Different Genetic Resistance Resulted in Distinct Response to Newcastle Disease Virus

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Summary and Implications

As one of the most severe infectious diseases in the poultry industry, Newcastle disease (ND) causes a significant economic loss worldwide even with the extensive implementation of vaccine. To find targets to improve genetic resistance to ND to enhance protection in chickens, gene expression was analyzed in spleen of two chicken lines which differed in their resistance to ND. The comparison of gene expression between two treatments (challenged or non-challenged) in the two chicken lines at 2 and 6 days post-inoculation (dpi) suggests that that the most dramatic changes of gene expression occurred in Leghorn chickens at 2dpi. The identified differentially expressed genes that regulate splenic response to NDV provide potential avenues to breed NDV-resistant chickens in the future.

Introduction

Despite the prevalent use of vaccination to prevent outbreaks of ND, the virus genetic diversity, continuous evolution of NDV, and improper management of vaccines can still lead to failure to control of the disease. Therefore, enhancing genetic resistance of chickens through genetic selection and breeding is necessary. In this study, two inbred chicken lines - Fayoumi and Leghorn – were inoculated with NDV or phosphate-buffered saline (PBS). Fayoumi chickens, which are derived from Egyptian village fowl, serve as a resistant model because of their robust resistance to some avian diseases, while Leghorn chickens serve as a disease-susceptible model. RNA-seq is a high-throughput technology that facilitates comprehensive gene expression analysis throughout genome. The purpose of this study was to find out how the gene expression in the chicken spleen was affected by NDV treatment and genetic resistance through RNA-seq study.

Materials and Methods

Fayoumi and Leghorn chickens (n=24 for each line) were raised at the same condition with free access to feed and water after hatch. At 21 days-post-hatch, each line was randomly divided into two groups (n=12 in each group), one group was treated with a mild LaSota NDV strain through nasal and ocular inoculation routes, while the other group was treated with Phosphate-buffered saline (PBS). At 2 and 6dpi, the spleen tissues (n=6/group/time point) were collected for RNA isolation and the viral load in lachrymal fluid was measured by real-time PCR. Using RNA-seq technology, the expressed mRNA from each gene in each spleen sample was converted to cDNA and the fragments were counted after subsequent amplification, and then the magnified counts were used for comparison of gene expression between different treatments and chicken lines. The genes with significantly different amount of mRNA between two different groups were then defined as differentially expressed genes (DEGs), and their enriched gene ontology and related pathways were identified through Ingenuity Pathway Analysis (IPA).

Results and Discussion

Both Fayoumi and Leghorn lines showed fewer DEGs for NDV vs. PBS contrast at 6dpi than those at 2dpi as the viral load decreased along with time. However, Fayoumi chickens seem to have higher resistance to and faster elimination of the virus than Leghorn chickens. When the viral loads in challenged birds were similar between the two lines at 2dpi, Fayoumi chickens showed much less DEGs number than Leghorn chickens. When Fayoumi chickens showed much less viral load than Leghorn chickens at 6dpi, their gene expressions have dropped to the same level as PBS group (Table 1). Seven DEGs involved in regulation of interferon signaling and innate immune response are upregulated in NDV challenged birds in Leghorn at 2 & 6dpi and Fayoumi at 2dpi, indicating that these genes play universal roles in regulation of immune response to NDV (Table 2). On the other hand, seven DEGs involved in glutamine synthesis, complement activation and immune regulation are unique in Fayoumi at 2dpi, suggesting that these genes may play key roles to enhance genetic resistance to NDV (Table 3). These results provide some potential targets for future breeding of NDV-resistant chickens to improve the production of poultry industry.

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Table 1. NDV viral loads in lachrymal fluid and number of DEGs in spleen in the two chicken lines at two time points

	NDV viral load in Challenged Birds (Log Copy number)	Number of DEGs (adjusted P<0.1)
Leghorn 2dpi	6.873±0.115	124
Fayoumi 2dpi	6.866±0.133	25
Leghorn 6dpi	5.694±0.136	49
Fayoumi 6dpi	5.123±0.176	0

Note: NDV viral load is shown as mean±standard error.

Table 2. Full names and functions of the seven shared genes among three different groups

Gene	Full Name	Main Function
DDX60	Dead Box Protein 60	Promote activation of retinoic acid-inducible gene I and viral RNA degradation
IFIT5	Interferon-Induced Protein With Tetratricopeptide Repeats 5	Enhance innate immune signaling pathways
Mx	Interferon-Induced GTP-binding protein	Participates in the cellular antiviral response
ZNFX1	Zinc Finger, NFX1-Type Containing 1	Involved in regulation of disease resistance
CMPK2	Cytidine Monophosphate (UMP-CMP) Kinase 2	Participates in terminal differentiation of monocytic cells
OASL	2'-5'-Oligoadenylate Synthetase-Like	An interferon induced enzyme with antiviral activity
USP18	Ubiquitin Specific Peptidase 18	A major negative regulator of IFN signaling

Table 3. Full names and functions of the seven unique genes in Fayoumi chickens at 2dpi

Gene	Full Name	Main Function
GDA	Guanine Deaminase	Catalyzes synthesis of xanthine and ammonia from deamination of guanine
PLA2R1	Phospholipase A2 Receptor 1	Contribute to antibody formation and complement activation
GLUL	Glutamate-Ammonia Ligase	Catalyzes the synthesis of glutamine from glutamate and ammonia
PID1	Phosphotyrosine Interaction Domain Containing 1	Interact with nuclear factor-κB which is a key regulator of immune system
MRC1	Mannose Receptor, C Type 1	Mediates the endocytosis of glycoproteins by macrophages
GPT2	Glutamic Pyruvate Transaminase 2	Catalyze the synthesis of pyruvate and glutamate from alanine and ketoglutarate
CFHR2	Complement Factor H-Related 2	Regulates complement activation