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WEIGHTED GENE CO-EXPRESSION NETWORK ANALYSIS IDENTIFIES BIOLOGICAL PATHWAYS AND BIOMARKER GENES ASSOCIATED WITH CHICKENS' ADAPTATION TO BOTH LOW AND HIGH ALTITUDES

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ABSTRACT:

The main aim of the study was to identify modules, hub genes, and possible pathways linked with hypoxia adaptation in six types of tissues and organs (heart, kidney, liver, lung, muscle, and spleen) at altitudes ranging from 2,300 to 3,500 meters above sea level. On a transcription dataset from hypoxia-sensitive tissues, we performed weighted gene co-expression network analysis on 13,940 selected genes, and 10 transcriptional modules in total were detected (Turquoise 196 genes, Purple 27 genes, Blue 196, Brown 182, Yellow 108, Green 79 genes, Red 69 genes, Black 50 genes, Pink 44 genes, and Magenta 37 genes). Furthermore, we discovered that the majority of variable genes were screened by sub-setting 1000 genes; samples belonging to the same tissue clearly clustered together, and the expression in the liver and lung was more associated than in the heart and spleen. Functional enrichment analysis of all genes in 12 selected modules revealed that 9 KEGG pathways were considerably enriched, 13 Gene ontology terms were significantly enriched in the biological process and cellular component pathways, and 15 gene ontology terms were significantly enriched in the molecular function pathway. Through weighted gene co-expression network analysis, the results of this study expand our knowledge of the molecular pathway of catalytic and metabolic activity as a biomarker pathway

KEYWORDS: Poultry; Co-expression network; Molecular pathway; Altitude adaptation; Hypoxia

1. INTRODUCTION

Among the most popular and extensively dispersed highland breeds, Tibetan chicken is frequently utilized as a model organism to comprehend genetic adaptability to harsh environments in Tibet (Li *et al.*, 2023). However, native chickens in both high and low altitude environments exhibit a remarkable genetic variety (Sha *et al.*, 2020; Li *et al.*, 2023). Currently, published research has identified the genes PPARA, EGLN1, and EPAS1 as being crucial for adaptation to both high and low altitude (Shen *et al.*, 2020). Exploring the molecular pathways driving hypoxia adaptation has long sparked interest. It is critical to investigate the similarities in gene expression regulatory patterns related to environmental adaptability between high and low altitude in hypoxia. Weighted Gene Co-expression Network Analysis (WGCNA) is a technique for identifying patterns of gene expression across various samples that classifies genes with comparable expression profiles into modules based on their relationships. These modules can then be linked to certain characteristics or situations, allowing researchers to better understand the biological processes, molecular function and cellular component. It is extensively used in genomics to investigate complicated diseases, gene functions, and regulatory mechanisms (Langfelder *et al.*, 2008). WGCNA is increasingly being utilized in environmental research to investigate concerns such as adaptation to severe environments, biodiversity, and community ecology. It assists in connecting species or traits to environmental conditions, identifying ecological process networks, and investigating their responses to environmental changes. In a study in *Bos* species, with regard to hypoxic adaptation, hub genes were discovered based on connectivity by establishing a weighted gene co-expression network, and two hypoxia-related specialized modules were produced (Bao *et al.*, 2021). The current study aims to apply a weighted gene co-expression network in the Tibet chicken in six types of organs (heart, kidney, liver, lung, muscle, and spleen) to discover the relevant regulatory gene modules connected to the functions of each tissue and examine the primary driver genes. The current study will provide thorough investigation and new insights into how genes are constantly expressed in high and low altitude conditions.

2. MATERIALS AND METHODS

Raw data processing:

The Normalized counts dataset (fragments per kilobase of transcript (FPKM) per million mapped reads) was collected from the National Center for Biotechnology Information. Genes having an FPKM greater than 0.5 in more than 80% of the samples were chosen for further analysis using log2-transformed (FPKM + 1) values. The RNA dataset is available through BioSample: accession experiment number SAMN06242699; in the archives, GEO accession (GSM2464087 to GSM2464119). The details of the experiment, library preparation, and analysis were previously disclosed (Tang *et al.*, 2017). In brief, total RNA was isolated from six tissues (heart, kidney, liver, lung, muscle, and spleen) obtained from a semi-wild maintained field at an altitude of 2,300-3,500 meters in the Drung and Nujiang river basins. Raw data were aligned with the chicken maternal broiler

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reference genome (bGalGal1.mat.broiler.GR-Cg7b (GCA 016699485.1).

Construction of Gene Co-Expression Network:

WGCNA (Langfelder *et al*., 2008), iDEP.96 (Ge *et al*., 2018) packages were used to reveal patterns of gene expression in six types of tissues and organ Tibetan chicken in a hypoxic environment. The topological overlap matrix was analyzed using average linkage hierarchical clustering. Genes that were functionally connected were visualized using MF (Molecular Function), BP (Biological Process), CC (Cellular Component), and KEGG pathway (Kyoto Encyclopedia of Genes and Genomes). Furthermore, the most variable genes were identified by sub-setting 1000 genes, and their expression was examined and shown in a heatmap tree. All of these analyses were performed utilizing tools inside the Rstudio program (Team, 2014).

3. RESULTS

Weighted Gene Co-expression Analysis:

In WGCNA analysis, 15,496 genes from the transcription dataset were processed for co-expression analysis. The soft-power threshold of 14 (scale-free R2 of 0.85) was chosen for further analysis (Fig. 1 A), after which 13,940 genes passed the filter, the expression pattern across all samples and conditions was normalized, and no artifact genes remained in the dataset (Fig. 1 B). We created branches of strongly related genes and assigned each one a unique color. Finally, ten transcriptional modules (Turquoise 196 genes, Purple 27 genes, Blue 196, Brown 182, Yellow 108, Green 79 genes, Red 69 genes, Black 50 genes, Pink 44 genes, and Magenta 37 genes) were discovered (Fig. 1c). According to regulatory network analysis, SLC27AS and SLC22A1B genes are required to express other highest cluster module genes (transcriptional modules of Turquoise 196 genes) (Fig. 1d).

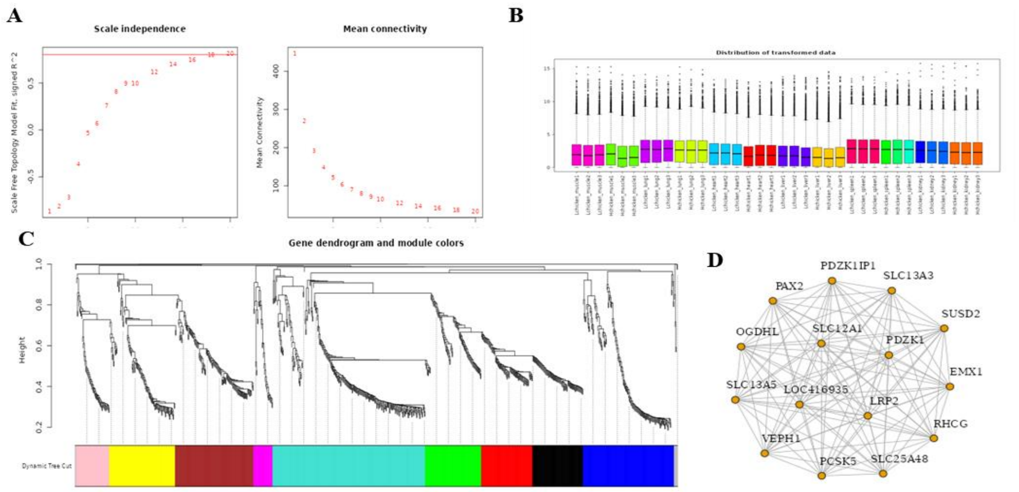

Figure 1: WGCNA analysis of 13,940 genes from the transcription dataset. (A) A plot of scale-free topology and mean connectivity in terms of soft-thresholding power for samples. (B) Expression pattern across all samples of processed data. (C) A hierarchical clustering tree (dendrogram) of genes created using co-expression network analysis. (D) Regulatory network analysis of the top 15 genes subset from 192 genes.

The most variable genes for 1000 genes were selected, and samples from the same tissue were clearly clustered together; expression in the liver and lung was more closely related than in the heart and spleen. As demonstrated in Figure 2, over 250 genes in the liver (in both low and high-altitude environments) are significantly up-regulated in gene expression when compared to other organs. In addition, muscular tissues express a subset of genes differently than other types of tissues.

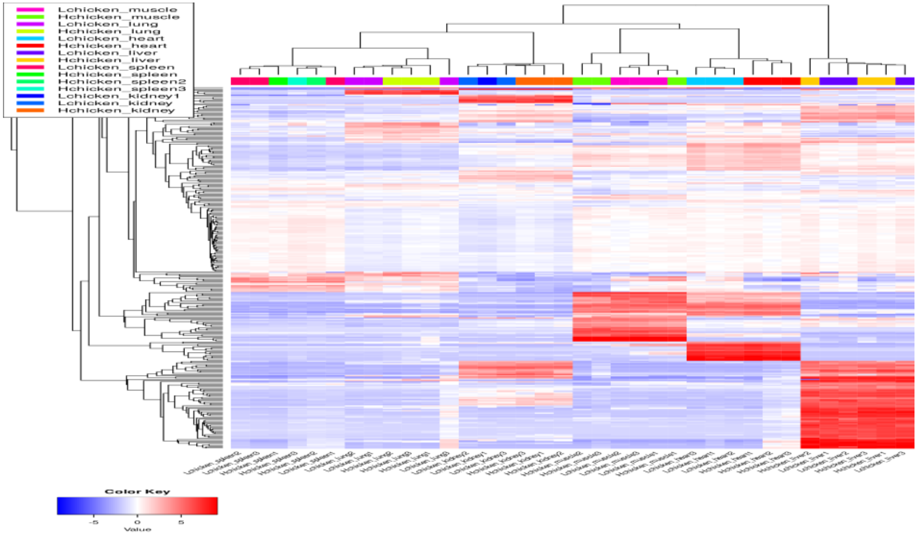

Figure 2: Expression Heatmap tree showing 1000 genes. The sample and the expression count are columns and rows, respectively. The color bar on the bottom indicates the scaled expressions of these genes in each sample. The red means high level of gene expression while blue shows reduced relative gene expression.

Gene enrichment analysis:

The functional enrichment analysis of all genes in 12 selected modules revealed that 15 GO terms were considerably enriched in the MF pathway, 13 GO terms were significantly enriched in the BP and CC pathways, and 9 KEGG pathways were significantly enriched at adj.Pval less than 0.001 (Fig. 2). Catalytic activity was the most important pathway at the MF, with over 70 genes involved, dramatically increasing the p.adj value to 0.00017. Serine activity is also considerably enriched (p.adj value 0.000000056) by 13 genes in the MF pathway, as is peptidase activity, and endopeptidase inhibitor activity is significantly enriched (p.adj value < 0.00001) in more than 15 genes. BP pathways investigation revealed numerous intriguing pathways that were enriched, such as immunity and defense response in over 30 genes with a p.adj value less than 0.00001. Metabolic pathways were also strongly and significantly enriched in more than 50 genes, as demonstrated by KEGG pathway analysis (p.adj value < 0.000012).

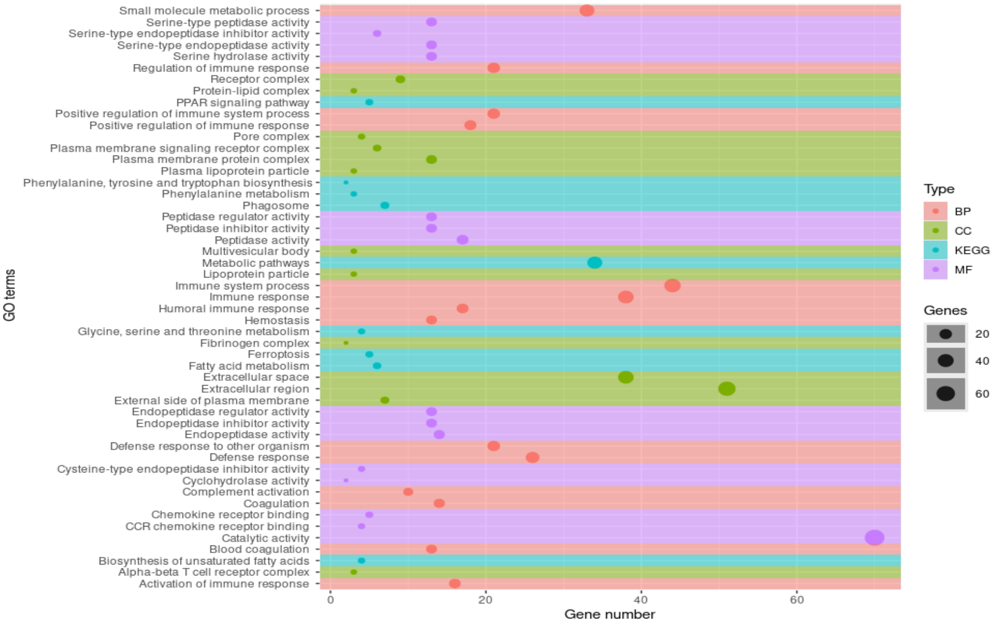

Figure 3: GO term enrichment pathway analysis with P adj value < 0.001. The X-axis depicts gene numbers, whereas the Y-axis represents GO terms. Red dot (BP), pink dot (MF), green dot (CC), and blue dot (KEGG) represent pathways.

4. DISCUSSION

As was pointed out in the introduction, WGCNA is increasingly being utilized in exploring adaptation of organisms to the environment. This study found that SLC27AS and SLC22A1B genes are required to be on the highest cluster module genes in Tibetan chickens in a hypoxic environment. The SLC27A5 gene produces the amino acid coding of fatty acid transport protein 5 (FATP5), which has a dual role as an acyl-CoA synthetase and a fatty acid transporter (essential for activating fatty acids for metabolism) (Wang *et al*., 2012). FATP5 is abundantly expressed in most organs, and the SLC27AS gene affects bile acid conjugation and lipid metabolism (Anderson *et al*., 2013). This study implies that at both high and low altitudes, adaptation to hypoxia by the heart, kidney, liver, lung, muscle, and spleen may stabilize FATP5 activity, so supporting overall metabolic balance. Another important finding is that many important GO terms were enriched in level BP, MF, CC, and KEGG pathways. Most interesting and highly enriched pathways are stability of catalytic and serine activity. The gene expression of these pathways across tissue types and altitudes can be attributed mostly to changes in air pressure, oxygen availability, and temperature. These results support data from prior studies suggesting catalytic and serine activity play a role in adaptation to atmospheric pressure, reduced oxygen levels, and temperature changes (Cheviron *et al*., 2012; Fu *et al*., 2022). Immunity and defense response were enriched in over 30 genes. These findings imply that chickens in high and low altitudes have evolved distinct adaptations to help preserve immunity during hypoxia stress. According to these findings, we can conclude that these 30 genes serve as biomarkers in the chicken immune system. However, this finding has not been previously reported. Several other investigations have detected these genes (Mx1, CCL5, TLR3, IL-8, IRF1, and STAT1) in the chicken, notably in response to bacterial and viral infections Heidari *et al*., 2010; Rue *et al*., 2011; Matulova *et al*., 2013; Cheng *et al*., 2014; Kang *et al*., 2016; Ranaware *et al*., 2016; S. *et al*., 2017; Schilling *et al*., 2018). These findings may suggest that adaptation to high altitude entails a number of metabolic processes and physiological challenges that allow individuals to adapt to harsh environments. All of these could be through oxygen transport and use, mitochondrial and cellular respiration, glycolysis and glucose, lipid and protein metabolism, decreased fat oxidation, and enhanced proteolysis (Papandreou *et al*., 2006; O'Brien *et al*., 2020).

CONCLUSIONS

We provide a comprehensive comparative transcriptome landscape of co-expression between low- and high-altitude chicken populations across multiple organs. This study revealed ten transcriptional modules in the Tibetan genome, including 196 genes in one module and 37 cluster genes in another. The second key finding was that the gene family SLC27AS regulates the expression of 196 genes in all organ types, with the gene's primary function supporting systemic metabolic balance through ATP5 action. Another significant discovery from this study was that 30 genes were continually expressed in immune system control. Finally, we discovered metabolic pathways; adaptation to high altitude entails a number of metabolic pathways and physiological challenges.

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Author Contributions

All authors contributed to the design and execution of this study. P.S.A.B., methodology. P.Y., data analysis. S.I.M., revise and writing. S.M., writing.

Ethical Statement

Department of Medical laboratory, College of Science Approved this work (Approval Code: CSML-2024-034).

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