Infection, Genetics and Evolution 21 (2014) 351–358

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

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

A genome-wide association study identifies major loci affecting the immune response against infectious bronchitis virus in chicken

Chenglong Luo [a,c,1], Hao Qu [a,c,1], Jie Ma [a,c], Jie Wang [a,c], Xiaoxiang Hu [b], Ning Li [b], Dingming Shu [a,c,*]

[a] Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, China
[b] State Key Laboratory for Agro-Biotechnology, China Agricultural University, Beijing 100193, China
[c] State Key Laboratory of Livestock and Poultry Breeding, Guangzhou 510640, China

ARTICLE INFO

Article history:
Received 23 August 2013
Received in revised form 6 November 2013
Accepted 3 December 2013
Available online 11 December 2013

Keywords:
Chicken
Coronavirus
Infectious bronchitis virus
Immune response
Genome-wide association study
Single nucleotide polymorphism

ABSTRACT

Coronaviruses are a hot research topic because they can cause severe diseases in humans and animals. Infectious bronchitis virus (IBV), belonging to gamma-coronaviruses, causes a highly infectious respiratory viral disease and can result in catastrophic economic losses to the poultry industry worldwide. Unfortunately, the genetic basis of the host immune responses against IBV is poorly understood. In the present study, the antibody levels against IBV post-immunization were measured by an enzyme-linked immunosorbent assay in the serum of 511 individuals from a commercial chicken (*Gallus gallus*) population. A genome-wide association study using 43,211 single nucleotide polymorphism markers was performed to identify the major loci affecting the immune response against IBV. This study detected 20 significant ($P < 1.16 \times 10^{-6}$) effect single nucleotide polymorphisms for the antibody level against IBV. These single nucleotide polymorphisms were distributed on five chicken chromosomes (GGA), involving GGA1, GGA3, GGA5, GGA8, and GGA9. The genes in the 1-Mb windows surrounding each single nucleotide polymorphism with significant effect for the antibody level against IBV were associated with many biological processes or pathways related to immunity, such as the defense response and mTOR signaling pathway. A genomic region containing a cluster of 13 *beta-defensin* (*GAL1–13*) and *interleukin-17F* genes on GGA3 probably plays an important role in the immune response against IBV. In addition, the major loci significantly associated with the antibody level against IBV on GGA1 and GGA5 could explain about 12% and 13% of the phenotypic variation, respectively. This study suggested that the chicken genome has several important loci affecting the immune response against IBV, and increases our knowledge of how to control outbreaks of infectious bronchitis.

1. Introduction

Since severe acute respiratory syndrome (SARS), caused by a coronavirus (CoV), emerged and caused human mortality in China in 2002, CoVs have received relatively much attention as human pathogens (Saif, 2004). The emergence of SARS-CoV also suggested that the evolution of CoVs has broken through the species barrier (Woo et al., 2006; Dong et al., 2007). Consequently, research has focused on CoV infections in animals, including wild and domestic animals.

Avian infectious bronchitis virus (IBV) belongs to the gamma-CoVs. Infectious bronchitis (IB), which is caused by IBV, was first reported in the United States in the 1930s (Cavanagh, 2007). IB began as a disease of young birds, but as time progressed, the disease spread to older birds worldwide (Sjaak et al., 2011). IBV can cause a severe respiratory viral disease of chickens and shows high mortality. IBV can also replicate in some epithelial cells of the gut, kidney and oviduct, resulting in poor weight gain in broilers, and serious egg yield drop and poor egg quality in layers and breeders (Ignjatovic and Sapats, 2000; Cavanagh, 2001, 2007). Thus, IB has a significant economic impact on the modern poultry industry. In addition, the CoV causing SARS most likely recombined genomic sequences from mammalian-like and avian-like CoVs (e.g. IBV), according to the molecular genetic data (Stavrinides and Guttman, 2004). The impact of SARS on public health has led to the epidemiology of IB receiving much attention in many countries (Bourogaa et al., 2009; Pohuang et al., 2009; Rimondi et al., 2009; Kulkarni and Resurreccion, 2010; Villarreal et al., 2010; Chacon et al., 2011; Ji et al., 2011; Abdel-Moneim et al., 2012; Acevedo et al.,

* Corresponding author. Address: Institute of Animal Science, Guangdong Academy of Agricultural Sciences, No. 1 Dafeng 1st Street, Wushan, Tianhe District, Guangzhou 510640, Guangdong, China. Tel.: +86 20 61368838; fax: +86 20 38765373.
E-mail addresses: chenglongluo1981@163.com (C. Luo), qhw03@163.com (H. Qu), zzitmj@126.com (J. Ma), wangjie030@126.com (J. Wang), huxx@cau.edu.cn (X. Hu), ninglcau@cau.edu.cn (N. Li), shudm@263.net (D. Shu).
[1] These authors contributed equally to the study.

1567-1348/$ - see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.meegid.2013.12.004

2012; Ma et al., 2012a,b). Studying IBV alone to develop IB vaccines is insufficient to control IB outbreaks because IBV is highly variable (Sjaak et al., 2011). Viruses and hosts show coevolution, so increasing our knowledge of host responses against IBV is helpful to protect the poultry industry from IB. However, there are few studies of the host responses against IBV, especially their genetic basis.

Fortunately, host immune responses to pathogens, such as Marek's disease virus and *Escherichia coli*, can be heritable in chickens (Yonash et al., 1996; Pitcovski et al., 2001; Sarson et al., 2008). Thus, we believed that it would be useful to explore the immune response of chicken to IBV by high-resolution mapping of loci affecting the antibody levels against IBV. Genome-wide association studies (GWASs) have become one of the most commonly used strategies for identifying genes for complex traits in humans, as well as in animals. In chickens, some major loci associated with growth (Gu et al., 2011; Xie et al., 2012), egg production (Liu et al., 2011; Wolc et al., 2012), resistance to Marek's disease (Li et al., 2012) and immune response to Newcastle disease virus (Luo et al., 2013) were identified by GWASs.

This study aimed to identify major genomic regions associated with the immune response against IBV using a GWAS based on a 60 k single nucleotide polymorphism (SNP) chip (Groenen et al., 2011) in chickens. We hoped to increase our knowledge of methods for controlling outbreaks of IB from a host perspective.

2. Materials and methods

2.1. Animals and phenotypic measurements

The Animal Care Committee of Institute of Animal Science, Guangdong Academy of Agricultural Sciences (Guangzhou, People's Republic of China) approved the study (Approval No. GAAS-IAS-2009-73).

All experimental birds were from an F_2 population, which was built from the full-sib intercross of two divergent lines (23 P and 51 F_1). The first line was a fast-growing Chinese yellow broiler, which had undergone more than 10 generations of selection for high growth rate. The second line was the Huiyang bearded chicken, a Chinese local breed with a low growth rate and high meat quality tailored to Chinese tastes. The resistance to IBV of the second line is better than that of the first line (unpublished results). The population included 511 individuals from six hatches. All experimental birds were weighed at 28 and 91 days, and were immunized with a commercial IBV live attenuated vaccine of the H120 strain (Intervet International B.V., Boxmeer, Netherlands), using the standard dose given in the instructions of the vaccine, by eye drop at day 30 and serum was collected at day 91. Venous blood was collected into centrifuge tubes containing anticoagulant and stored in −80 °C for SNP genotyping. The antibody levels against IBV (*S*/*P* values) were determined using an indirect enzyme-linked immunosorbent assay (ELISA) test according to the instructions of a commercial ELISA kit (BioChek, Inc., Foster City, CA, USA). The ELISA kit measures IBV-specific immunoglobulin Y, but not immunoglobulin A or immunoglobulin M. All of the experimental birds were positive for the IBV-specific immunoglobulin Y post-immunization.

2.2. SNP genotyping and selection

Genomic DNA of all birds was extracted from venous blood by a phenol–chloroform method. DNA LandMarks Inc. (Quebec, Canada) helped with the genotyping of the chicken 60 k SNP chips from Illumina Inc. (Groenen et al., 2011) using 75 μL of approximately 50 ng/μL genomic DNA. Six of 511 samples were excluded because more than 5% of the SNP genotypes were missing. Of 57,636 SNPs in the 60 k chip, 43,211 SNPs with a minor allele frequency (MAF) of 5% or greater, a call rate of 95% or greater, and having exact chromosome position in the 505 samples were selected for use in the current study. The mean distance between adjacent SNPs was 23.39 kb.

2.3. Statistical analysis

Population stratification can influence accuracy of a GWAS; therefore, we conducted a principal component analysis (PCA), which approximately explains population structure, in the experimental population using the validated information SNPs by GAPIT (Lipka et al., 2012). Simultaneously, the kinship for the 505 birds was estimated by program TASSEL version 3.0 (Bradbury et al., 2007). The phenotypic data for the antibody levels against IBV were initially adjusted for sex and hatch effects by the general linear model $Y_{ij} = \mu + \mathrm{SEX}_i + H_j + e_{ij}$, where Y_{ij} is the phenotypic value for the antibody response against IBV, μ is the overall mean, SEX_i is the effect of the ith sex, H_j is the effect of the jth hatch, and e_{ij} is the residual effect. The adjusted phenotypic value was calculated as $\mu + e_{ij}$. The adjusted phenotypic values, including the PCA and the kinship information were then used to perform the GWAS, as well as genetic parameters estimation for the antibody response against IBV, with a mixed linear model implemented in TASSEL version 3.0 (Bradbury et al., 2007). The model was described as follows:

$$\boldsymbol{y} = \boldsymbol{Xb} + \boldsymbol{Za} + \boldsymbol{e}$$

where $\boldsymbol{y}$ is the vector of the adjusted phenotypic values; $\boldsymbol{b}$ is the vector of fixed effects including the SNP and population structure (PCA); $\boldsymbol{a}$ is the vector of random additive genetic effects from multiple background quantitative trait loci (QTLs) for individuals; $\boldsymbol{e}$ is the vector of random residuals; and $\boldsymbol{X}$ and $\boldsymbol{Z}$ are the corresponding incidence matrices. The variance of random effects were $\mathrm{var}(\boldsymbol{a}) = \boldsymbol{G} = \boldsymbol{K}\sigma_{\boldsymbol{a}}^{\boldsymbol{2}}$ and $\mathrm{var}(\boldsymbol{e}) = \boldsymbol{R} = \boldsymbol{I}\sigma_{\boldsymbol{e}}^{\boldsymbol{2}}$, and the distributions for the random effects were assumed as: $\boldsymbol{a} \sim N(0, \boldsymbol{G})$ and $\boldsymbol{e} \sim N(0, \boldsymbol{R})$, where $\boldsymbol{K}$ is the kinship matrix; σ_a^2 is the animal additive genetic variance; $\boldsymbol{I}$ is an identity matrix; and σ_e^2 is the residual variance.

The threshold *P*-value for declaring genome-wide significance was $0.05/43{,}211 = 1.16 \times 10^{-6}$ $(-\log_{10}(P) = 5.94)$, according to Bonferroni correction. Haploview (Barrett et al., 2005) was used to calculate the linkage disequilibrium (LD), whose parameter was r^2 in this study, for SNPs on each chicken (*Gallus gallus*) chromosome (GGA). The extent of LD was calculated under the standard with $r^2 = 0.1$. Each candidate genomic region related to the antibody response against IBV was predicted to cover a length of the genome-wide significant SNP position ± the extent of LD in the corresponding GGA.

2.4. Bioinformatics analysis

Information on the genes in the candidate genomic regions was obtained from Ensembl (Ensembl Genome Browser, 2011) and NCBI (National Center for Biotechnology Information, 2011). The Database for Annotation, Visualization and Integrated Discovery (DAVID) (Dennis et al., 2003; Huang et al., 2009) was used to conduct pathway analysis for the candidate genes. Further global analysis for the identified pathways was performed through the KEGG pathway database and the KEGG Atlas (Kanehisa and Goto, 2000; Okuda et al., 2008; Kanehisa et al., 2012; Kotera et al., 2012), including comparisons with other species.