

More MHC-like Class I Y mRNA Detected in Relatively Resistant Fayoumis than Susceptible Leghorns

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Melissa S. Deist, Graduate Research Assistant, ISU;
Rodrigo A. Gallardo, Assistant Professor, UC Davis;
David A. Bunn, Assistant-Adjunct Professor, UC Davis;
Terra R. Kelly, Senior Research Scientist, UC Davis;
Jack C.M. Dekkers, Distinguished Professor, ISU;
Huaijun Zhou, Distinguished Professor, UC Davis;
Susan J. Lamont, Distinguished Professor, ISU

Summary and Implications

The inbred chicken lines Fayoumi and Leghorn have been shown to be relatively resistant and susceptible, respectively, to Newcastle disease virus (NDV). In this experiment we detected higher gene expression levels of MHC-like class I Y (MHCIY) in the Fayoumi trachea, lung, and Harderian gland of NDV challenged and nonchallenged birds across multiple time points. MHC region is important to the immune system and is often associated with resistance to pathogens. This gene may be associated with the Fayoumis' resistant phenotype.

Introduction

The Fayoumi and Leghorn are known to differ in their response to many pathogens (including NDV), with the Fayoumi tending to respond more favorably, resulting in their 'resistant' label. Examining the gene expression of an immune related gene may give insights into the differences in the immune system between these two lines.

Gene expression experiments measure and compare amounts of mRNA transcribed from particular genes. A gene's mRNA expression level tends to correlate with its protein level. Studying the expression levels of genes gives insight into their function. Gene expression levels are dynamic and change in response to stimuli; they can be impacted by time, tissue, and treatment.

The MHC region contains many genes that are very important to the immune system and it has previously been associated with resistance to pathogens in many species. The MHC-like class I Y (MHCIY) gene is assumed to be important because of its sequence similarity to MHC class I, however, very little is known about the specific function of MHCIY.

This study examined the expression levels of MHCIY in the trachea, lung, and Harderian gland tissues of nonchallenged (control) birds and birds challenged with NDV, at three time points post infection, in both the Fayoumis and Leghorns. We expected to see differences between the two lines and higher expression of MHCIY in the challenged birds compared to the nonchallenged

chickens, because increased levels of this gene are expected to lead to increased immune function.

Materials and Methods

In this study, Fayoumis and Leghorns were either challenged with a highly concentrated NDV vaccine or administered saline-solution (nonchallenged) via the eyes and nares. At 2, 6, and 10 days post infection (dpi), challenged and nonchallenged birds were euthanized for collection of the trachea, lung, and Harderian gland. The epithelial lining of the trachea was removed and used for further analysis. The RNA was isolated from all samples and used to quantify the amount of MHCIY mRNA present using Fluidigm Biomark to perform quantitative PCR. The following primers were used to bind and amplify MHCIY mRNA: forward (GCCGGAACGCTACAACAAA) and reverse (TCCAGGATGTCACAGCCAAA). For each sample, the cycle threshold (CT) value for a housekeeping gene was subtracted from MHCIY CT value to calculate the Δ CT values. The Δ CT values were analyzed using JMP statistical software to test for the main effects of line, tissue, challenge, and dpi.

Results and Discussion

There was a significant impact of line ($p < 0.001$), treatment ($p = 0.0056$), and tissue ($p < 0.001$) on the expression level of MHCIY (Figure 1). MHCIY levels did not differ between time points post infection ($p > 0.05$). The challenged birds expressed more MHCIY than the nonchallenged birds, which suggests this gene may have a role in host defense against NDV.

Higher levels of MHCIY were detected in the Fayoumis regardless of tissue, treatment, or dpi (Figure 1). A duplication event may have occurred in the historical development and evolution of the Fayoumis, resulting in two copies of this gene, leading to higher mRNA levels. Previously, duplications of other important immune genes were identified in the Fayoumi.

Also, the Fayoumi and Leghorn produced different melting curves (data not shown), suggesting differences in the amplicon, which is the region targeted by the qPCR primers. The amplicon for each genetic line may include different structural variations in the DNA (insertions, deletions, etc.), which could impact the function of this gene.

This study warrants future experiments to investigate the structural and possible copy number variation of the MHCIY gene and determine if and how that variation in MHCIY contributes to the differences in the relative resistance and susceptibility of the Fayoumi and Leghorn.

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higher the $-\Delta\text{CT}$ value (y-axis) the more MHCIY was detected for each individual (black dot). The x-axis separates individuals by dpi (2, 6, or 10), challenged (NDV) or Nonchallenged (NON) group, Fayoumi (white) or Leghorn (gray), and tissue (trachea, lung, Harderian gland). Box plots show the amount of variation among samples within each treatment group.

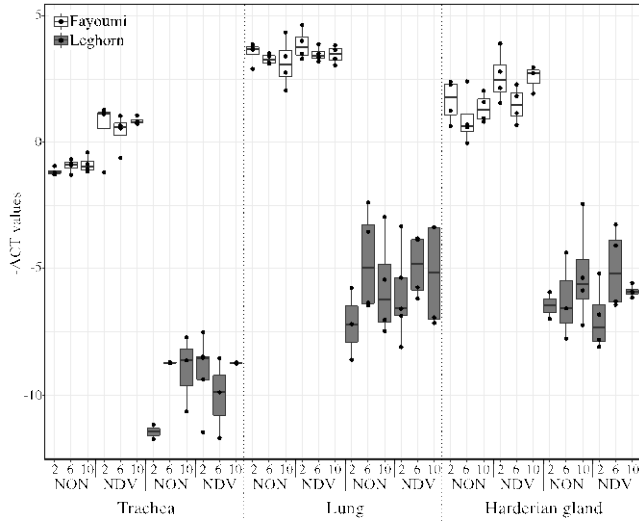


Figure 1: Box plot of the $-\Delta\text{CT}$ values of the MHCIY gene across three tissues in Fayoumi and Leghorn chickens. The