

Reverse transcription quantitative real-time polymerase chain reaction

The cDNA synthesis was conducted utilizing the Evo M-MLV RT Mix Kit and gDNA Clean reagent (Accurate Biotechnology Co., Ltd, Changsha, China), with 1 μg of extracted total RNA as the starting material. Post-synthesis, the cDNA was diluted at a 1:3 ratio with nuclease-free water and subsequently stored at −20 °C. The reverse transcription quantitative real-time polymerase chain (**RT-qPCR**) was executed using a Bio-Rad Light Cycler 96 Real-Time PCR system, employing the SYBR Green Premix Pro TaqHS qPCR Tracking Kit II (Accurate Biotechnology) for analysis. The specific reagent components and PCR protocols for RT-qPCR have been previously reported (Chen et al., 2020; Zheng et al., 2022). Based on the cloned cDNA sequences, primers for avian *NPC1L1* RT-qPCR were designed using Primer Premier 5.0. The primers for the reference genes *TBP* and *RPL13* in chicken and duck were adopted from previous studies (Na et al., 2021; Shu et al., 2024) and synthesized by Shangya Biotechnology. The detailed information about these primers is provided in Table S2.

Intestinal epithelial cells isolation and culture

The isolation of chicken intestinal epithelial cells was conducted based on a previous report with modifications (Ghiselli et al., 2021). In brief, small intestine tissue was isolated from 19-day-old chick embryos, washed repeatedly with PBS, and then cut into 1-2 mm pieces using sterile scissors. The tissue was digested in Dulbecco's Modified Eagle Medium/ Nutrient Mixture F-12 (**DMEM/F12**) containing 1 mg/mL type I collagenase at 37 °C for 30 minutes with shaking every 5 minutes. Digestion was neutralized using DMEM/F12 medium supplemented with 10 % fetal bovine serum (Wuhan Pricella Biotechnology Co., Ltd, Wuhan, China), followed by gentle pipetting to resuspend cells and filtration through three layers of sterile gauze to remove undigested debris. Centrifugation is then performed at 800 rpm. The obtained cells are washed with PBS, centrifuged again, collected, and then cultured in DMEM/F12 plus 10 % superfine fetal bovine serum (Pricella Biotechnology), 1 % penicillin/streptomycin at 37 °C/5 % CO_2 in an incubator. For siRNA transfection, cells were plated into 12-well plates (NEST Biotechnology, Wuxi, China).

Oligonucleotides, transient transfection

The siRNA sequences targeting chicken *NPC1L1*, which were designed and obtained from Sangon (Sangon Biotech Co., Ltd, Shanghai, China) in this study, adhered to stringent selection criteria: 19 bp in length, with a GC content ranging from 30-55 %, and designed to exclude off-target matches through BLASTn analysis against the chicken genome. These sequences included two chemically double-stranded siRNA oligonucleotides targeting chicken *NPC1L1*, each with 3′ dTdT overhangs, detailed in Table S3. Transient transfection of siRNA oligonucleotides was performed using RNATransMate (Sangon Biotech) as per the manuscript's guidelines. The transfection efficiency was assessed by RT-qPCR analysis. A total of 6 plates of 12-well cells were transfected, with 1 plate dedicated to the RT-qPCR experiment and the remaining 5

plates all used for medium-to-cell cholesterol transit in cells experiment.

Medium-to-cell cholesterol transit in intestinal epithelial cells

The medium-to-cell cholesterol transit experiment was performed as described in a previous study (Nakano et al., 2016). Briefly, 40 hours after transfection with siRNA of chicken *NPC1L1*, we discarded the culture medium, added 1 mL DMEM/F12 containing 0.05 mM cholesterol, 0.5 mM chenodeoxycholic acid, and incubated the cells for 2 hours. Remove the culture medium and wash the cells twice with PBS, then lyse the cells with 2 % triton solution. Total cholesterol (**TC**) in cells was assessed using commercial kits in accordance with the manufacturer's guidelines (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The protein concentration, determined by the BCA protein quantification kit (Beyotime Biotechnology, Shanghai, China), was utilized to normalize the TC content. Absorbance readings were obtained using a BioTek Epoch 2 microplate auto-reader (BioTek, Winooski, USA).

Statistical analysis

The Sanger sequencing data were analyzed using Chromas v2.6.5, Chromas Pro v1.33, and sequences were aligned using DNAMAN v6.0 software. During the RT-qPCR analysis, the cycle threshold (CT) values of the genes were exported to Microsoft Excel for further analysis. The relative gene expression levels were normalized using the $2^{-\triangle\triangle CT}$ method. Two reference genes, *TBP* and *RPL13*, were used to normalize gene expression levels, with the geometric mean of their expression

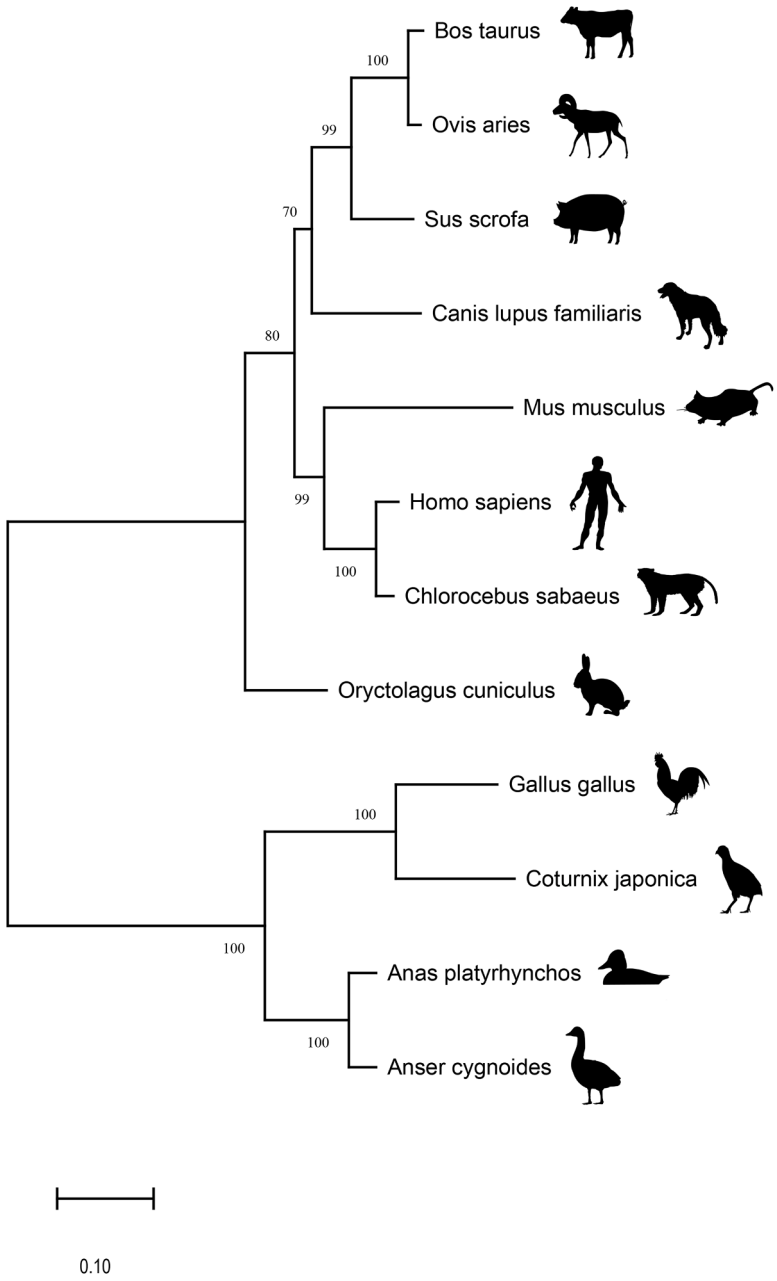

Fig. 1. Phylogenetic analysis of *NPC1L1* sequences in different species. The accession numbers of *NPC1L1* are as follows: *NPC1L1* sequences of *Gallus gallus, Coturnix japonica,* and Anas platyrhynchos are cloned in this study; *Anser cygnoides* (XM_066983451.1), *Homo sapiens* (NM_001101648.2), *Sus scrofa* (XM_005673340.3), *Bos taurus* (XM_002686890.6), *Chlorocebus sabaeus* (XM_037988233.1), *Mus musculus* (NM_207242.2), *Canis lupus familiaris* (NM_001097550.1), *Ovis aries* (XM_004008262.6), *Oryctolagus cuniculus* (NM_001082228.1). Silhouettes of vertebrates were taken from PhyloPic (http://phylopic.org/).

values serving as the final normalized value. This approach aligns with established methodologies for analyzing tissue-specific gene expression profiles in chickens (Zheng et al., 2022). To evaluate statistical significance among different groups, a one-way ANOVA was conducted following the assessment of homogeneity of variance, with significance determined at $P < 0.05$.

RESULTS

Full-length cDNA cloning of NPC1L1

To clone the complete mRNA sequences of chicken *NPC1L1*, we first aligned the *NPC1L1* sequences from various species and designed primers targeting highly conserved regions. This approach enabled us to clone partial *NPC1L1* sequences from chickens, quails, and ducks. Utilizing these partial sequences, we employed RACE technology to successfully clone the full-length *NPC1L1* mRNA sequences for poultry. The mRNA sequence lengths were determined to be 4940 bp for chicken, 4222 bp for quail, and 3938 bp for duck. Although the mRNA sequence lengths among the three species are generally comparable, analysis of the CDS regions revealed that quail possesses the longest NPC1L1 coding region, whereas duck has the shortest. Notably, the duck NPC1L1 sequence lacks approximately 100 amino acids at the N-terminus compared to those of chicken and quail. All nucleotide sequences have been submitted to the NCBI database and are accessible under accession numbers PQ845754, PQ845755, and PQ845756.

Phylogenetic and protein sequence alignment analysis of NPC1L1

In order to elucidate the genetic relationships of NPC1L1 across various species, this study utilized the cloned sequence in conjunction with mRNA and protein sequences of NPC1L1 from goose, human, pig, cattle, green monkey, mouse, dog, sheep, and rabbit, as sourced from the NCBI database, to construct a phylogenetic tree using MEGA 11 software. The phylogenetic analysis of *NPC1L1* mRNA revealed a distinct bifurcation between poultry and mammalian species, with chicken and quail forming a closer cluster, and duck and goose forming another (Fig. 1). The phylogenetic tree analysis of NPC1L1 protein sequences yielded results consistent with those of the mRNA analysis (Fig. S1). Among the 12 species examined, the NPC1L1 protein sequences exhibited a similarity of 70.20 %. Notably, the cholesterol-sensing peptide sequences demonstrated a high degree of conservation, with a similarity of 82.45 %. Within the four poultry species, the NPC1L1 protein sequences shared approximately 78.15 % similarity, and the cholesterol-sensing peptides were highly conserved, with a similarity of 95.08 %. These findings suggest that, based on sequence similarity, poultry NPC1L1 may be capable of transporting cholesterol. The results of transmembrane regions prediction, obtained using the TMHMM 2.0 online software, indicated that the NPC1L1 of chicken, quail, duck, human, green monkey, and mouse are all anticipated to possess multiple transmembrane structures (Fig. 2). Among these, the NPC1L1 proteins of quail and duck have the highest predicted number of transmembrane helices, which is 14.

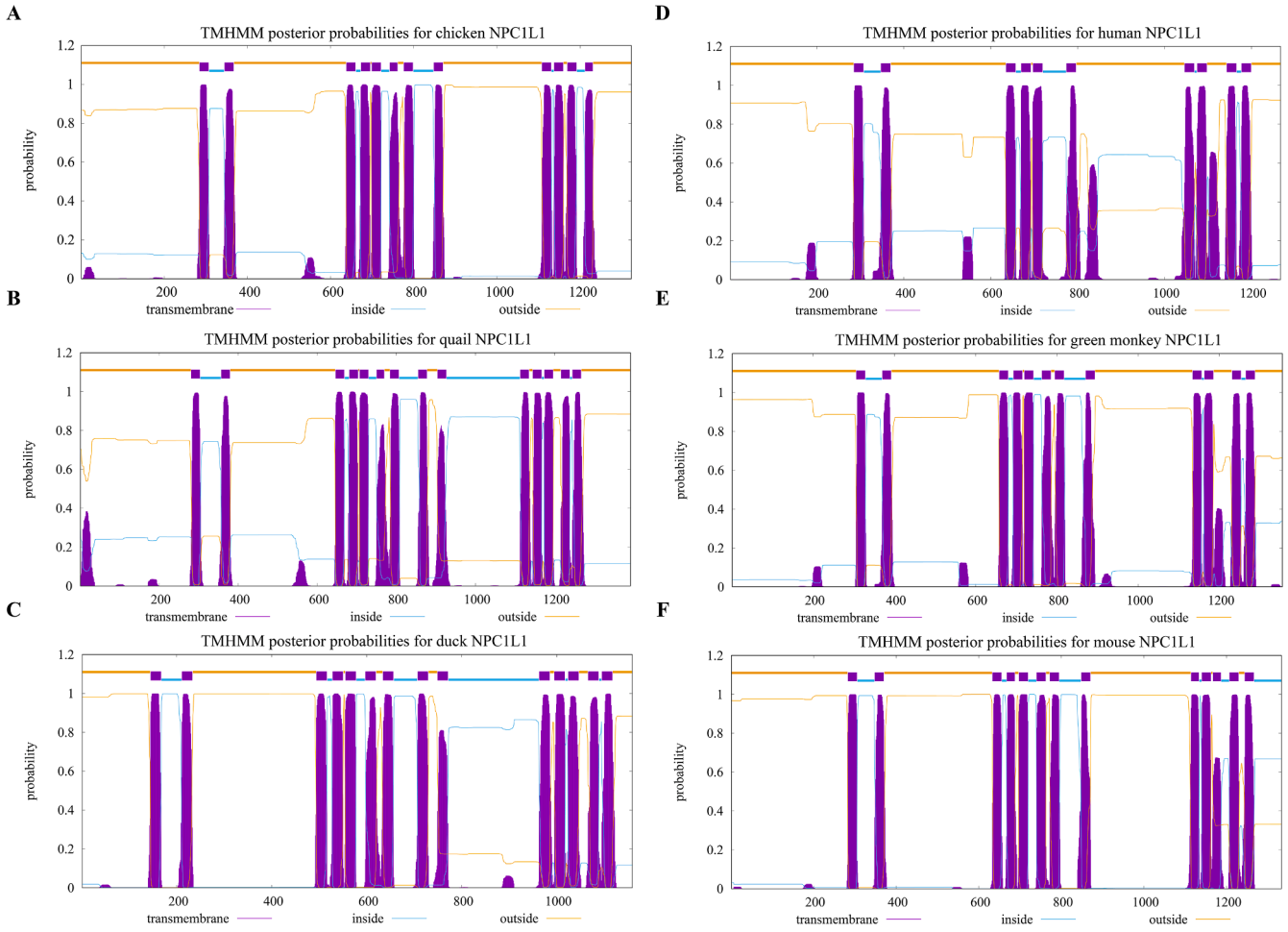

Fig. 2. Transmembrane region prediction of NPC1L1 in chicken (A), quail (B), duck (C), human (D), green monkey (E), and mouse (F) using TMHMM 2.0 (http://www.cbs.dtu.dk/services/TMHMM/).

Gene expression profile analysis of NPC1L1

To investigate the primary tissues of expression for *NPC1L1* in chickens, quails, and ducks, quantitative primers were developed based on the newly identified mRNA sequences in this study (Table S2). We analyzed the gene expression profiles of major tissues for *NPC1L1* in chickens, quails, and ducks. The findings reveal that chicken *NPC1L1* exhibited elevated expression levels in the duodenum, jejunum, liver, and lungs, with the highest expression observed in the duodenum (Fig. 3A). Similarly, quail *NPC1L1* demonstrated high expression in the duodenum, jejunum, liver, and lungs, with the highest levels observed in both the duodenum and jejunum (Fig. 3B). In contrast, the duck *NPC1L1* was predominantly expressed in the duodenum, jejunum, ileum, and liver tissues, with the duodenum showing the highest expression (Fig. 3C). Overall, the *NPC1L1* in chickens, quails, and ducks is predominantly expressed in the small intestine, particularly in the duodenum, and the liver.

Functional analysis of NPC1L1 in the transport of cholesterol

We isolated intestinal epithelial cells from chicken embryos as a representative model to demonstrate whether the cloned avian NPC1L1

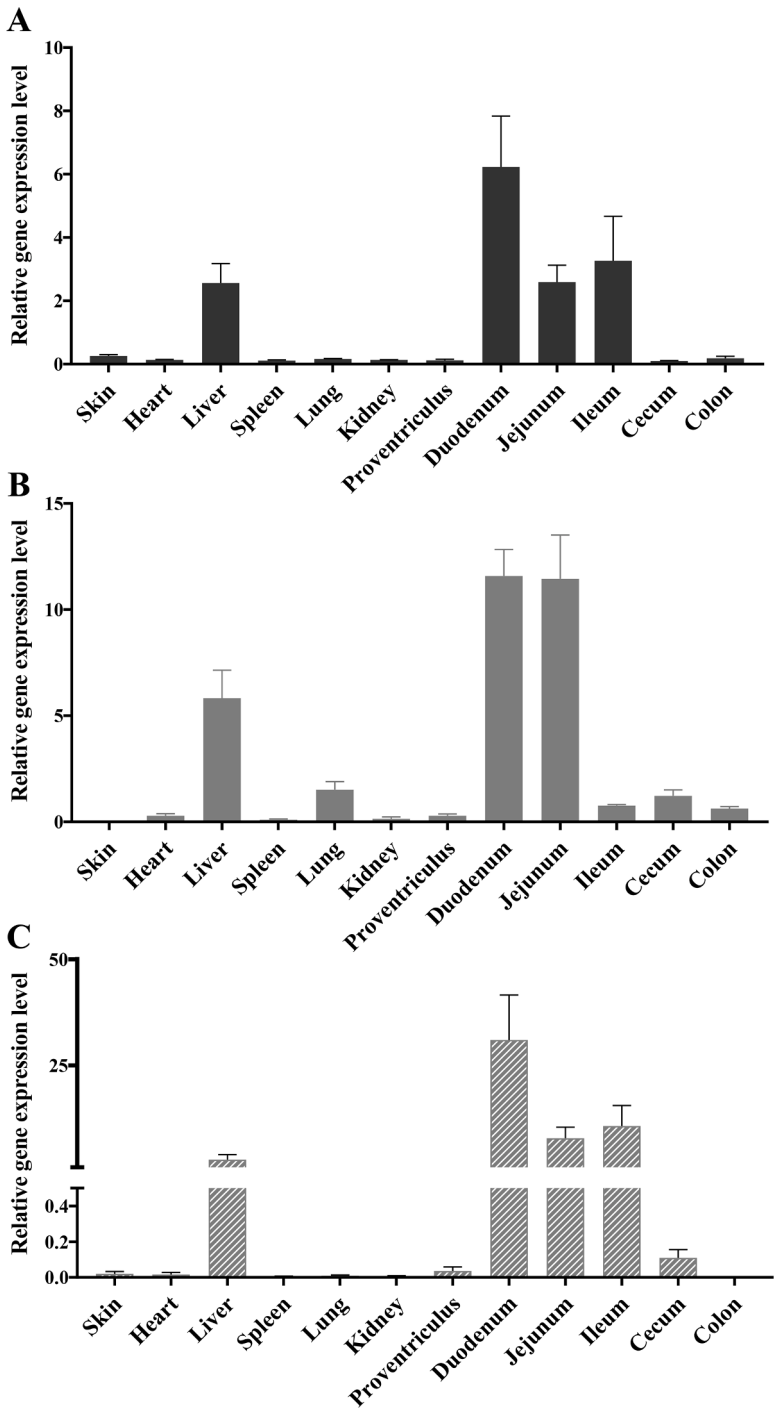

Fig. 3. Relative expression levels of *NPC1L1* in the main tissues. (A), (B), and (C) represent relative gene expression profiles of *NPC1L1* in chicken, quail, and duck in 12 tested tissues respectively.

in this study can transport cholesterol. During the isolation process, we observed the formation of cell colonies that gradually expand and eventually aggregate into a monolayer. At 24 hours, these cells are mostly flattened, polygonal, or elliptical in shape, with well-defined boundaries and a paving-stone-like appearance (Fig. 4A). RT-qPCR results showed that the designed siRNA targeting chicken *NPC1L1* could knockdown its expression by approximately 30 % at 40 hours post-transfection (Fig. 4B). The results of medium-to-cell cholesterol transit in intestinal epithelial cells demonstrate that low expression of *NPC1L1* can significantly reduce the transport of TC into cells (Fig. 4C), proving that chicken NPC1L1 also has the ability to transport cholesterol.

Discussion

NPC1L1 has emerged as a critical functional gene that is associated with cholesterol absorption in both the intestine and the liver (Jia et al., 2011; Pramfalk et al., 2011; Xiao et al., 2023); however, research and reporting on NPC1L1 in poultry studies remain limited, and its existence in poultry has not been fully confirmed. This is primarily due to the incomplete sequencing of *NPC1L1*, which hinders the ability to conduct functional gene studies.

Previous research has indicated the absence of a large number of protein-coding genes with crucial functions in birds (Friedman-Einat et al., 1999; Daković et al., 2014; Lovell et al., 2014), such as *leptin* and tumor necrosis factor-α (***TNF-α***). Over several generations, advancements have been made in the chicken genome, resulting in the successful annotating of previously uncloned or presumed non-existent genes (Wang et al., 2021; Zhu et al., 2023). Notably, *leptin*, a hormone from adipose tissue crucial for regulating appetite, energy expenditure, and fat metabolism, (Obradovic et al., 2021), ovocledin-17, which plays a vital role in eggshell formation by converting calcium carbonate into the calcite crystals that comprise the shells (Mann and Siedler, 1999; Reyes-Grajeda et al., 2004), and TNF-α, a pleiotropic cytokine with critical roles in host defense (Idriss and Naismith, 2000; Aggarwal et al., 2012), have all been cloned (Zhu et al., 2023). Despite these achievements, the chicken *NPC1L1* gene has yet to be cloned. Recent pan-genomic research has identified that the chicken *NPC1L1* exists on chromosome 22, but its sequence remains incomplete (Ren et al., 2024). Furthermore, while the *NPC1L1* in goose and quail can be determined through high-throughput sequencing, this suggests that the current gene assembly strategies in chicken may be imperfect, potentially leading to the omission of key genes. Alternatively, the complexity of the chicken *NPC1L1* sequence may necessitate the use of specialized algorithms during assembly. These hypotheses warrant further investigation in future research endeavors.

In this study, we successfully cloned the complete *NPC1L1* sequences from chicken, quail, and duck using experimental methods. Prior research has established a close phylogenetic relationship between chickens and quails (Shu et al., 2024); however, our cloned chicken *NPC1L1* sequence exhibits notable differences from the quail *NPC1L1* sequence available in the current online database, particularly at the 3′ end of the gene. To verify the accuracy of the quail *NPC1L1* sequence in the database, we employed RACE technology to re-clone the full-length quail *NPC1L1* sequence. Subsequent sequence analysis revealed that the quail *NPC1L1* sequence in the online database was incomplete.

Most poultry are omnivorous animals, and their daily diets are supplemented with protein foods. These foods contain a certain amount of cholesterol, which, if not absorbed and utilized, will result in a loss of nutrients. Consequently, it is theoretically plausible that poultry possess the NPC1L1, which facilitates cholesterol transport. Evolutionary tree analysis reveals that the sequence of NPC1L1 in birds is distinct from that in mammals (Fig. 1, Fig. S1), suggesting that the *NPC1L1* sequence has undergone long-term evolutionary selection. However, the core protein sequence of NPC1L1, including the cholesterol-sensing domain, exhibits high homology across different species. This indicates that despite undergoing distinct evolutionary pressures, poultry NPC1L1 retains the fundamental cholesterol-sensing component. Furthermore, studies predicting the transmembrane structure of NPC1L1 protein have shown that poultry NPC1L1 possesses multiple transmembrane helices, aligning with its role in transporting cholesterol into cells.

Tissue expression profiling in this study indicated that *NPC1L1* is predominantly expressed in small intestinal tissues, corroborating findings from other mammalian studies (Altmann et al., 2004). Overall, the *NPC1L1* in chickens, quails, and ducks is primarily expressed in the small intestine and liver, suggesting potential specific physiological functions. Previous research has demonstrated that dietary supplementation with lycopene in chickens significantly reduces both the cholesterol content of eggs and the intestinal expression of the NPC1L1 protein (Orhan et al., 2021). Lycopene is likely to reduce cholesterol absorption by downregulating the expression of NPC1L1 in the intestine. This finding highlights the role of chicken NPC1L1 in cholesterol transport. The study involved interfering with the expression of NPC1L1 in chicken intestinal epithelial cells, resulting in a significantly lower cholesterol content in the interference group compared to the control group (Fig. 4C). This directly confirms the ability of chicken NPC1L1 to transport cholesterol. However, further research is required to fully understand the role of NPC1L1 in cholesterol absorption in poultry. Furthermore, in this study, RT-qPCR conducted at 40 hours post-treatment revealed approximately 30 % interference effects. Had it been performed at 24 hours, the chicken *NPC1L1* mRNA expression

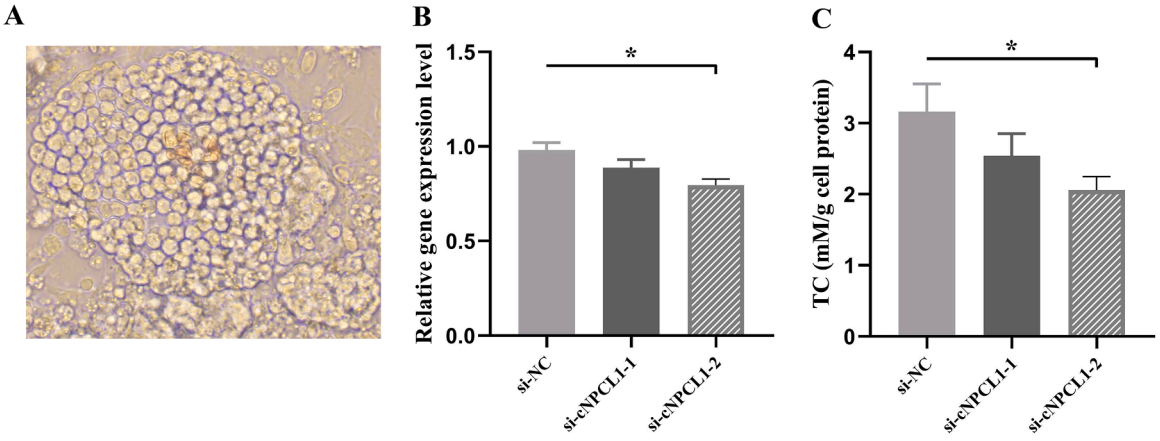

Fig. 4. The effect of chicken *NPC1L1* on cholesterol transport. (A) chicken embryo intestinal epithelial cells images with 40 X objective in Olympus IX-71 microscope. (B) show *NPC1L1* expression levels after transfection with siRNAs. (C) represent total cholesterol (TC) content of intestinal epithelial cells after transfection with siRNAs at 40 hours. cNPC1L1 means chicken NPC1L1. Significant differences were denoted by asterisks (*$P < 0.05$)

might have exhibited more pronounced interference. Nevertheless, the final transport tests proved effective, indicating a reduction in chicken NPC1L1 protein levels at the 40-hour mark. We endeavored to construct *NPC1L1* overexpression vectors in chickens and ducks, but our attempts failed due to the gene's extensive coding region and high GC content. Future research endeavors will concentrate on employing novel methods to construct avian *NPC1L1* overexpression vectors and elucidating its functions.

In mammals, NPC1L1 mediates dietary cholesterol uptake via clathrin-coated vesicle endocytosis in intestinal enterocytes (Ge et al., 2008; Li et al., 2014). It transports cholesterol to the Rab11-positive endocytic recycling compartment (**ERC**) through Flotillin-1/−2-enriched membrane microdomains, dynamically recycling between the plasma membrane and ERC via the LIMA1-myosin Vb complex (Ge et al., 2011; Zhang et al., 2018). Cholesterol is esterified by ACAT2 in the endoplasmic reticulum for chylomicron assembly and lymphatic secretion (Xie et al., 2012). Regarding the regulatory mechanisms, the expression of mouse *NPC1L1* in the intestine is down-regulated by liver X receptor activators (Duval et al., 2006; Srivastava et al., 2020). Since the gene sequence of *NPC1L1* in poultry has been cloned in this study and is predominantly expressed in the small intestine and liver, future efforts will primarily focus on investigating the impact of NPC1L1 on cholesterol transport and the regulatory mechanisms of its gene expression, both *in vivo* and *in vitro*. The ultimate goal is to identify endogenous and exogenous regulators that can modulate the expression of poultry NPC1L1, providing new theories and methods to improve lipid metabolism and enhance the health of poultry during farming. This endeavor aligns with previous research conducted on mammals (Yu et al., 2006; Cao et al., 2021; Shen et al., 2023; Xiao et al., 2023).

In conclusion, this study is the first to experimentally confirm the presence of *NPC1L1* in poultry and successfully generate full-length cDNA sequences of *NPC1L1* in chicken, quail, and duck, including both the 5′ and 3′ untranslated regions. The sequence of *NPC1L1* in major poultry is conserved and highly expressed in the small intestine and liver, and chicken NPC1L1 has the biological function of transporting cholesterol in intestinal epithelial cells. This study lays the foundation for further investigating the function of the poultry NPC1L1.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (32302725), the Natural Science Foundation of Jiangsu Province (BK20220648), the Natural Science Research of Jiangsu Higher Education Institutions of China (24KJB230001), and the Postgraduate Research and Practice Innovation Program of Jiangsu Province (SJCX24_2575).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2025.105032.

References

Aggarwal, B.B., Gupta, S.C., Kim, J.H., 2012. Historical perspectives on tumor necrosis factor and its superfamily: 25 years later, a golden journey. Blood 119 (3), 651–665.
Altmann, S.W., Davis, H.R., Zhu, L., Yao, X., Hoos, L.M., Tetzloff, G., Sai, P.N.I., Maguire, M., Golovko, A., Zeng, M., Wang, L., Murgolo, N., Graziano, M.P., 2004. Niemann-Pick C1 like 1 protein is critical for intestinal cholesterol absorption. Sci. (Am. Assoc. Advancem. Sci.) 303 (5661), 1201–1204.
Betters, J.L., Yu, L., 2010. NPC1L1 and cholesterol transport. FEBS Lett. 584 (13), 2740–2747.
Cao, K., Zhang, K., Ma, M., Ma, J., Tian, J., Jin, Y., 2021. Lactobacillus mediates the expression of NPC1L1, CYP7A1, and ABCG5 genes to regulate cholesterol. Food Sci. Nutr. 9 (12), 6882–6891.
Chen, J., Dalirsefat, S.B., Han, D., Dong, X., Hua, G., Zheng, X., Xia, T., Shao, T., Deng, X., Wu, C., 2020. An EAV-HP insertion in the 5′ flanking region of SLCO1B3 is associated with its tissue-expression profile in blue-eggshell Yimeng chickens (Gallus gallus). Poult. Sci. 99 (12), 6371–6377.
Daković, N., Térézol, M., Pitel, F., Maillard, V., Elis, S., Leroux, S., Lagarrigue, S., Gondret, F., Klopp, C., Baeza, E., Duclos, M.J., Roest Crollius, H., Monget, P., 2014. The loss of adipokine genes in the chicken genome and implications for insulin metabolism. Mol. Biol. Evol. 31 (10), 2637–2646.
Duval, C., Touche, V., Tailleux, A., Fruchart, J., Fievet, C., Clavey, V., Staels, B., Lestavel, S., 2006. Niemann–Pick C1 like 1 gene expression is down-regulated by LXR activators in the intestine. Biochem. Biophys. Res. Commun. 340 (4), 1259–1263.
Friedman-Einat, M., Boswell, T., Horev, G., Girishvarma, G., Dunn, I.C., Talbot, R.T., Sharp, P.J., 1999. The chicken leptin gene: has it been cloned? Gen. Comp. Endocrinol. 115 (3), 354–363.
Ge, L., Wang, J., Qi, W., Miao, H., Cao, J., Qu, Y., Li, B., Song, B., 2008. The cholesterol absorption inhibitor ezetimibe acts by blocking the sterol-induced internalization of NPC1L1. Cell Metab. 7 (6), 508–519.
Ge, L., Qi, W., Wang, L., Miao, H., Qu, Y., Li, B., Song, B., 2011. Flotillins play an essential role in Niemann-Pick C1-like 1-mediated cholesterol uptake. Proc. Natl. Acad. Sci. u S. a 108 (2), 551–556.
Ghiselli, F., Rossi, B., Felici, M., Parigi, M., Tosi, G., Fiorentini, L., Massi, P., Piva, A., Grilli, E., 2021. Isolation, culture, and characterization of chicken intestinal epithelial cells. BMC. Mol. Cell Biol. 22 (1), 12.
Idriss, H.T., Naismith, J.H., 2000. TNF alpha and the TNF receptor superfamily: structure-function relationship(s). Microsc. Res. Tech. 50 (3), 184–195.
Jia, L., Betters, J.L., Yu, L., 2011. Niemann-Pick C1-like 1 (NPC1L1) protein in intestinal and hepatic cholesterol transport. Annu. Rev. Physiol. 73 (1), 239–259.
Jones, D.T., Taylor, W.R., Thornton, J.M., 1992. The rapid generation of mutation data matrices from protein sequences. Bioinformatics. 8 (3), 275–282.
Li, P., Fu, Z., Zhang, Y., Zhang, J., Xu, C., Ma, Y., Li, B., Song, B., 2014. The clathrin adaptor Numb regulates intestinal cholesterol absorption through dynamic interaction with NPC1L1. Nat. Med. 20 (1), 80–86.
Lovell, P.V., Wirthlin, M., Wilhelm, L., Minx, P., Lazar, N.H., Carbone, L., Warren, W.C., Mello, C.V., 2014. Conserved syntenic clusters of protein coding genes are missing in birds. Genome Biol. 15 (12), 565.
Luo, J., Yang, H., Song, B., 2020. Mechanisms and regulation of cholesterol homeostasis. Nat. Rev. Mol. Cell Biol. 21 (4), 225–245.
Mann, K., Siedler, F., 1999. The amino acid sequence of ovocleidin 17, a major protein of the avian eggshell calcified layer. Biochem. Mol. Biol. Int. 47 (6), 997–1007.
Na, W., Wang, Y., Gong, P., Zhang, X., Zhang, K., Zhang, H., Wang, N., Li, H., 2021. Screening of reference genes for RT-qPCR in chicken adipose tissue and adipocytes. Front. Physiol. 12, 676864.
Nakano, T., Inoue, I., Takenaka, Y., Ono, H., Katayama, S., Awata, T., Murakoshi, T., 2016. Ezetimibe promotes brush border membrane-to-lumen cholesterol efflux in the small intestine. PLoS. One 11 (3), e0152207.
Obradovic, M., Sudar-Milovanovic, E., Soskic, S., Essack, M., Arya, S., Stewart, A.J., Gojobori, T., Isenovic, E.R., 2021. Leptin and Obes. Role Clin. Implic. Front Endocrinol. (Lausanne) 12, 585887.
Orhan, C., Kucuk, O., Sahin, N., Tuzcu, M., Sahin, K., 2021. Lycopene supplementation does not change productive performance but lowers egg yolk cholesterol and gene expression of some cholesterol-related proteins in laying hens. Br. Poult. Sci. 62 (2), 227–234.
Pramfalk, C., Jiang, Z., Parini, P., 2011. Hepatic Niemann–Pick C1-like 1. Curr. Opin. Lipidol. 22 (3), 225–230.
Ren, J., Kou, W., Xu, Y., Lu, M., Gong, M., Wang, X., Zhang, X., Liu, Z., Li, H., Yang, Q., Shah, A.M., Zhu, F., Hou, Z., Xu, N., Jiang, Y., Wang, F., 2024. Pan-genome analyses add ~1000 genes to the "complete" genome assembly of chicken. J. Genet. Genomics. 52 (1), 116–119.
Reyes-Grajeda, J.P., Moreno, A., Romero, A., 2004. Crystal structure of ovocleidin-17, a major protein of the calcified gallus gallus eggshell. J. Biol. Chem. 279 (39), 40876–40881.
Schade, D.S., Shey, L., Eaton, R.P., 2020. Cholesterol review: A metabolically important molecule. Endocr. Pract. 26 (12), 1514–1523.
Shen, W., Shao, W., Wang, Q., Wang, B., Zhao, G., Gu, A., Jiang, Z., Hu, H., 2023. Dietary diosgenin transcriptionally down-regulated intestinal NPC1L1 expression to prevent cholesterol gallstone formation in mice. J. Biomed. Sci. 30 (1), 44.
Shu, X., Chen, Z., Zheng, X., Hua, G., Zhuang, W., Zhang, J., Chen, J., 2024. Quail GHRL and LEAP2 gene cloning, polymorphism detection, phylogenetic analysis, tissue expression profiling and its association analysis with feed intake. Gene 918, 148479.
Srivastava, R.A.K., Cefalu, A.B., Srivastava, N.S., Averna, M., 2020. NPC1L1 and ABCG5/8 induction explain synergistic fecal cholesterol excretion in ob/ob mice co-treated with PPAR-α and LXR agonists. Mol. Cell Biochem. 473 (1-2), 247–262.
Tamura, K., Stecher, G., Kumar, S., 2021. MEGA11: molecular evolutionary genetics analysis version 11. Mol. Biol. Evol. 38 (7), 3022–3027.
Wang, K., Hu, H., Tian, Y., Li, J., Scheben, A., Zhang, C., Li, Y., Wu, J., Yang, L., Fan, X., Sun, G., Li, D., Zhang, Y., Han, R., Jiang, R., Huang, H., Yan, F., Wang, Y., Li, Z., Li, G., Liu, X., Li, W., Edwards, D., Kang, X., Wittkopp, P., 2021. The chicken pan-genome reveals gene content variation and a promoter region deletion in IGF2BP1 affecting body size. Mol. Biol. Evol. 38 (11), 5066–5081.
Wang, Q., Liu, M., Xu, L., Wu, Y., Huang, Y., 2018. Transcriptome analysis reveals the molecular mechanism of hepatic fat metabolism disorder caused by Muscovy duck reovirus infection. Avian Pathol. 47 (2), 127–139.

Xiao, J., Dong, L., Liu, S., Meng, F., Xie, C., Lu, X., Zhang, W.J., Luo, J., Song, B., 2023. Bile acids-mediated intracellular cholesterol transport promotes intestinal cholesterol absorption and NPC1L1 recycling. Nat. Commun. 14 (1), 6469.

Xie, C., Zhou, Z., Li, N., Bian, Y., Wang, Y., Wang, L., Li, B., Song, B., 2012. Ezetimibe blocks the internalization of NPC1L1 and cholesterol in mouse small intestine. J. Lipid Res. 53 (10), 2092–2101.

Xu, C., Fu, F., She, Y., Xu, C., 2023. NPC1L1 plays a novel role in nonalcoholic fatty liver disease. ACS. Omega 8 (51), 48586–48589.

Yu, L., Bharadwaj, S., Brown, J.M., Ma, Y., Du, W., Davis, M.A., Michaely, P., Liu, P., Willingham, M.C., Rudel, L.L., 2006. Cholesterol-regulated translocation of NPC1L1 to the cell surface facilitates free cholesterol uptake. J. Biol. Chem. 281 (10), 6616–6624.

Zhang, R., Zeng, J., Liu, W., Meng, J., Wang, C., Shi, L., Yang, S., Chang, J., Xing, D., 2022. The role of NPC1L1 in cancer. Front. Pharmacol. 13, 956619.

Zhang, Y., Fu, Z., Wei, J., Qi, W., Baituola, G., Luo, J., Meng, Y., Guo, S., Yin, H., Jiang, S., Li, Y., Miao, H., Liu, Y., Wang, Y., Li, B., Ma, Y., Song, B., 2018. A LIMA1 variant promotes low plasma LDL cholesterol and decreases intestinal cholesterol absorption. Science 360 (6393), 1087–1092.

Zheng, X., Chen, Z., Zhuang, W., Zhang, J., He, J., Xie, Y., Chen, J., 2022. Chicken LEAP2 level substantially changes with feed intake and may be regulated by CDX4 in small intestine. Animals 12 (24), 3496.

Zhu, F., Yin, Z., Zhao, Q., Sun, Y., Jie, Y., Smith, J., Yang, Y., Burt, D.W., Hincke, M., Zhang, Z., Yuan, M., Kaufman, J., Sun, C., Li, J., Shao, L., Yang, N., Hou, Z., 2023. A chromosome-level genome assembly for the Silkie chicken resolves complete sequences for key chicken metabolic, reproductive, and immunity genes. Commun. Biol. 6 (1), 1233.