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Genome-wide association study on reproductive traits in Jinghai Yellow Chicken

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ABSTRACT

To identify molecular markers and candidate genes associated with reproductive traits, a genome-wide analysis was performed in Jinghai Yellow Chickens to analyze body weight at first oviposition (BWF), age at first oviposition (AFE), weight of the egg at first oviposition (FEW), egg weight at the age of 300 days (EW300), number of eggs produced by 300 days of age (EN300), egg hatchability (HA) and multiple selection index for egg production (MSI). The results showed that seven single nucleotide polymorphisms (SNPs) were associated with reproductive traits ($P < 1.80E{-}6$, Bonferroni correction). The *P*-values of the seven SNPs were 5.62E−10, 3.45E−08, 9.76E−07, 8.90E−07, 1.12E−06, 1.42E−07 and 1.48E−07, respectively. These SNPs were located in close proximity to or within the sequence of the five candidate genes, including FAM184B, TTL, RGS1, FBLN5 and PCNX. An additional 46 SNPs that could be associated with reproductive traits were identified ($P < 3.59E{-}5$, Bonferroni correction). Identification of the candidate genes as well as genome-wide SNPs that may be associated with reproductive traits will greatly advance the understanding of the genetic basis and molecular mechanisms underlying reproductive traits and may have practical significance in breeding programs for the improvements of reproductive traits in the Jinghai Yellow Chicken.

1. Introduction

Reproductive traits are important economical traits that reflect animal production performance. Improving reproductive performance is, therefore, essential for a chicken breeding program. However, the conventional method of genetic selection is becoming more and more difficult to make improvements in reproductive traits. With advances in technologies of molecular genetics and availability of DNA markers, the identification of a quantitative trait locus (QTL) controlling chicken reproductive traits for application in marker-assisted selection (MAS) has been progressing rapidly (Wilkanowska et al., 2014; Qin et al., 2015).

A genome-wide association study (GWAS) using a high density genotyping platform represents a method for identifying genetic variations influencing various traits. As a statistical tool, GWAS is one of the most effective methods for identifying important SNPs and candidate genes. In recent years, many important SNPs associated with chicken body weight traits have been identified (Gu et al., 2011; Xie et al., 2012). For reproductive traits, Liu et al. (2011) performed a GWAS on both White Leghorn and dwarf brown-egg layers and identified eight SNPs that were associated with reproductive and egg quality traits. Wolc et al. (2014) performed a GWAS on egg production and quality for a brown egg layer line, and several QTLs

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were identified. However, there has not been a GWAS on reproductive traits of any of the Chinese chicken breeds.

The Jinghai Yellow Chicken is a national meat breed which has been developed by the closed breeding of this breed for seven generations. This chicken breed is characterized by high-quality meat, early maturation, high reproductive performance and its adaptability to poor quality feeds. In the present study, a GWAS was performed on 400 Jinghai Yellow Chickens to identify molecular markers and candidate genes affecting reproductive traits by using 60 K SNP Illumina chicken array. Results could potentially benefit research into chicken reproductive traits.

2. Materials and methods

2.1. Ethics statement

All experimental procedures were conducted in conformity with institutional guidelines for the care and use of laboratory animals, and protocols were approved by the Institutional Animal Care and Use Committee of Ethic Institute, Yangzhou University, China.

2.2. Experimental animals

Unrelated male chickens ($n = 19$) from the Jiangsu Jinghai Poultry Industry Group Co., Ltd. were selected to constitute 19 half-sib families, and each male chicken was mated with ten female chickens by use of the artificial insemination technology. There were 400 female offspring resulting from the tenth generation of selection that were chosen randomly from the same batch of birds. The chickens were reared in stair-step cages under consistent conditions. Records were kept for body weight at first oviposition (BWF), age at first oviposition (AFE), egg weight at first oviposition (FEW), egg weight at the age of 300 days (EW300) and number of eggs produced by 300 days of age (EN300). A multiple selection index (MSI) for egg production and hatchability (HA) were calculated. Johnson transformations were applied using Minitab (v16.1.1) for the seven traits that did not follow a normal distribution. The multiple selection index (MSI; Wang et al., 2001) was used during the breeding process of the Jinghai Yellow Chicken and it is calculated as follows:

$$I = 1.05 \times D + 2.86 \times N + 0.62 \times W$$

where I, multiple selection index; D, age at first oviposition; N, number of eggs produced by 300 days of age and W, egg weight at the age of 300 days.

HA in the present study was defined as the hatchability of fertilized eggs. This value was calculated based on the incubation records from the same batch which was hatched at the age of 300 days, and all hens had a record of producing at least five eggs from which hatching occurred.

2.3. Sample preparation

Blood samples were collected from the wing vein of 400 female offspring using heparin sodium as anticoagulants. Genomic DNA was extracted using the Dzup Genomic DNA Isolation Reagent Kit (Blood) as per manufacturers' instructions (Sangon Biotech Co., Ltd., Shanghai, China). DNA concentrations and purity were quantified by using an ND-1000 spectrophotometer (Nano Drop Co., Ltd., USA) and equilibrated to 50 ng/μl for genotyping. Genotyping services were provided by DNA LandMarks Inc. (Quebec, Canada) using the Illumina 60 K Chicken SNP Beadchip.

2.4. Data preparation

Quality control of the genotyping data was performed in PLINK (v1.07; Purcell et al., 2007). During this process, four individuals were rejected for low call rate (<90%), and 10,971 SNPs were rejected for low call frequency (<95%), low Hardy–Weinberg equilibrium (<1E−6) or low minor allele frequency (<3%). In total, 396 individuals and 46,665 SNPs were retained for further study. The SNP statistics for different chromosomes are shown in Table S1.

Supplementary Table S1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.anireprosci.2015.09.011.

2.5. Statistical analysis

The population structure was calculated by multidimensional scaling analysis (MDS) in PLINK. Autosomal linkage disequilibrium based on SNP pruning was determined using parameters of 25, 5 and 0.2, representing window size, window step, and the r^2 threshold, respectively. For the 12,877 independent SNPs that were identified, an identity-by-state (IBS) analysis was performed. The MDS was performed based on the IBS matrix. Moreover, a principal component analysis (PCA) for the independent 12,877 SNPs was performed in GCTA (version 1.24; Yang et al., 2011).

The mixed linear model (MLM (1) in Tassel; version 3.0; Bradbury et al., 2007) was used in this study. The model is as follows:

$$Y = X\alpha + P\beta + K\mu + e \quad (1)$$

where Y is the vector of observations; X is the genetic marker (46,665 SNPs) matrix; P is the population structure matrix including PCA1 and PCA2; K is the relative kinship matrix; α and β are vectors for genetic marker and population structure, respectively; μ is a vector of random additive genetic effects from genetic background (kinship); and e is the random error. The relative kinship matrix (K) was calculated using the 12,877 independent SNPs acquired by Plink (v1.07) software. The analyses were performed using TASSEL 3.0 software (version 3.0).

Significance thresholds were established from the estimated number of independent SNP markers and linkage disequilibrium (LD) blocks. LD block was defined as a set of contiguous SNPs having pairwise r^2 values >0.40 (Gu et al., 2011). Using this approach, a total of 27,824 independent SNP markers and LD blocks were found. The threshold P-value of the 5% Bonferroni genome-wide significance was set at 1.80E−6 (0.05/27,824), and the threshold P-value for significance was *3.59E−5* (1/27,824).