RELATIONSHIP BETWEEN CANDIDATE GENES OF INNATE IMMUNITY AND RESISTANCE TO VIRAL DISEASES IN CHICKEN

By

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B.Sc. Agric. Sc. (Poultry Production), Ain Shams University, 2001

M.Sc. Agric. Sc. (Poultry Breeding), Ain Shams University, 2006

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ABSTRACT

Haitham Ahmed Mohamed Ahmed Yacoub: Relationship Between Candidate Genes of Innate Immunity and Resistance to Viral Diseases in Chicken. Unpublished Ph.D. Dissertation, Department of Poultry Production, Faculty of Agriculture, Ain Shams University, 2010.

Gallinacins are antimicrobial peptides that play a significant role in innate immunity in chicken. The aim of this study was to determine the relationship between candidate genes of innate immunity and resistance to Marek's disease and to predict whether the amino acids substitutions lead to produce new phenotypes. We used in current study two inbred lines of White Leghorn chickens, line 6 which selected for resistant to Marek's disease and line 7 which selected to susceptible to Marek's disease from ADOL, ARS,USDA. We examined gallinacins (1-5) and Gal-10 in current study by sequenced a 3.68 kb in two directions from two inbred lines (6 and 7). A total of 17 SNPs were identified within the sequenced regions. This equates to an SNP rate of 4.61 SNPs/kb, nearly to the previously reported 5 SNPs/kb across the entire chicken genome. The current study showed that the gallinacin genes are polymorphic because there are many single nucleotide polymorphisms (SNPs) in both inbred lines of White Leghorn chickens and some of these SNPs are nonsynonymous and others are synonymous and some of them are located in intronic region and the rest are in exonic region. All identified SNPs were intronic, except for Gal-1 and Gal-4, were exonic resulting in an amino acids changes but that is for Gal-1 only which have a non-synonymous SNP resulting in amino acids changes of asparagine to serine, histidine to tyrosine and tyrosine to serine, respectively. From SIFT (Sorting Intolerant from tolerant) program which used to predict whether an amino acids substitutions can affect protein function resulting in phenotypic effect , that is may be made the inbred line 7 of White Leghorn chickens are susceptible to Marek's disease rather than line 6. We are concluded that a new chromosomal region with effects on the response to Marek's disease in chickens was characterized in this study. Within this region, the SNPs in the gallinacin candidate genes could potentially be used

in a marker assisted selection program to enhance the response to Marek's disease. Analysis of the gallinacin genes in the protective pathways of disease resistance has also opened the possibilities for therapeutic strategies using endogenous antimicrobial peptides.

Key Words: single nucleotide polymorphisms, Gallinacin, genes, Marek's disease, resistance

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LIST OF ABBREVIATIONS

A	Adenine
ADOL	Avian Disease and Oncology Laboratory
AFLP	Amplified Fragment Length Polymorphism
ALV	Avian Leukosis Virus
AMP	Antimicrobial Peptide
BNBD	Bovine Neutrophil β-Defensins
Bp	Base Pair
C	Cytosine
cDNA	Complementary DNA
CEF	Chicken Embryo Fibroblast
CHP	Chicken Heterophil Peptides
DNA	Deoxy Nucleic Acid
E.coli	*Escherichia Coli*
EBV	Epstein- Barr Virus
ED 18	Embryonic Date 18
EST	Expressed Sequence Tag
FAO	Food and Agriculture Organization
G	Guanine
Gal	Gallinacin
GH	Growth Hormone
GPV	Gallopavin
HIV	Human immunodeficiency virus
HSV	Herpes Simplex Virus
HVT	Turkey Herpesvirus
IBDV	Infectious Bursal Disease Virus
Line 6	MD Resistant Line
Line 7	MD susceptible Line
MAS	Marker Assisted Selection
MD	Marek's Disease
MDV	Marek's Disease Virus
MHC	Major Histocompatibility Complex

MI	Michigan State
MIV	Multivalent *in vo* vaccine
NDV	Newcastle Disease Virus
OSP	Ostricacin From Ostrich
PCR	Polymerase Chain Reaction
QTL	Quantitative Trait Loci
RAPD	Randomly Amplified Polymorphic DNA
REFLP	Restriction Fragment Length Polymorphism
REV	Recombinant Fowlpox Virus
RNA	Ribo nucleic Acid
RNP	Rabbit Neutrophil Peptide
RPV	Reticuloendothelioss Virus
SCA 2	Stem Cell Antigen 2
SIFT	Sorting Intolerant from Tolerant
SNP	Single Nucleotide Polymorphism
SSCP	Single Strand Conformation Polymorphism
T	Thymine
THP	Turkey Heterophil Peptide
TSA1	Thymic Shared Antigen 1
USDA	United States Department of Agriculture
VZV	Varicella-Zoster Virus
α-Defensins	Alpha Defensins
β-Defensins	Beta- Defensins
θ-Defensins	Ceuta Defensins

I. INTRODUCTION

Global production of chickens has experienced massive change and growth over the past 50 years. The commercial broiler and layer markets produce more than 50 billion birds annually to meet current worldwide consumer demands of more than 74 million metric tons of meat and more than 66 million metric tons of eggs. In fact, poultry has become the leading meat consumed in the United States and most other countries and is the most dynamic animal commodity in the world; production has increased by 436% since 1970, more than 2.3 times and 7.5 times the corresponding growth in swine and beef, respectively **(http//faostat.fao.org).** Unfortunately, the poultry industry continues to be confronted with new and emerging infectious diseases such as Newcastle disease, avian leucosis, avian influenza and Marek's disease that can led to significant economic losses.

Marek's disease (MD) is a lymphoproliferative disease, caused by a member of the herpesvirus family, that is estimated to cost the poultry industry nearly $1 billion annually. Diseased chickens infected by the Marek's disease virus (MDV), the causative pathogen, commonly exhibit paralysis, blindness, and visible lymphoid tumors that result in condemnation of the birds. Although vaccination programs have effectively reduced the incidence of MD, there is evidence that current vaccines do not protect well against some highly pathogenic MDV strains that have emerged in recent years, Also, MD vaccines control rather than eliminate losses from MD because they do not block MDV infection, thus as a result, MDV is ubiquitous on poultry farms, and all chickens are exposed to the pathogenic agent at 1 day of age.

All these factors point to the need to complement vaccinal protection with alternative methods such as genetic resistance and even if a specific disease has been controlled through vaccination, genetic resistance is of value because it represents a safeguard against heavy losses in the case of disease outbreaks.

One such class of genes that may play a role in resistance to Marek's disease are gallinacin genes, one family of antimicrobial peptides (AMP). Antimicrobial peptides (AMP) are relatively small molecules that are less than 100

amino acids in length and have a broad spectrum of antimicrobial activity **(Ma *et al.*, 2008).**

The main objectives of this study is

1. To screen candidate (gallinacin genes) in the inbred White Leghorn Lines 6 subline 3 (6_3) and 7 subline 2 (7_2), which are Marek's disease resistant and susceptible, respectively.
2. To predict whether an amino acid substitution in a protein will have a phenotypic effect on Marek's disease.

II. REVIEW OF LITERATURE

1. Overview of immune system

The immune system provides protection against infectious diseases that are caused by various microorganisms including viruses, bacteria, pathogenic fungi and parasites, and can be broadly divided into two categories namely the innate or non-specific immune system and the acquired or specific immune system. Innate immunity is the basic defense mechanism for an animal provided by non-discriminatory barriers of anatomic, physiologic, endocytic and phagocytic and inflammatory mechanisms. The acquired immunity is a specialized response against infectious agents, and is characterized by specificity, diversity, memory and self/non-self recognition **(Kuby *et al*., 1997)**. The effector cells involved in the innate immune response include granulocytes. However, there is not a complete demarcation between these two types of immunities and cells involved in the innate immune response take part in effector actions of the adaptive immune response **(Janeway *et al*., 2001).**

2. Genetic markers in the chicken

The majority of genetic markers for chicken are molecular DNA-based markers. The DNA markers are of two types: within genes (Type I) or anonymous DNA segments (Type II), which include microsatellites, randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLP), CR1 retrotransposon elements, and others **(Emara and Kim, 2003).** SNPs are the genetic marker of choice now. The most current SNP “chip” can genotype 60 K markers.

Currently, there are approximately 350 Type I markers present in chicken genes **(Groenen *et al.,* 2000).** In the past, Type I markers didn’t receive extensive applications in QTL mapping due to the laborious RFLP analysis and the limited number of RFLP that were observed within Type I loci. In contrast, the Type II markers have received considerably more attention and they have been the marker of choice for genetic mapping and QTL searches **(Emara and Kim, 2003).**

In fact, microsatellite markers have been referred to as the "second-generation of markers" for gene mapping studies, whereas the genes are