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Identification of 19 loci for reproductive traits in a local Chinese chicken by genome-wide study

Q.C. Fan[1]*, P.F. Wu[1]*, G.J. Dai[1], G.X. Zhang[1], T. Zhang[1], Q. Xue[1], H.Q. Shi[2] and J.Y. Wang[1]

[1]College of Animal Science and Technology, Yangzhou University, Yangzhou, Jiangsu, China
[2]Jiangsu Jinghai Poultry Industry Group CD, LTD, Nantong, Jiangsu, China

*These authors contributed equally to this study
Corresponding author: G.X. Zhang
E-mail: gxzhang@yzu.edu.cn

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ABSTRACT. Reproductive traits have long been studied and have an important influence on chicken breeding. To identify quantitative trait loci affecting reproductive traits, a genome-wide analysis of a Chinese chicken breed was performed to analyze age at first egg body weight at first egg, first egg weight, egg weight at the age of 300 days, egg weight at the age of 462 days, egg number at the age of 300 days, egg number between the ages of 300 and 462 days and egg number at the age of 462 days. Nineteen SNPs related to reproductive traits were presented (P < 1.80E-6). Nine of the 19 SNPs had a significant effect on BWF, six SNPs were significantly associated with egg weight, and four SNPs were significantly associated with egg number. These SNPs were located near to or in 17 genes including *FAM184B*, *HTT*, *KCNH7*, *CDC42BPA*, *KCNIP4*, *GJA5*, *CBFB*, and *GPC6.* The present results may be beneficial for reproductive research and may be used in marker-assisted selection in future studies. These results

could potentially benefit further breeding programs, especially in Jinghai Yellow Chicken.

Key words: Reproductive traits; Jinghai Yellow Chicken; GWAS; SNP; Marker-assisted selection

INTRODUCTION

Reproductive traits have been studied since breeding work began and are very important for chicken breeding. Marker-assisted selection (MAS) has been shown to improve outcomes in chicken breeding programs. Thus, quantitative trait loci (QTLs) associated with reproductive traits are increasingly important in modern breeding work (Cui et al., 2006), and remarkable progress has been made towards identifying effective genes and markers (Li et al., 2011; Xiao et al., 2011; Xu et al., 2011). Xu et al. (2011) found that five single nucleotide polymorphisms (SNPs), that is, C1704887T and C1715301T of VIPR-1, T5841629C of DRD2, and T32742468C and G32742603A of SH3GL2 were associated with egg number at the age of 300 days (EN300).

Genome-wide association studies (GWAS) are used to identify SNPs and functional genes that affect quantitative traits (Jin et al., 2015). This technique is more efficient at identifying genetic characteristics for economic traits than the candidate gene approach. Success in chicken body weight and reproductive traits has been achieved through GWAS in recent years (Liu et al., 2011; Xie et al., 2012; Liu et al., 2013; Zhang et al., 2015). SNPs showing genome-wide significance ($P < 2.59E-6$) for nine out of 10 body composition traits studied were identified by Liu et al. (2013). Additionally, a consistent region on chicken (*Gallus gallus*) chromosome 4 (GGA4), including seven significant SNPs and four candidate genes (LCORL, LAP3, LDB2, and TAPT1), were found to be associated with carcass weight and eviscerated weight. Xie et al. (2012) found that the region 169-179 Mb on GGA1 was significantly associated with 23 growth traits. Liu et al. (2011) found eight SNPs showing genome-wide significance ($P < 1.51E-06$) associated with egg production and quality traits under the Fisher's combined probability method. Zhang et al. (2015) found that two SNPs reached 5% Bonferroni genome-wide significance ($P < 1.8E-6$) and seven SNPs reached "suggestive" genome-wide significance ($P < 3.59E-6$) for meat quality.

In this study, a GWAS was performed to identify effective SNPs and functional genes for reproductive traits in Jinghai Yellow Chicken using a 60 K SNP Illumina chicken array. The results of this study may be of benefit in further breeding programs.

MATERIAL AND METHODS

Ethics statement

Chicken blood samples were collected from the brachial vein by a standard venipuncture procedure that was approved by the Animal Welfare Committee of Yangzhou University.

Experimental animals

The experimental chickens included 19 half-sib families. Four-hundred chickens were kept under the same conditions in step cages and fed commercial diets meeting requirements

of United States National Research Council (NRC). Records were kept and the following traits were calculated: age at first egg (AFE), body weight at first egg (BWF), first egg weight (FEW), egg weight at the age of 300 days (EW300), egg weight at the age of 462 days (EW462), egg number at the age of 300 days (EN300), egg number between the ages of 300 and 462 days (ENA300), and egg number at the age of 462 days (EN462).

Basic reproductive traits are shown in Table 1. Johnson transformations were applied to traits that did not conform the normal distribution using Minitab (v16.1.1).

Table 1. Recorded data for reproductive traits.

Trait	Sample	Max	Min	Average	Standard deviation
BWF (g)	400	2284.0	1110.0	1657.2	208.6
AFE (days)	400	166.0	115.0	143.6	9.4
FEW (g)	400	56.0	18.0	32.6	5.4
EW300 (g)	397	61.7	37.5	50.3	3.6
EN300	400	154.0	110.0	126.3	9.2
ENA300	396	138.0	1.0	85.3	27.3
EW462 (g)	314	65.0	36.7	50.9	4.0
EN462	394	281.0	119.0	211.3	29.6

BWF = body weight at first egg; AFE = age at first egg; FEW = first egg weight; EW300 = egg weight at the age of 300 days; EN300 = egg number at the age of 300 days; ENA 300 = egg number between the ages of 300 and 462 days; EW462 = egg weight at the age of 462 days; EN462 = egg number at the age of 462 days.

Sample preparation

DNA was extracted from the samples using a Dzup Genomic DNA Isolation Reagent Kit (Blood) from Sangon Biotech Co., Ltd. (Shanghai, China). Following use in a concentration and purity assay, the DNA was sent to DNA LandMarks Inc. (Quebec, Canada) for genotyping analysis.

Data preparation

Plink (v1.07) was used for the quality control of genotyping data (Purcell et al., 2007). During this process, 10,971 SNPs were rejected for having low Hardy-Weinberg equilibrium (<1E-6), low call frequency (<95%), low minor allele frequency (<3%), or low call rate (<90%). Therefore, 396 individuals and 46,665 SNPs were used for further research. Multidimensional scaling analysis (MDS) was used to calculate the population structure in PLINK. SNP pruning can result in autosomal linkage disequilibrium, which was determined using window size, window step, and the r^2 threshold (25, 5, and 0.2, respectively). Finally, 12,877 independent SNPs were identified in the present study. In addition, a principal component analysis (PCA) was conducted for the 12,877 SNP loci in GCTA (version 1.24). In order to reduce the influence of population stratification, the first and second components of the PCA were used in the model.

Statistical analysis

The general linear regression model (GLM) in PLINK was used in this study. The model is as follows:

$$Y = G\alpha + X\beta + e \quad \text{(Equation 1)}$$

where Y is the vector of observations; X is a matrix containing all other fixed effects, including the population structure (PCA1 and PCA2) effect, *G* is the genetic marker (46,665 SNPs) matrix, α and β are the incidence matrix, and e is the the random error

The P value was adjusted by Bonferroni correction based on linkage disequilibrium (Wang et al., 2009). To ensure the accuracy of the results, the r^2 value was set to 0.4. In total, 27,824 independent SNPs and linkage disequilibrium (LD) blocks were found. The Bonferroni significance level of the P value was 1.8E-6 (0.05/27824).

RESULTS

Nineteen SNPs were significantly associated with six reproductive traits, which are shown in Table 2. No SNPs associated with AFE and EN300 were found. The Manhattan plots of the six reproductive traits are presented in **Figure S1**.

Table 2. Statistics for significant single nucleotide polymorphisms SNPs and proximal genes.

Traits	SNP ID	Chr	Pos (bp)[1]	Alleles[2]	MAF[3]	P-adj[4]	Proximal genes[5]
BWF	GGaluGA049990	1	151372832	TC	0.2058	1.99E-07	67 D GPC6
	rs13713113	1	151458029	TC	0.0596	2.47E-08	53 U LOC101751412
	rs13957061	1	154795787	CT	0.3023	1.19E-08	238 U LOC101748963
	rs13713351	1	154823490	TC	0.3114	2.85E-07	210 U LOC101748963
	rs15498187	1	173718815	GA	0.2121	1.20E-08	8 D MIR15A
	rs13973774	1	175116535	CT	0.2273	6.07E-07	63 U COG6
	rs14710787	4	78797460	GA	0.3548	7.12E-09	FAM184B
	rs16023603	4	78802461	AC	0.3750	9.75E-08	FAM184B
	GGaluGA267974	4	85148698	CT	0.4508	1.62E-06	HTT
FEW	rs14714701	7	22380561	AG	0.2150	7.47E-07	KCNH7
EW300	rs14085822	3	13239671	CT	0.3611	1.87E-07	CDC42BPA
	GGaluGA265645	4	76554919	AG	0.2967	7.21E-08	62 U PPARGC1A
	GGaluGA265806	4	77456527	TC	0.2538	1.74E-06	KCNIP4
EW462	rs13929546	1	122953030	AG	0.2741	6.08E-07	318 U CNKSR2
	rs14254270	2	141172740	GT	0.2828	5.43E-07	57 U MED30
ENA300	rs13905010	1	95793916	CT	0.2273	1.28E-06	GJA5
	rs15938574	2	31333604	AG	0.4205	4.31E-08	20 D STK31
	rs15602813	11	2338929	CT	0.3835	1.22E-06	CBFB
	rs13628422	11	2350952	GA	0.302	1.40E-06	CBFB
EN462	rs13905010	1	95793916	CT	0.2273	1.86E-08	GJA5
	rs15938574	2	31333604	AG	0.4205	6.86E-07	20 D STK31

[1]The positions are based on WADHUC2. [2]The first allele is the minor allele. [3]Minor allele frequency. [4]P-adj represents the P value adjusted by Bonferroni correction. [5]U = upstream, D = downstream. Distance is measured in kb. Proximal genes were obtained from the National Center for Biotechnology Information (NCBI). BWF = body weight at first egg; FEW = first egg weight; EW300 = egg weight at the age of 300 days; EW462 = egg weight at the age of 462 days; ENA 300 = egg number between the ages of 300 and 462 days.

Body weight at first egg

Nine of the 19 SNPs had a significant effect on BWF. Six of the nine SNPs were found separately in the regions 151.3-154.9 Mb (4) and 173.0-175.2 Mb (2) on chromosome 1 (GGA1). The three remaining SNPs were located in the region 78.7-85.2 Mb on GGA4. The nearest genes to these nine significant SNPs were glypican 6 (*GPC6*), *LOC101751412*, *LOC101748963*, microRNA mir-15a (*MIR15A*), component of oligomeric golgi complex 6 (*COG6*), family with sequence similarity 184, member B (*FAM184B*), and huntingtin (*HTT*).

Egg weight

Six SNPs were significantly associated with egg weight. One had an effect on FEW, was located at 22.4 Mb on GGA7, and was proximal to the gene potassium voltage-gated channel, subfamily H (eag-related), member 7 (*KCNH7*). Three SNPs were associated with EW300, of which, one was located at 76.6 Mb on GGA4 and the nearest gene was peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (*PPARGC1A*). The remaining two SNPs were located at 13.2 Mb on GGA3 and 77.5 MB on GGA4, and were proximal to the genes CDC42 binding protein kinase alpha (*CDC42BPA*) and Kv channel interacting protein *4* (*KCNIP4*). Two SNPs were identified as having effects on EW462. They were located at 141.2 Mb on GGA 2 and 123.0 Mb on GGA1 and were proximal to the genes mediator complex subunit 30 (*MED30*) and connector enhancer of kinase suppressor of Ras 2 (*CNKSR2*).

Egg number

Four SNPs were significantly associated with egg number. Two of the four SNPs affecting ENA300 were located in the region 23.3-23.5 Mb on GGA11, and shared the same closest gene core-binding factor, beta subunit (*CBFB*). The other two SNPs with effects on both ENA300 and EN462 were located at 95.8 Mb on GGA1 and 31.3 Mb on GGA2, and were proximal to the genes gap junction protein, alpha 5 (*GJA5*), and serine/threonine kinase 31 (*STK31*).

DISCUSSION

Body weight at first egg

Xie et al. (2012) performed a GWAS with F_2 chickens and found that the region 169-179 Mb on GGA1 was significantly related to 23 growth traits. Besnier et al. (2011) found that QTLs in the region 169-175 Mb on GGA1 had an effect on BW56. Jin et al. (2015) found that 18 SNPs reached 5% Bonferroni genome-wide significance with growth traits in Yancheng chickens, and these SNPs, which were located on four different chromosomes and in a region of 72.3-82.1 Mb on GGA4, had a significant effect on growth traits. In the present study, six SNPs significantly associated with BWF were detected on GGA1, of which two (173.0-175.2 Mb) were within the region where SNPs were identified by Xie et al. (2012) and Besnier et al. (2011). Gu et al. (2011) found that the region 71.6-80.2 Mb on GGA4 was associated with bodyweight and average daily weight gain over 7-14 weeks. Two SNPs that significantly affect BWF were found in the present study, and they were located at 78.8 and 78.9 Mb on GGA4. Seven genes were proximal to the nine SNPs that had significant effects on BWF. However, the functions of *LOC101751412* and *LOC101748963* were not found. *GPC6* encodes a protein of the Glypican family. Glypican regulates the growth and differentiation of cells. The protein encoded by this gene is a hypothetical cell-surface receptor for growth factors (Paine-Saunders et al., 1999). A previous study found that GPC6 could regulate human endochondral ossification (Campos-Xavier et al., 2009). *MIR15A,* which is expressed in many tissues of adult chicken, is involved in tumorigenesis (Xu et al., 2006; Aqeilan et al., 2010). *COG6* encodes a subunit of the COG complex, which maintains the normal form and function of the Golgi apparatus, the processing center of proteins (Lübbehusen et al., 2010). There are

few reports on *FAM184B*. In one study, *FAM184B* was found to influence ingestion, daily weight gain, and carcass weight of bovine (Lindholm-Perry et al., 2011). *HTT* encodes the protein huntingtin (Luo and Rubinsztein, 2009), and wild type *HTT* can protect neurons and combine with *Pak2* to regulate apoptosis (Leavitt et al., 2006).

Egg weight

Tuiskula-Haavisto et al. (2002) found that a region related to egg weight at 40 weeks was located on chromosome 4. Liao et al. (2016) discovered an SNP (ss1985401190) located on GGA4, which was significantly associated with EW. In the present study, six SNPs were significantly associated with FEW (1), EW300 (3), and EW462 (2), two of which were mapped to chromosome 4. A total of six proximal genes were found, and most encoded signal transducers and regulatory factors. *KCNH7* encodes a member of the voltage-gated potassium channel subfamily H (Martínez et al., 2008), and *KCNIP4* encodes a voltage-gated potassium (Kv) channel-interacting protein (Bonne et al., 2007). Voltage-gated potassium channels regulate diverse functions such as insulin secretion and smooth muscle contraction (Danielsson et al., 2013), and these channels may affect egg weight by regulating the transfer of necessary materials. *CDC42BPA* encodes a protein that can combine with cell division cycle 42 (*CDC42*). *CDC42* encodes a variation of GTPase that regulates signaling pathways and controls diverse cellular functions (Gong et al., 1997). *CDC42BPA* may play a role in egg formation through the function of CDC42. *PPARGC1A* encodes a transcriptional coactivator that is involved in energy metabolism (Ling et al., 2008). This protein can reportedly regulate cellular cholesterol homoeostasis and excessive obesity in humans. *PPARGC1A* may affect egg weight by regulating energy and cholesterol metabolism.

Egg number

Yuan et al. (2015) detected nine genome-wide loci that significantly affected egg number during the laying phases of 21-26, 27-36, and 37-72 weeks using GWAS technology. Liao et al. (2016) found a genome-wide significant locus (ss1985401199) located on the sex chromosome Z, which was associated with egg number in hens at 25-45 weeks. In the present study, six SNPs were significantly associated with ENA300 (4) and EN462 (2). Three proximal genes (*STK31, CBFB, and GJA5*) were also found, although most have not been previously reported in chicken. *STK31* encodes a member of the TDRD family, which localizes exclusively to male germ cells and associates with germinal granules that are essential for germline development in mice (Chuma et al., 2006). One study showed that *STK31* was confined to granule-like structures in the cytoplasm of mid-to-late spermatocytes (Bao et al., 2012). *STK31* may have the same function in female chickens. *CBFB* encodes a beta subunit of a member of the PEBP2/CBF transcription factor family. This family regulates the expression of many genes, especially those required for hematopoiesis and osteogenesis, which are necessary during egg formation (Komori, 2003). *GJA5* encodes a member of the connexin gene family. This gene is a component of gap junctions, which allow small molecules to move between cells (Schmucker and Chen, 2009). Gap junctions are important in the regulation of cellular proliferation and differentiation (Liu et al., 2009).

Age at first egg (AFE) and egg number at the age of 300 days (EN300)

No SNPs associated with AFE and EN300 were found. Two reasons may account for this result. First, the size of the experimental group was limited, with only 396 individuals in the present study, which results in no significant phenotypic separation for the two traits, and preventing significant loci from being detected. The second may be that the Bonferroni correction was too strict, resulting in some of the regions with QTLs not being detected.

In conclusion, a GWAS for reproductive traits was performed in Jinghai Yellow Chicken. Nineteen SNPs had significant effects on six reproductive traits. Seventeen proximal genes were identified including *FAM184B*, *HTT*, *KCNH7*, *CDC42BPA*, *KCNIP4*, *GJA5*, *CBFB*, and *GPC6*. These results may benefit the further study of reproductive traits in chickens, and breeding programs in Jinghai Yellow Chicken.

Conflicts of interest

The authors declare no conflict of interests.

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Supplementary material

Figure S1. Manhttan plots for the six reproductive traits with genome-wide significant SNPs. 1-28 on the x-axis indicate chromosomes 1-28, and 29, 30 and 31 indicate LGE22, LGE64 and chromosome Z respectively. The magenta horizontal line shows the potential significance threshold: -log10 (3.59E-5), and the black one shows the potential significance genome-wise significance threshold: -log10 (1.80E-06).