Identification of Candidate Genomic Regions for Chicken Egg Production Traits Based on Genome Wide Association Study

Xiurong Zhao
Department of Animal Genetics and Breeding, National Engineering Laboratory for Animal Breeding, College of Animal Science and Technology, China Agricultural University, Beijing 100193

Changsheng Nie
Department of Animal Genetics and Breeding, National Engineering Laboratory for Animal Breeding, College of Animal Science and Technology, China Agricultural University, Beijing 100193

Jinxin Zhang
Department of Animal Genetics and Breeding, National Engineering Laboratory for Animal Breeding, College of Animal Science and Technology, China Agricultural University, Beijing 100193

Xinghua Li
Department of Animal Genetics and Breeding, National Engineering Laboratory for Animal Breeding, College of Animal Science and Technology, China Agricultural University, Beijing 100193

Tao Zhu
Department of Animal Genetics and Breeding, National Engineering Laboratory for Animal Breeding, College of Animal Science and Technology, China Agricultural University, Beijing 100193

Zi Guan
Department of Animal Genetics and Breeding, National Engineering Laboratory for Animal Breeding, College of Animal Science and Technology, China Agricultural University, Beijing 100193

Yu Chen
Beijing Municipal General Station of Animal Science, Beijing 100107

Liang Wang
Beijing Municipal General Station of Animal Science, Beijing 100107

XueZe Lv
Beijing Municipal General Station of Animal Science, Beijing 100107

Weifang Yang
Beijing Municipal General Station of Animal Science, Beijing 100107

Yaxiong Jia
Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing 100193

Zhonghua Ning
Department of Animal Genetics and Breeding, National Engineering Laboratory for Animal Breeding, College of Animal Science and Technology, China Agricultural University, Beijing 100193

Haiying Li
College of Animal Science, Xinjiang Agricultural University, Urumqi 830000
Changqing Qu
Engineering Technology Research Center of Anti-aging Chinese Herbal Medicine of Anhui Province, Fuyang Normal University, Fuyang, Anhui, 236037
Huie Wang
College of Animal Science, Tarim University, Alar, Xinjiang, 843300
Lujiang Qu (quluj@163.com)
Department of Animal Genetics and Breeding, National Engineering Laboratory for Animal Breeding, College of Animal Science and Technology, China Agricultural University, Beijing 100193

Research Article

Keywords: chicken, egg production, GWAS, SNPs, genomic region

Posted Date: January 27th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-148037/v1

Version of Record: A version of this preprint was published at BMC Genomics on August 10th, 2021. See the published version at https://doi.org/10.1186/s12864-021-07755-3.

Abstract

Background: Since the domestication of chicken, various chicken breeds have been developed for food production, entertainment, and so on. Compared to indigenous chicken breeds which generally do not show elite production performance, commercial breeds or lines are selected intensely for meat or egg production. In the present study, in order to understand the molecular mechanisms underlying the dramatic egg yielding differences between commercial egg-type chickens and indigenous chickens, we performed a genome-wide association study (GWAS) in a mixed linear model.

Results: We obtained 148 single nucleotide polymorphisms (SNPs) associated with egg production traits or reproductive traits (57 significantly, 91 suggestively). Among them, 18 SNPs overlapped with previously reported quantitative trait loci (QTL), including 13 for egg production and 5 for reproductive traits. Three SNPs were significantly associated with multiple egg production traits, such as egg number, age at first egg, and egg production rate in chickens. Furthermore, we identified 32 candidate genes based on the function of the screened genes. These genes were found to be mainly involved in regulating hormones, playing a role in the formation, growth, and development of follicles, and in the development of the reproductive system. Some genes such as ***NELL2***, ***KITLG***, ***GHRHR***, ***NCOA1***, ***ITPR1***, ***GAMT***, and ***CAMK4*** deserve our attention and further study since they have been reported to be closely related to reproductive traits. In addition, the most significant genomic region obtained in this study was located at 48.61-48.84Mb on GGA5. In this region, we have repeatedly annotated four genes, in which ***YY1*** and ***WDR25*** have been shown to be related to oocytes and reproductive tissues, respectively, which implies that this region may be a candidate region underlying egg production traits.

Conclusion: Our study utilized the genomic information from various chicken breeds or populations differed in egg production to understand the molecular genetic mechanisms involved in reproduction traits. We identified a series of SNPs, candidate genes, or genomic regions that associated with reproductive traits, which could help us in developing egg production in chickens.

Background

Reproduction traits, especially egg production, are the most important economic cares in chickens [1]. Laying performance usually reflects a chicken's reproductive performance [2]. As an important source of animal protein, the consumption of poultry eggs worldwide has increased significantly over the past few decades [3]. Each person consumes approximately 12.5 kg of eggs per year [4]. Egg consumption may continue to increase with accretion in urban populations [5, 6]. Therefore, it is of great practical and economic significance to understand the genetic mechanisms of chicken reproductive traits. However, egg production is a polygenic genetic trait with low to medium heritability and is affected by both genetic components and environmental factors [7, 8]. Also, its improvement by traditional breeding methods is vulnerable to environmental influences, which eventually leads to errors and difficulty in the estimation of heritability and genetic improvement [9].

It is possible to analyze the genetic mechanisms of complex traits by using genome-wide association study (GWAS) with the development of sequencing technology. GWAS can not only take full advantage of molecular markers at the genome level, but, owing to the use of whole genome sequences, avoid the effects of linkage imbalance between SNPs and underlying genes [10]. At present, a few candidate genes and regions related to egg production have been reported based on GWAS technology [9, 11–16]. According to the quantitative trait loci (QTL) database [17], 12,508 QTLs related to chicken economic traits have been identified, 982 of which are associated with reproductive traits such as egg production.

Some commercial lines or populations are intensively selected for their production traits. Rhode Island Red and White Leghorn chickens are well known for their distinguished egg productivity. Dwarf chickens in China have also been developed for egg production. The average annual egg production of them are approximately about 300 eggs. Chinese indigenous chickens grow relatively slowly and known to produce less than 200 eggs per year. Therefore, we performed a GWAS based on egg production performance differences between egg-type chickens and local chickens to explore the underlying molecular genetic mechanisms and identify candidate genes or genomic regions related to egg production traits. The results of this study are supposed to be beneficial for layer breeding.

Results

Population structure testing

The PCA results showed that there was a certain stratification phenomenon in the distribution of these 13 different breeds. At the same time, we found that Tibetan chicken was mixed with White Leghorn (Figure 1). In GWAS, population stratification might lead to false-positive results. Therefore, we added the first principal component as a covariable to minimize this impact.

Admixture software was used to analyze the population structure. We displayed a bar plot based on the cross-validation error rate (Additional file 4: Figure S1). When K=2, Rhode Island Red and one of the White Leghorn groups (WL_YQ) appeared as two differentiated clusters. When K= 3-4, two White Leghorn populations (WL_CAU, WL_YQ) gathered in the same group. When K=5, two White Leghorn populations were separated. When K= 6-9, the high productivity layers from four populations (WL_CAU, WL_YQ, RIR, DW) were separated, indicating that the genetic backgrounds of these layers were different (Figure 2). These results reinforce the subsequent analyses.

Genome wide association study

The QQ plot reflects the impact of population stratification on the GWAS [18]. The QQ plot (Figure 3b) showed that the selection of the model in this study was reasonable, effectively avoiding population stratification, and suggested that the GWAS results were reliable. In this study, λ was 1.004, which showed that there was no population stratification phenomenon and it was consistent with the QQ plot, which verified our further analysis.

The effective number of independent tests was 127,862. Hence, the threshold P value was adjusted to 3.91×10^{-7} for a genome-wide significance level, and 7.82×10^{-6} for a genome-wide suggestive significance level. This means that SNPs with P values below 7.82×10^{-6} are considered and may be associated with egg production traits.

After correction, we found 148 SNPs that could be associated with egg production (57 significantly, 91 suggestively) (Additional file 1: Table S1). The global view of P-values (in terms of -log10 (P-value)) for all SNPs was represented by a Manhattan plot, as shown in Figure 3a. Table 2 lists the SNP information that was emphasized in this study. Using Ensembl to annotate related SNPs, we found a total of 68 genes around significant peaks, and identified 32 candidate genes associated with egg production according to their functions (Table 3). Some genes such as ***NELL2***, ***KITLG***, ***GHRHR***, ***NCOA1***, ***ITPR1***, ***GAMT***, and ***CAMK4***, which have been proved to be related to egg production, litter size, or reproductive traits, are worth a deeper exploration [19-32].

In addition, the most significant peak in this study was located at 48.61-48.84Mb on chromosome 5. The chi-square test was carried out to compare the allele frequencies of the significant SNPs identified in this region between the high and low productivity groups. The results showed that the allele frequencies of these 20 SNPs were significantly different between the two groups (Additional file 2: Table S2). At the same time, we annotated 4 genes many times in this region. ***YY1*** (YY1 transcription factor) is involved in oocyte growth and maturation [33]. ***SLC25A29*** is a member of the solute carrier family 25 and is involved in the transport of amino acids. ***WDR25*** (WD repeat domain 25) may be related to the reproductive tissues [34]. Brain enriched guanylate kinase (***BEGAIN***) is a gene specifically expressed in the brain and is involved in the regulation of postsynaptic neurotransmitter receptor activity [35].

Comparing with previously reported QTLs

Through Animal QTLdb, we detected 18 QTLs that overlapped with SNPs obtained from this study. Thirteen of these 18 QTLs were associated with egg production, including 3 with egg number, 8 with age at first egg, 3 with egg production rate, 1 with ovarian follicle weight, and 3 with small yellow follicle number. The remaining five QTLs were related to reproductive traits, including four with ovary weight, three with oviduct length, and two with oviduct weight. Some QTLs (e.g., AX-75225234, AX-76713110, and AX-76715084) were related to multiple traits (Additional file 3: Table S3).

Discussion

GWAS and QTL overlapping

An important condition for GWAS to achieve better results is to eliminate false associations caused by differences in allele frequencies arising from population stratification, recessive kinship, and genotyping errors [36]. The GEMMA adopted in this study considers the group stratification and sample structure. At the same time, we also added the first principal component as a covariate to reduce the group

stratification effect. The results of the QQ plot and λ show that the correction effect is good, and there is no population stratification phenomenon.

Eighteen of the 148 QTLs in this study were those identified in previous studies. Among these overlapping QTLs, 13 QTLs were associated with egg production. This reinforces the results of our study. Moreover, it is worth noting that AX-75225234, AX-76713110, and AX-76715084 are related to multiple traits, including egg number, age at first egg, egg production rate, and small yellow follicle number. AX-75225234 is located 0.1Mb downstream of the ***ENSGALG00000036169*** gene on chromosome 1. AX-76713110 is located 0.029Mb downstream of the ***ENSGALG00000041624*** gene and 0.042Mb downstream of the ***GABRA4*** (gamma-aminobutyric acid type A receptor alpha4 subunit) gene on chromosome 4. AX-76715084 is located 0.046Mb upstream of the ***GRXCR1*** (glutaredoxin and cysteine-rich domain containing 1) gene on chromosome 4. Although these genes have not been very well studied in chickens, and their functions have not been fully elucidated, they provide a reference and idea to understand the molecular mechanism for egg production traits.

Candidate genes

As far as we know, this study has the largest variety of breeds so far in the research of reproductive traits in chicken, which not only improves the detection ability of related QTLs, but also allows us to detect some QTLs related to fat and heat resistance. We speculate that this was due to the relatively slow fat formation and deposition [37, 38] and the relatively high heat generation of layers [39]. Thus, we identified 32 candidate genes based on their function. These genes mainly affect egg production in three ways (Table 3). Some genes regulate hormone levels, including gonadotropin-releasing hormone (GNRH), oxytocin (OXT), growth hormone (GH), and thyroid hormone (TH). All these hormones play a vital role in the female reproductive system [40-45]. Some genes affect egg production traits by affecting the growth and development of follicles. It is well known that the growth and development of follicles are critical for reproductive function, especially in chickens. The remaining genes directly affect reproductive system development. Among these 32 candidate genes, we also found 7 important genes that have been identified as related to reproductive traits such as egg production and litter size in previous studies, which further validates our findings. ***NELL2*** (neural EGFL like 2) and ***KITLG*** (KIT ligand) are located on GGA1. ***NELL2*** not only affects the synthesis and secretion of GNRH [46], but has also been shown to be involved in maintaining the normal female reproductive cycle of mammals [19]. ***KITLG*** plays an important role in the growth and development of follicles and is an essential gene for ovarian development and survival of primordial follicles [20, 47]. It plays a significant role in the reproductive process of animals and has been considered an excellent candidate gene for reproductive traits of humans and livestock [21]. At the same time, it has been shown to be related to the litter size of goat and sheep [20-22]. Therefore, it is reasonable to speculate that ***KITLG*** has an important impact on egg production traits. Growth hormone releasing hormone receptor (***GHRHR***) located on GGA2 participates in the secretion and synthesis of GH. It is believed to be involved in the growth and reproduction of livestock [48]. Liu et al. identified three SNPs in the ***GHRHR*** promoter that are significantly related to egg production traits in Beijing You chickens [23]. ***NCOA1*** (Nuclear receptor coactivator 1), located on GGA3, is involved in regulating signal pathways

mediated by TH and estrogen. Both TH and estrogen play major roles in reproductive processes. Furthermore, ***NCOA1*** has been shown to be an important gene that influences reproductive traits in pigs and sheep [24-27]. Also, Mahmood Gholami et al. and Huang et al. have proved that ***NCOA1*** is related to egg production, fertility, and reproductive traits in chicken [28, 29]. ***ITPR1*** repeatedly annotated in the 18.56-18.57Mb on chromosome 12 can not only participate in the signaling pathway of GnRH, estrogen, and the synthesis and secretion of GH and TH, but also affects the growth and differentiation of follicles. Both are key events in the reproductive process. The ***ITPR1*** gene was also located in a region previously reported reproduction-related (Additional file 3: Table S3). In addition, ***ITPR1*** has been reported to be involved in the transport of Ca^{2+} and may be associated with egg number [30]. This suggests that this region located on chromosome 12 and the ***ITPR1*** gene may be important for chicken egg production traits. ***GAMT*** (guanidinoacetate N-methyltransferase) located on GGA28 has been shown to be associated with the reproductive system and development [31]. ***CAMK4*** (calcium/calmodulin-dependent protein kinase IV) located on chromosome Z is involved in the signaling pathway of OXT and may play a role in the development of follicles and ovulation [49]. It is believed to play a significant role in the reproductive processes of females [32]. However, ***NELL2***, ***GAMT***, and ***CAMK4*** have not been studied before in chickens, and the results of this study may pave the way for future researchers to explore the relationship between these genes and egg production traits.

Our results provide avenues for further exploration of the genetic mechanisms underlying egg production traits. Also, the specific functions of these genes need to be further verified.

Candidate region

In this study, the most significant peak obtained was located at 48.61-48.84Mb region on GGA5. In this region, ***YY1***, ***SLC25A29***, ***WDR25***, and ***BEGAIN*** were annotated. Among them, ***YY1*** and ***WDR25*** have been shown to be related to oocytes and reproductive tissues, respectively [33, 34]. However, there is no concrete literature to prove that they are associated with egg production traits in chickens, thus further research is still required. At the same time, interestingly enough, a number of studies have detected regions associated with egg production traits on chromosome 5 [12, 15, 50]. The region identified in our study was about 1.2 Mb away from the QTL reported by Zhang et al. [15]. Although the results are different, it has once again proved that chromosome 5 is an important candidate region that affects the reproductive traits of chickens.

Conclusions

In this study, we performed a GWAS based on the egg production performance difference between high productivity layers and Chinese indigenous chickens and identified a series of SNPs and candidate genes related to reproductive traits. Eighteen of the SNP effects overlapped with previously reported QTL regions, which supports the results of this study. These results may help us to better understand the molecular mechanisms underlying reproductive traits in chickens and even other species .

Materials And Methods

Experimental animals

We selected egg-type chickens and Chinese indigenous chickens to represent the high and low egg production groups, respectively. Among them, Rhode Island Red, White Leghorn, and Dwarf layer chickens producing about 300 eggs per year were placed in the high egg productivity group. Ten Chinese indigenous breeds laying less than 200 eggs annually were classified into the low egg productivity group. The details of the samples are presented in Table 1.

Genotyping and Quality Control

In total, blood samples from 442 chickens from the high and low egg production groups were collected by standard venipuncture. After DNA extraction using the standard phenol/chloroform method [51], the chickens were genotyped using a 600K Affymetrix Axiom Chicken Genotyping Array with a total of 580,861 SNPs [52]. Quality control was performed using Plink v1.9 [53]. SNPs with a minor allele frequency ≥ 1% and genotyping rate ≥ 98% were retained. Individuals with a genotype deletion rate of >5% were excluded. SNPs with Hardy-Weinberg equilibrium $P < 10^{-6}$ were eliminated. After filtering, 439 chickens, including 218 in high and 221 in low egg production groups, and 456,647 SNPs were retained for further analyses.

Population structure analysis

Pink1.9 was used for principal component analysis (PCA) to determine the population structure, and the "ggplot2" package in R studio was used to visualize the results of PCA. We selected the first two principal components with the largest variance interpretation rate as the horizontal and vertical coordinates to create a PCA plot to check the stratification status of the population. The principal component contribution rate was calculated based on eigenvalues.

We retained relatively dependent SNPs with the plink '–indep-pairwise 25 5 0.2' command. The genetic structure was estimated using Admixture software [54]. We calculated the ancestor coefficient matrix, simulated the situation of genetic clusters (K) from 1 to 20, and computed the cross-validation error rate. Furthermore, we used an online pophelper to display a population structure bar plot (http://pophelper.com/) [55].

Genome wide association study

We performed a GWAS by using a univariate mixed linear model in GEMMA [56]. The model is as follows:

$$y=W\alpha+x\beta+u+\varepsilon$$

where $\boldsymbol{y}$ denotes a phenotypic value vector of 440 individuals, $\boldsymbol{W}$ is a matrix of covariates (fixed effects that contain a column of 1s and the first principal component), $\boldsymbol{\alpha}$ represents a vector of the corresponding

coefficients consisting of intercepts, $\boldsymbol{x}$ is a vector of marker genotypes, $\boldsymbol{\beta}$ is the effect size of a marker, $\boldsymbol{u}$ is a random-effects vector of an individual, and $\boldsymbol{\varepsilon}$ is a vector of random residuals. In this study, the Wald statistic was used to test each SNP.

Manhattan and Quantile-Quantile (QQ) plot were made by R package "CMplot" and "qqman" respectively, and we also calculated the genomic inflation factors (λ) to judge the degree of false-positive [57].

The traditional Bonferroni correction is too strict, resulting in a higher false-negative rate and omission of some SNPs truly associated with the target trait [58]. Therefore, in this study, we calculated the sum of the number of independent SNPs and LD blocks for correction [59].

Bioinformatics analysis of candidate regions

Ensembl (http://www.ensembl.org/index.html) databases were used to annotate the screened associated SNPs to identify possible candidate genes or regions. We then checked the biological functions of these genes in PubMed (https://pubmed.ncbi.nlm.nih.gov).

Overlap with known QTLs

In addition, regions within 5 Mb of a candidate SNP were searched for previously reported QTLs with egg production or reproductive traits in the chicken QTL database (https://www.animalgenome.org/cgi-bin/QTLdb/GG/index).

List Of Abbreviations

GWAS: Genome-wide association study

SNPs: Single nucleotide polymorphisms

QTL: Quantitative trait loci

PCA: Principal component analysis

λ: Genomic inflation factors

Declarations

Ethics approval

We confirm that standard techniques were performed to collect blood samples from animals and all experimental protocols related to animal experimentation in this study were reviewed and approved by the Animal Welfare Committee of China Agricultural University. The study was carried out in compliance with the ARRIVE guidelines.

Consent for publication

Not applicable.

Availability of data and materials

All data generated and/or analysed during this study are not publicly available due study is still being continuous, but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was funded by the earmarked fund for the Beijing Innovation Team of the Modern Agro-industry Technology Research System (BAIC04-2021); Anhui Province Science and Technology Major Special Project (202003a06020020, 201904f06020018); Key Natural Science Research Project of Anhui Province, Department of Education (KJ2020A0520); Cooperative Innovation Project of universities in Anhui Province (GXXT-2019-049).

Authors' contributions

LQ and ZN conceived and designed the experiment. XZ performed bioinformatics analyses. CN, JZ, XLi, TZ, ZG, WY and XZ performed the experiments and interpreted the result data. YC, LW, XLv, YJ, HL, CQ and HW contributed resources and funding. LQ and XZ led the manuscript writing. All authors read and approved the final manuscript.

Acknowledgments

We are grateful for all of our colleagues of the Poultry Team of the National Engineering Laboratory for Animal Breeding of China Agricultural University.

References

1. Liu WJ, Sun DX, Yu Y, Li G, Tang SQ, Zhang Y, Wang YC, Zhang Y: **Association of Janus kinase 2 polymorphisms with growth and reproduction traits in chickens**. *Poultry Science* 2010, **89**(12):2573–2579.
2. Du Y, Liu L, He Y, Dou T, Jia J, Ge C: **Endocrine and genetic factors affecting egg laying performance in chickens: a review**. *Br Poult Sci* 2020, **61**(5):538–549.
3. Mueller S, Kreuzer M, Siegrist M, Mannale K, Messikommer RE, Gangnat IDM: **Carcass and meat quality of dual-purpose chickens (Lohmann Dual, Belgian Malines, Schweizerhuhn) in comparison to broiler and layer chicken types**. *Poult Sci* 2018, **97**(9):3325–3336.

4. Hjellnes V, Šližyte R, Rustad T, Carvajal AK, Greiff K: **Utilization of egg-laying hens (Gallus Gallus domesticus) for production of ingredients for human consumption and animal feed**. *BMC Biotechnol* 2020, **20**(1):22.
5. Yang Z, Rose SP, Yang HM, Pirgozliev V, Wang ZY: **Egg production in China**. *World's Poultry Science Journal* 2018, **74**(3):417–426.
6. Goto T, Fernandes AFA, Tsudzuki M, Rosa GJM: **Causal phenotypic networks for egg traits in an F(2) chicken population**. *Mol Genet Genomics* 2019, **294**(6):1455–1462.
7. Zhang L, Li DY, Liu YP, Wang Y, Zhao XL, Zhu Q: **Genetic effect of the prolactin receptor gene on egg production traits in chickens**. *Genet Mol Res* 2012, **11**(4):4307–4315.
8. Qin N, Liu Q, Zhang YY, Fan XC, Xu XX, Lv ZC, Wei ML, Jing Y, Mu F, Xu RF: **Association of novel polymorphisms of forkhead box L2 and growth differentiation factor-9 genes with egg production traits in local Chinese Dagu hens**. *Poult Sci* 2015, **94**(1):88–95.
9. Liu Z, Yang N, Yan Y, Li G, Liu A, Wu G, Sun C: **Genome-wide association analysis of egg production performance in chickens across the whole laying period**. *BMC Genet* 2019, **20**(1):67.
10. Wang J, Yuan X, Ye S, Huang S, He Y, Zhang H, Li J, Zhang X, Zhang Z: **Genome wide association study on feed conversion ratio using imputed sequence data in chickens**. *Asian-Australas J Anim Sci* 2019, **32**(4):494–500.
11. Dong X, Li J, Zhang Y, Han D, Hua G, Wang J, Deng X, Wu C: **Genomic Analysis Reveals Pleiotropic Alleles at EDN3 and BMP7 Involved in Chicken Comb Color and Egg Production**. *Front Genet* 2019, **10**:612.
12. Wolc A, Arango J, Jankowski T, Dunn I, Settar P, Fulton JE, O'Sullivan NP, Preisinger R, Fernando RL, Garrick DJ *et al*: **Genome-wide association study for egg production and quality in layer chickens**. *J Anim Breed Genet* 2014, **131**(3):173–182.
13. Liu W, Li D, Liu J, Chen S, Qu L, Zheng J, Xu G, Yang N: **A genome-wide SNP scan reveals novel loci for egg production and quality traits in white leghorn and brown-egg dwarf layers**. *PLoS One* 2011, **6**(12):e28600.
14. Yuan J, Sun C, Dou T, Yi G, Qu L, Qu L, Wang K, Yang N: **Identification of Promising Mutants Associated with Egg Production Traits Revealed by Genome-Wide Association Study**. *PLoS One* 2015, **10**(10):e0140615.
15. Zhang GX, Fan QC, Wang JY, Zhang T, Xue Q, Shi HQ: **Genome-wide association study on reproductive traits in Jinghai Yellow Chicken**. *Anim Reprod Sci* 2015, **163**:30–34.
16. Liao R, Zhang X, Chen Q, Wang Z, Wang Q, Yang C, Pan Y: **Genome-wide association study reveals novel variants for growth and egg traits in Dongxiang blue-shelled and White Leghorn chickens**. *Anim Genet* 2016, **47**(5):588–596.
17. Hu ZL, Park CA, Wu XL, Reecy JM: **Animal QTLdb: an improved database tool for livestock animal QTL/association data dissemination in the post-genome era**. *Nucleic Acids Res* 2013, **41**(Database issue):D871-879.

18. Jiang Y, Tang S, Wang C, Wang Y, Qin Y, Wang Y, Zhang J, Song H, Mi S, Yu F *et al*: **A genome-wide association study of growth and fatness traits in two pig populations with different genetic backgrounds**. *J Anim Sci* 2018, **96**(3):806–816.
19. Ryu BJ, Kim HR, Jeong JK, Lee BJ: **Regulation of the female rat estrous cycle by a neural cell-specific epidermal growth factor-like repeat domain containing protein, NELL2**. *Mol Cells* 2011, **32**(2):203–207.
20. An XP, Hou JX, Li G, Song YX, Wang JG, Chen QJ, Cui YH, Wang YF, Cao BY: **Polymorphism identification in the goat KITLG gene and association analysis with litter size**. *Anim Genet* 2012, **43**(1):104–107.
21. An XP, Hou JX, Lei YN, Gao TY, Song YX, Wang JG, Cao BY: **Two mutations in the 5'-flanking region of the KITLG gene are associated with litter size of dairy goats**. *Anim Genet* 2015, **46**(3):308–311.
22. An XP, Hou JX, Gao TY, Lei YN, Song YX, Wang JG, Cao BY: **Association analysis between variants in KITLG gene and litter size in goats**. *Gene* 2015, **558**(1):126–130.
23. Liu W, Yu Y, Li G, Tang S, Zhang Y, Wang Y, Zhang S, Zhang Y: **Single-nucleotide polymorphisms in the promoter of the growth hormone-releasing hormone receptor gene are associated with growth and reproduction traits in chickens**. *Anim Genet* 2012, **43**(5):564–569.
24. Kamei Y, Xu L, Heinzel T, Torchia J, Kurokawa R, Gloss B, Lin SC, Heyman RA, Rose DW, Glass CK *et al*: **A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors**. *Cell* 1996, **85**(3):403–414.
25. Razeto A, Ramakrishnan V, Litterst CM, Giller K, Griesinger C, Carlomagno T, Lakomek N, Heimburg T, Lodrini M, Pfitzner E *et al*: **Structure of the NCoA-1/SRC-1 PAS-B domain bound to the LXXLL motif of the STAT6 transactivation domain**. *J Mol Biol* 2004, **336**(2):319–329.
26. Glass CK: **Differential recognition of target genes by nuclear receptor monomers, dimers, and heterodimers**. *Endocr Rev* 1994, **15**(3):391–407.
27. Yuan Z, Zhang J, Li W, Wang W, Li F, Yue X: **Association of Polymorphisms in Candidate Genes with the Litter Size in Two Sheep Breeds**. *Animals (Basel)* 2019, **9**(11).
28. Gholami M, Erbe M, Gärke C, Preisinger R, Weigend A, Weigend S, Simianer H: **Population genomic analyses based on 1 million SNPs in commercial egg layers**. *PLoS One* 2014, **9**(4):e94509.
29. Huang HY, Li SF, Zhao ZH, Liang Z, Zhang J, Ding YR: **Association of polymorphisms for nuclear receptor coactivator 1 gene with egg production traits in the maternal line of Shaobo hens**. *Br Poult Sci* 2011, **52**(3):328–332.
30. Tarsani E, Kranis A, Maniatis G, Avendano S, Hager-Theodorides AL, Kominakis A: **Deciphering the mode of action and position of genetic variants impacting on egg number in broiler breeders**. *BMC Genomics* 2020, **21**(1):512.
31. Singh V, Bala R, Chakraborty A, Rajender S, Trivedi S, Singh K: **Duplications in 19p13.3 are associated with male infertility**. *J Assist Reprod Genet* 2019, **36**(10):2171–2179.
32. Zhang H, Yu JQ, Yang LL, Kramer LM, Zhang XY, Na W, Reecy JM, Li H: **Identification of genome-wide SNP-SNP interactions associated with important traits in chicken**. *BMC Genomics* 2017, **18**(1):892.

33. Griffith GJ, Trask MC, Hiller J, Walentuk M, Pawlak JB, Tremblay KD, Mager J: **Yin-yang1 is required in the mammalian oocyte for follicle expansion**. *Biol Reprod* 2011, **84**(4):654–663.
34. Jin F, Dai J, Ji C, Gu S, Wu M, Qian J, Xie Y, Mao Y: **A novel human gene (WDR25) encoding a 7-WD40-containing protein maps on 14q32**. *Biochem Genet* 2004, **42**(11–12):419–427.
35. Deguchi M, Hata Y, Takeuchi M, Ide N, Hirao K, Yao I, Irie M, Toyoda A, Takai Y: **BEGAIN (brain-enriched guanylate kinase-associated protein), a novel neuronal PSD-95/SAP90-binding protein**. *J Biol Chem* 1998, **273**(41):26269–26272.
36. Raeesi V, Ehsani A, Torshizi RV, Sargolzaei M, Masoudi AA, Dideban R: **Genome-wide association study of cell-mediated immune response in chicken**. *J Anim Breed Genet* 2017, **134**(5):405–411.
37. Willson NL, Forder REA, Tearle RG, Nattrass GS, Hughes RJ, Hynd PI: **Evaluation of fatty acid metabolism and innate immunity interactions between commercial broiler, F1 layer × broiler cross and commercial layer strains selected for different growth potentials**. *J Anim Sci Biotechnol* 2017, **8**:70.
38. Griffin HD, Butterwith SC, Goddard C: **Contribution of lipoprotein lipase to differences in fatness between broiler and layer-strain chickens**. *Br Poult Sci* 1987, **28**(2):197–206.
39. Sato M, Tachibana T, Furuse M: **Heat production and lipid metabolism in broiler and layer chickens during embryonic development**. *Comp Biochem Physiol A Mol Integr Physiol* 2006, **143**(3):382–388.
40. Maggi R, Cariboni AM, Marelli MM, Moretti RM, Andrè V, Marzagalli M, Limonta P: **GnRH and GnRH receptors in the pathophysiology of the human female reproductive system**. *Hum Reprod Update* 2016, **22**(3):358–381.
41. Ma Y, Ladisa C, Chang JP, Habibi HR: **Multifactorial control of reproductive and growth axis in male goldfish: Influences of GnRH, GnIH and thyroid hormone**. *Mol Cell Endocrinol* 2020, **500**:110629.
42. Prapaiwan N, Manee-In S, Olanratmanee E, Srisuwatanasagul S: **Expression of oxytocin, progesterone, and estrogen receptors in the reproductive tract of bitches with pyometra**. *Theriogenology* 2017, **89**:131–139.
43. Ji H, Liu N, Yin Y, Wang X, Chen X, Li J, Li J: **Oxytocin inhibits ovarian cancer metastasis by repressing the expression of MMP-2 and VEGF**. *J Cancer* 2018, **9**(8):1379–1384.
44. Hull KL, Harvey S: **Growth hormone and reproduction: a review of endocrine and autocrine/paracrine interactions**. *Int J Endocrinol* 2014, **2014**:234014.
45. Cai YY, Lin N, Zhong LP, Duan HJ, Dong YH, Wu Z, Su H: **Serum and follicular fluid thyroid hormone levels and assisted reproductive technology outcomes**. *Reprod Biol Endocrinol* 2019, **17**(1):90.
46. Zhou SS, Li P: **Effects of NELL2 on the regulation of GnRH expression and puberty in female rats**. *Genet Mol Res* 2014, **13**(3):6672–6682.
47. Sun YX, Zhang YX, Zhang D, Xu CM, Chen SC, Zhang JY, Ruan YC, Chen F, Zhang RJ, Qian YQ *et al*: **XCI-escaping gene KDM5C contributes to ovarian development via downregulating miR-320a**. *Hum Genet* 2017, **136**(2):227–239.

48. Dettori ML, Pazzola M, Paschino P, Amills M, Vacca GM: **Association between the GHR, GHRHR, and IGF1 gene polymorphisms and milk yield and quality traits in Sarda sheep**. *J Dairy Sci* 2018, **101**(11):9978–9986.
49. Wu JY, Gonzalez-Robayna IJ, Richards JS, Means AR: **Female fertility is reduced in mice lacking Ca2+/calmodulin-dependent protein kinase IV**. *Endocrinology* 2000, **141**(12):4777–4783.
50. Goraga ZS, Nassar MK, Brockmann GA: **Quantitative trait loci segregating in crosses between New Hampshire and White Leghorn chicken lines: I. egg production traits**. *Anim Genet* 2012, **43**(2):183–189.
51. Nie C, Zhang Z, Zheng J, Sun H, Ning Z, Xu G, Yang N, Qu L: **Genome-wide association study revealed genomic regions related to white/red earlobe color trait in the Rhode Island Red chickens**. *BMC Genetics* 2016, **17**(1):115.
52. Kranis A, Gheyas AA, Boschiero C, Turner F, Yu L, Smith S, Talbot R, Pirani A, Brew F, Kaiser P *et al*: **Development of a high density 600K SNP genotyping array for chicken**. *BMC Genomics* 2013, **14**:59.
53. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ *et al*: **PLINK: a tool set for whole-genome association and population-based linkage analyses**. *Am J Hum Genet* 2007, **81**(3):559–575.
54. Alexander DH, Novembre J, Lange K: **Fast model-based estimation of ancestry in unrelated individuals**. *Genome Res* 2009, **19**(9):1655–1664.
55. Francis RM: **pophelper: an R package and web app to analyse and visualize population structure**. *Molecular Ecology Resources* 2017, **17**(1):27–32.
56. Zhou X, Stephens M: **Genome-wide efficient mixed-model analysis for association studies**. *Nat Genet* 2012, **44**(7):821–824.
57. Shen M, Qu L, Ma M, Dou T, Lu J, Guo J, Hu Y, Yi G, Yuan J, Sun C *et al*: **Genome-Wide Association Studies for Comb Traits in Chickens**. *PLoS One* 2016, **11**(7):e0159081.
58. Bland JM, Altman DG: **Multiple significance tests: the Bonferroni method**. *BMJ* 1995, **310**(6973):170.
59. Nicodemus KK, Liu W, Chase GA, Tsai Y-Y, Fallin MD: **Comparison of type I error for multiple test corrections in large single-nucleotide polymorphism studies using principal components versus haplotype blocking algorithms**. *BMC Genetics* 2005, **6**(1):S78.

Tables

Due to technical limitations, table 1,2,3 is only available as a download in the Supplemental Files section.

Figures

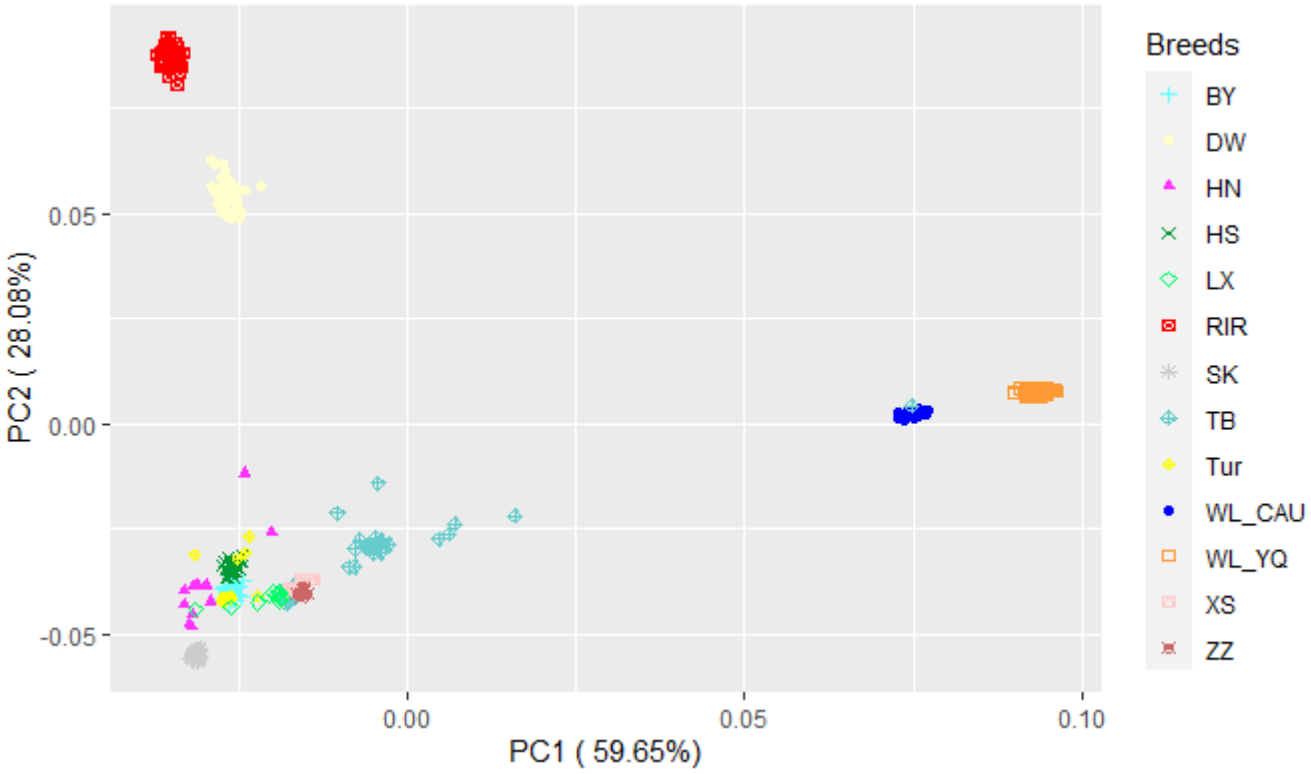

Figure 1

PCA plot of chicken populations in this study. Each color represents a breed and the abbreviations are as defined in Table1. PC1, principal components one; PC2, principal components two. The contribution rates of PC1 and PC2 are 59.65% and 28.08 % respectively.

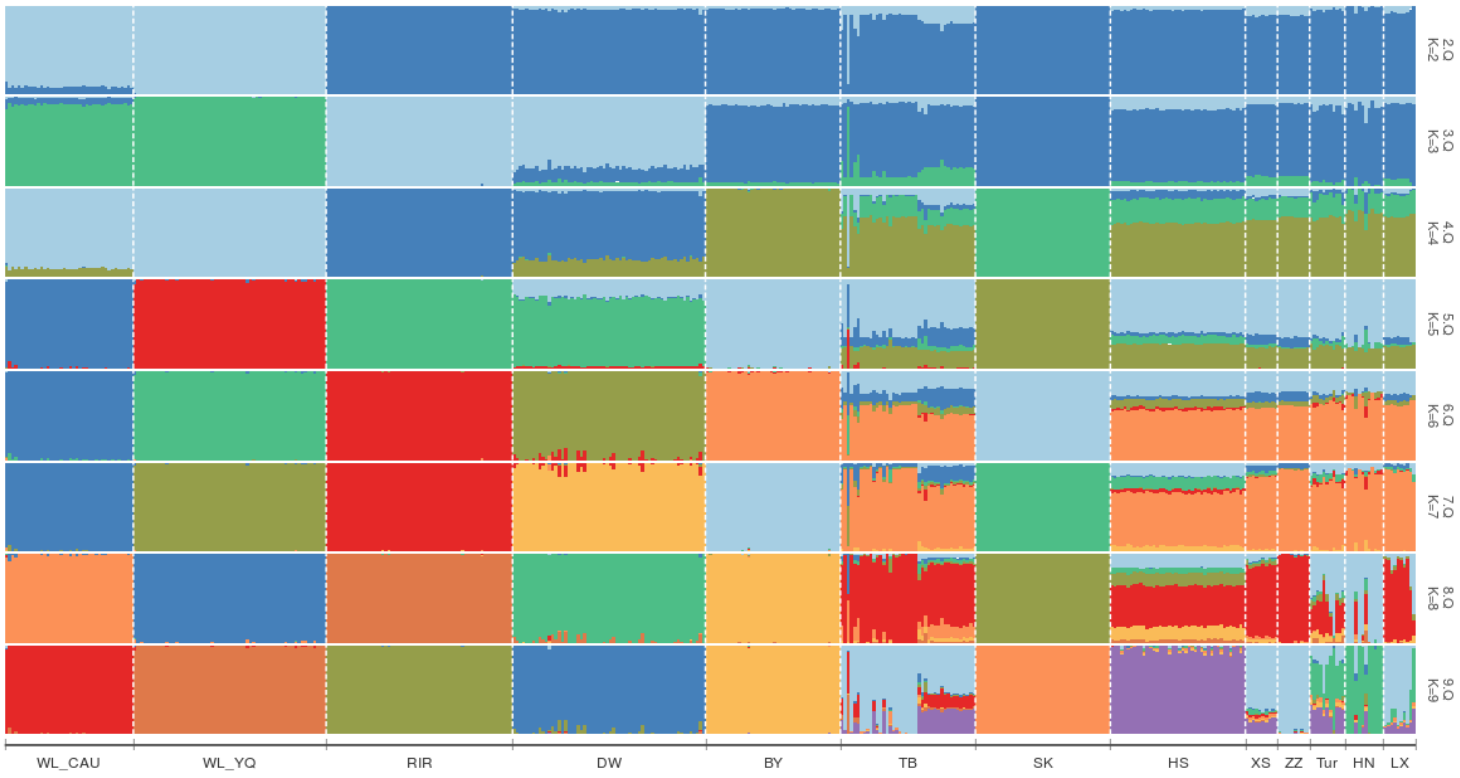

Figure 2

Admixture plot. Each color represents separate groups, each line represents a group value.

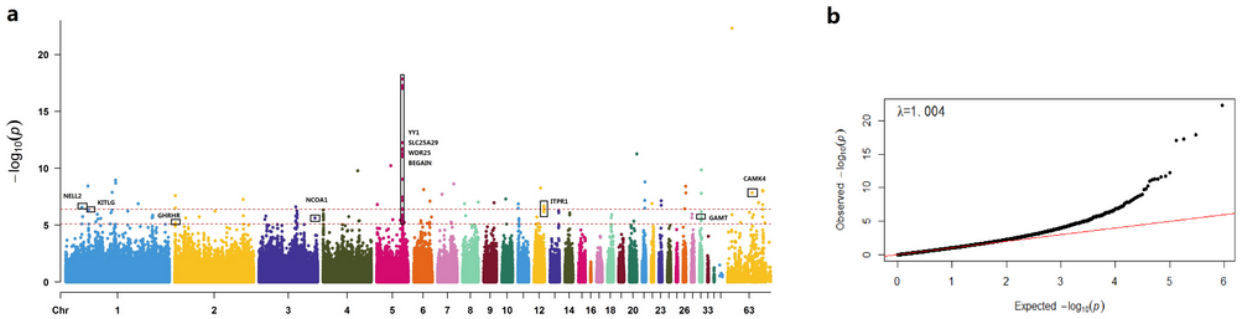

Figure 3

Manhattan and QQ plot of the reproductive traits. a In Manhattan plot, the x-axis is the position of each SNP on the chicken chromosomes (34, 40, 63 indicate LEG64, W, and Z respectively), and the y-axis is the -log10 P-value. The horizontal red dotted line at the top indicates the genome-wide significant thresholds is 3.91 × 10−7, line at the bottom represents the genome-wide suggestively thresholds is 7.82 × 10−6. b The QQ plot shows the expected -log10 P-value (the x-axis) plotted against the observed -log10 P-value (y-axis). In the top left of the QQ plot, the genomic inflation factors (λ) is shown as 1.004.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.xlsx
- Table2.xlsx
- Table3.xlsx
- AdditionalFile.xlsx